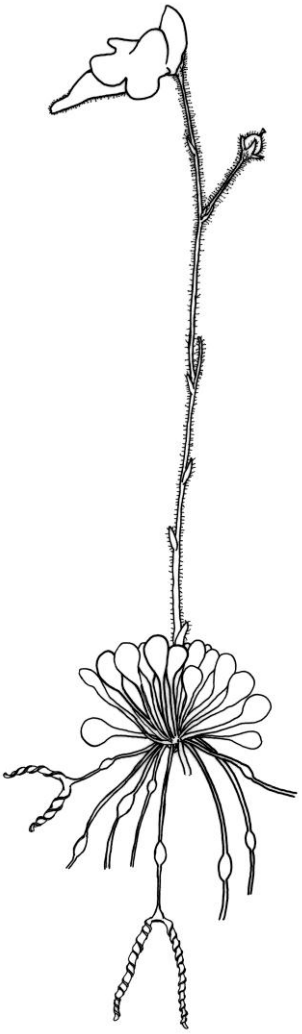
Phylogenetic relationships, systematics, and biology of carnivorous Lamiales, with special focus on the genus *Genlisea* (Lentibulariaceae)



Dissertation zur Erlangung des Doktorsgrades der Naturwissenschaften (Dr. rer. nat.) der Fakultät für Biologie der Ludwig-Maximilians-Universität München

vorgelegt von
Andreas Fleischmann
München, 28.11.2011

Erstgutachter: Prof. Dr. Günther Heubl
Zweitgutachter: Prof. Dr. Susanne Renner

Tag der mündlichen Prüfung: 29.03.2012

Eidesstattliche Versicherung und Erklärung

Eidesstattliche Versicherung

Ich, Andreas Fleischmann, versichere hiermit an Eides statt, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Beihilfe angefertigt ist.

München, den 28. 11. 2011 ……..…….....
Andreas Fleischmann

Erklärung

Hiermit erkläre ich, Andreas Fleischmann, dass ich mich anderweitig einer Doktorprüfung nicht unterzogen habe, und dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist.

München, den 28. 11. 2011 ……..…….....
Andreas Fleischmann

Declaration of contribution as co-author

In this cumulative thesis, the results from Andreas Fleischmann's doctoral research are presented, which was carried out under the supervision of Prof. Günther Heubl at the Ludwig-Maximilians-University of Munich. The following six articles have been published in international peer-reviewed journals, and are presented in the appendix of the thesis. All of them have resulted from collaborations with other scientists, and the author's contributions to each of them were as follows:

Contribution to Article I:
Schäferhoff, B., **Fleischmann, A.**, Fischer, E., Albach, D.C., Borsch, T., Heubl, G., Müller, K.F., 2010. Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. BMC Evolutionary Biology 10: 352–374.

A. Fleischmann contributed to the taxon sampling, DNA extraction, and sequence generating, wrote parts of the manuscript and provided photographs for the figures.
B. Schäferhoff generated sequence data, made the data analyses and drafted the manuscript. E. Fischer improved the manuscript and contributed to the conception of the study during its initial phase. D.C. Albach provided data, contributed to the conception and improved the manuscript. T. Borsch provided plant material and contributed during manuscript preparation. G. Heubl improved the manuscript in its final phase. K.F. Müller was responsible for the conception of the study and helped writing the manuscript.

Contribution to Article II:
Fleischmann, A., Schäferhoff, B., Heubl, G., Rivadavia, F., Barthlott, W., Müller, K.F., 2010. Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae). Molecular Phylogenetics and Evolution 56: 768–783.

A. Fleischmann planned, coordinated and conducted the sampling of plant material on joint field trips to Sierra Leone, South Africa, Zambia, Venezuela and Brazil. He conducted the laboratory work, including DNA extraction, PCR amplification, PCR product purification, subsequent sequence editing and alginment, and phylogenetic analyses. The design and preparation of the figures and diagrams, and the manuscript concept and writing was done by A. Fleischmann.
B. Schäferhoff conducted the amplification and sequencing of the *trnK* intron, and contributed to the data analyses. G. Heubl supervised the laboratory work and phylogenetic analyses, and improved the manuscript. F. Rivadavia provided herbarium specimens for DNA extraction and morphometric analyses, and contributed to the manuscript. W. Barthlott improved the manuscript in its final stage. K.F. Müller supervised the data analysis and corrected the manuscript.

Contribution to Article III:
Fleischmann, A., Rivadavia, F., Gonella, P.M., Heubl, G., 2011. A revision of *Genlisea* subgenus *Tayloria* (Lentibulariaceae). Phytotaxa 33: 1-40.

A. Fleischmann wrote the manuscript (including the taxonomic treatments, the Latin diagnoses, and he designed the identification key to the species), and drew all figures, maps, and botanical illustrations. He made the morphometric measurements and analyses based on examination of herbarium specimens in B, K, M, RB, SPF, UEC, and plants studied *in situ* in Brazil. All but four of the flower photographs illustrating the plants at their natural habitats were taken by A. Fleischmann. He conducted the microscopic seed preparations and SEM images.

F. Rivadavia conducted the initial fieldwork, provided herbarium specimens, and contributed to the manuscript. P.M. Gonella helped with measurements of herbarium specimens, contributed to the manuscript, provided photographs, and helped with fieldwork in Brazil. G. Heubl improved the manuscript.

Contribution to Article IV:
Beck, S.G., **Fleischmann, A.**, Huaylla, H., Müller, K.F., Borsch, T., 2008. *Pinguicula chuquisacensis* (Lentibulariaceae), a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula*. Willdenowia 38: 201–212.

Andreas Fleischmann wrote the entire taxonomic part of the article, including the species description, the Latin diagnosis, and the identification key for the Bolivian species of *Pinguicula*. He examined the herbarium material and made the morphological analyses for the species description, and he made the botanical line drawing based on the studied type specimens. He wrote major parts of the introduction and discussion parts. Further, he extracted DNA from herbarium specimens or plants from his private living collection for all newly sampled taxa of *Pinguicula* for this article.
S.G. Beck introduced the new species to the knowledge of the other co-authors, and improved the manuscript. H. Huaylla contributed habitat description and observations made on living plants at the locus classicus. K.F. Müller performed the data analysis, and wrote the material and methods part. T. Borsch was responsible for DNA amplification and generated the molecular sequences, and contributed to all parts of the manuscript.

Contribution to Article V:
Fleischmann, A., Rivadavia, F., 2009. *Utricularia rostrata* (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil. Kew Bulletin 64: 155–159.

A. Fleischmann wrote the manuscript and taxonomical treatment of the new species (including the Latin diagnosis and the identification key), made the morphological measurments and microscope analyses, the SEM images of the seed, and drew the botanical illustration.
F. Rivadavia discovered the new species, provided herbarium specimens for morphological analysis, drew the distribution map, and contributed to the manuscript.

Contribution to Article VI:
Fleischmann, A., Heubl, G., 2009. Overcoming DNA extraction problems from carnivorous plants. Anales del Jardín Botánico de Madrid 66: 209–215.

A. Fleischmann developed the concept of the study, provided plant material, made the laboratory work, prepared the figures and wrote the manuscript.
G. Heubl supervised the laboratory work and improved the manuscript.

All photographs, images, and textual illustrations in this work were made by Andreas Fleischmann.

„*Die Resultate der eigenen Untersuchung seien im Folgenden geschildert, wobei sich zeigen wird, dass Genlisea eine der merkwürdigsten unter allen tierfangenden Pflanzen ist.*“
Karl von Goebel, 1891, Pflanzenbiologische Schilderungen II, p. 121.

Contents

1. Introduction

1.1. Carnivorous Plants

In 1875, Charles Darwin provided conclusive evidence in his seminal work "Insectivorous Plants" to validate the theory of plants that trap and digest animals. Ever since, evolutionary botanists have attempted to address the question of how carnivory could have evolved in the plant kingdom. Darwin (1875) himself was convinced that there had been several independent origins of carnivory, however tracing the relationships of carnivorous genera was often clouded by both convergent and parallel evolution of carnivorous traits among angiosperms (Ellison & Gotelli 2009).

The "carnivorous syndrome" is reflected in many different specialised morphological characters and physiological adaptations, and generally comprises the ability of a plant to attract, retain, trap, kill, and digest animals and finally absorb and exploit the nutrients gained from digestion of the prey (Givnish et al. 1984; Juniper 1986; Givnish 1989; Juniper et al. 1989; Adamec 1997; the latter establishing a more precise ecophysiological circumscription of carnivory). Different methods and ways of prey attraction and trapping were established in carnivorous plants, resulting in what can be distinguished as six different trap types: 1. adhesive traps ("flypaper traps" or "sticky traps") constituting glandular leaves; 2. pitcher traps ("pitfall traps") formed by tubular leaves or rosettes (in case of two carnivorous tank-forming bromeliads); 3. snap traps ("spring traps" or "bear traps") formed by rapidly closing laminar lobes; 4. eel traps ("lobster pot traps") formed by narrow tubular leaves that are internally lined with retrorse hairs; and 5. suction traps ("bladder traps") which are complex modified tubular leaves that can actively inhaul small prey by creating a water current from low pressure. Some of these trapping principles are realized only in a single genus (suction traps are exclusively found in *Utricularia*), while others are present in several carnivorous plant genera. However similar or identical trap types often evolved analogous in unrelated plant groups, or closely related plant groups convergently developed different trapping methods. Modern research methods of phylogenetic reconstructions showed evidence that carnivory has evolved several times independently among flowering plants (Albert et al. 1992; Williams et al. 1994; Müller et al. 2004, 2006; Heubl et al. 2006; Chase et al. 2009; Ellison & Gotelli 2009; APG III 2009), confirming Darwin's initial hypothesis of a polyphyletic origin and convergent evolution of carnivorous plant lineages (Darwin 1875).

Reconstructing the phylogenetic relationships of flowering plants (APG III 2009) revealed that the 17 known genera of carnivorous plants did not evolve from a single common carnivorous ancestor (like eg. proposed by Croizat 1960), but fall into ten different angiosperm families that belong to five separate botanical orders (see Table 1), all of which evolved at least six times independently from non-carnivorous plants (at least two times in Lamiales, see Chapter 2.1), and none of them is comprising exclusively carnivorous families (Albert et al. 1992; Müller et al. 2004, 2006; Heubl et al. 2006; Ellison & Gotelli 2009).

In Caryophyllales, carnivory is a monophyletic trait (Albert et al. 1992; Meimberg et al. 2000, 2001; Cameron et al. 2002; Cuénoud et al. 2002; Rivadavia et al. 2003; Heubl et al. 2006), but three different trap types developed in this lineage (adhesive traps, snap traps, and pitcher traps). Interestingly, carnivory most likely got lost again at least twice in this lineage, in two members of Dioncophyllaceae and in the entirely non-carnivorous, monogeneric Ancistrocladaceae (Ellison & Gotelli 2009; Fleischmann 2010).

In Ericales, carnivory most likely has evolved two times independently (Fleischmann 2010), one lineage with pitcher traps leading to the American pitcher plant family Sarraceniaceae, and a sister lineage with resinous adhesive traps to the African Roridulaceae (Albert et al. 1992; Conran & Dowd 1993; Bayer et al. 1996; Anderberg et al. 2002; Neyland & Merchant 2006).

Oxalidales only comprise a single carnivorous plant taxon, the enigmatic, monospecific Australian pitcher plant family Cephalotaceae (Albert et al. 1992; APG III 2009).

The phylogenetic relationsships of the carnivorous Lamiales taxa are treated in more detail in the present thesis. Carnivory has evolved at least twice in this order, in the just distantly-related families Lentibulariaceae and Byblidaceae (Müller et al. 2004, 2006; Schäferhoff et al. 2010). Additionally, several other glandular members of this affinity have repeatedly been suspected to be carnivorous or "proto-carnivorous", including Martyniaceae (*Ibicella*, *Proboscidea*, carnivory assumed by Beal 1875; Mameli 1916, and others), Plantaginaceae/Gratiolaceae (*Philcoxia*, carnivory suspected by Taylor et al. 2000), and Orobanchaceae (*Lathraea*, for speculation about carnivory see Groom 1897; Mannagetta 1897; Heslop-Harrison 1976). However nutrient uptake from casually caught animals has not been detected in any of these genera (Studnička 1982; Juniper et al. 1989; Rice 1999; Fritsch et al. 2007; Płachno et al. 2009), therefore they are not considered carnivorous plants here, as they are not fulfilling the essential criteria of the carnivorous syndrome as circumscribed by Adamec (1997).

In the monocot order Poales, carnivory was demonstrated for two tank-forming species of the bromeliad genus *Brocchinia* (Givnish et al. 1984; Benzing et al. 1985; Benzing 1986; Płachno et al. 2006), and it is suspected for one species of the tillandsoid Bromeliaceae *Catopsis* (Fish 1976; Frank & O'Meara 1984; Benzing 1986).

Adaptations to carnivory must therefore have evolved repeatedly on multiple separate occasions in the plant kingdom, to give rise to the extant carnivorous plant families and genera (see Table 1).

More than 700 species of carnivorous plants are known today (see Table 1; McPherson 2010), however over 95% of the species diversity is entirely made up by the carnivorous Caryophyllales and Lamiales (Ellison & Gotelli 2009).

Angiosperm order	**family [number of genera / carnivorous genera]**	**genus**	**number of species [non-carnivorous species]**	**trap type**	**distribution**
Caryophyllales	Droseraceae [3/3]	*Drosera*	at least 194	adhesive trap	cosmopolitan
		Dionaea	1	snap trap	North America: eastern USA
		Aldrovanda	1	snap trap	Old World
	Drosophyllaceae[1/1]	*Drosophyllum*	1	adhesive trap	western Mediterranean
	Dioncophyllaceae [3/1]	*Triphyophyllum*	1	adhesive trap	tropical western Africa
	Nepenthaceae [1/1]	*Nepenthes*	at least 129	pitfall trap	Southeast Asia, India, Australia, Madagascar, Seychelles
Ericales	Sarraceniaceae [3/3]	*Sarracenia*	8	pitfall trap	North America: eastern USA + Canada
		Darlingtonia	1	pitfall trap	North America: western USA
		Heliamphora	at least 23	pitfall trap	South America: Guayana Highlands
	Roridulaceae [1/1]	*Roridula*	2	adhesive trap	South Africa
Oxalidales	Cephalotaceae [1/1]	*Cephalotus*	1	pitfall trap	Western Australia
Lamiales	Byblidaceae [1/1]	*Byblis*	7	adhesive trap	Australia
	Lentibulariaceae [3/3]	*Pinguicula*	at least 101	adhesive trap	cosmopolitan, excluding Australia
		Genlisea	at least 32	eel trap	tropical Africa, Neotropics
		Utricularia	at least 228	suction trap	cosmopolitan
Poales	Bromeliaceae [ca. 50/2]	*Brocchinia*	2 [19]	pitfall trap	South America: Guiana Highlands
		Catopsis	1 [20]	pitfall trap	Neotropics

Table 1. Carnivorous plant genera and species. An earlier version of this table has been prepared by myself for McPherson (2010). Species numbers updated from Holst (1997), Taylor (1989), McPherson (2010), McPherson et al. (2011).

1.2. Lentibulariaceae

The family Lentibulariaceae Richard (syn. Utricolariaceae, Pinguiculaceae) consists of herbaceous small plants, predominately hygrophytes (at least when in active growth), and some aquatics. It has been proposed as a natural group based on morphological characters (eg. Casper 1966; Cronquist 1981), and its monophyly was also shown in molecular phylogenetic reconstructions (Albert et al. 1992; Jobson et al. 2003; Müller et al. 2004). All members of Lentibulariaceae are carnivorous plants, and the family comprises three genera of markedly distinct morphology: the two sister genera *Utricularia* and *Genlisea* are rootless plants that trap microscopic aquatic or subsoil animals, while their common sister genus *Pinguicula* still possesses true roots and catches its prey with flypaper traps (Albert et al. 1992; Jobson et al. 2003; Müller et al. 2004, 2006; Müller & Borsch 2005). The largest and most diverse genus is *Utricularia* L. (including *Biovularia* Kamieński and *Polypompholyx* Lehm.), with over 200 species, (see Table 1; Taylor 1989; McPherson 2010).

Morphology

Roots (and even a radicle) are fully absent in *Genlisea* and *Utricularia* (Warming 1874; Darwin 1875; Goebel 1891, 1893; Lloyd 1942), but still present and functional in *Pinguicula* (Casper 1966). In the two other genera, the roots have been functionally[1] replaced by modified leaves (rhizophylls) in *Genlisea*, and by stolons (not quite appropriately termed "rhizoids" by Taylor (1989)) in *Utricularia*. In *Utricularia*, the actual leaves are modified to bladder traps (see below), and real photosynthetic leaves are absent in most species (but still present in certain species, see below). The foliar organs in the majority of *Utricularia* species constitute modified stolons (Brugger & Rutishauser 1989; Juniper et al. 1989; Sattler & Rutishauser 1990; Rutishauser 1999). Often, however, the distinction between leaf and stem is ambiguous (Lloyd 1942), and the plants display a "fuzzy morphology" (Rutishauser 1999; Rutishauser & Isler 2001). Taylor (1989) quite adequately circumscribed this peculiar organization of the vegetative organs of Lentibulariaceae as the "nonconforming nature of the[ir] vegetative morphology as a whole".

The generative organs, in contrast, virtuously follow the conventional bauplan of angiosperms. In *Pinguicula*, the flowers are borne solitary on long pedicels from the rosette (rarely bifurcate in a single species, *P. ramosa*); in *Genlisea* and *Utricularia* the inflorescence is a single or (rarely) double raceme. The calyx consists of five (*Pinguicula*, *Genlisea*), or two (occasionally four) (*Utricularia*) connate sepals. The sympetalous, bilabiate corolla is tubular with a pronounced spur, and usually possesses a gibbous palate, forming a masked flower of the "snap-dragon" type. The androeceum is reduced to the two anterior stamens, which are bithecate with curved filaments that clasp around the ovary. The superior ovary shows a central placentation, the style is short and persistent in fruit, with bilabiate stigma (the upper lobe shorter than the lower one, which is hiding the thecae). The stigma is chemotactile in some *Utricularia* species, but not in *Genlisea* and *Utricularia*. The fruit is a dry capsule, rarely indehiscent or fleshy in a few aquatic *Utricularia* species (see Taylor, 1989).

Carnivory

Three different carnivorous trap types can be found in Lentibulariaceae, each confined to one of the three genera: *Pinguicula* has adhesive traps (sticky flypaper traps), *Genlisea* has eel traps (lobster pot traps), and in *Utricularia* we find suction traps (bladder traps). In all three genera the leaves constitute the carnivorous traps, although in *Genlisea* and *Utricularia* they are highly modified and not easily recognised as such. In *Pinguicula*, the glandular sticky leaves both act as photosynthetic organs and carnivorous traps, whereas in the heterophyllous *Genlisea*, both functions are separated to the two types of leaves (see Chapter 1.3).

[1] the rhizophylls do not only serve to anchor the plant in the substrate, but also to take up nutrients from the soil, as Adamec (2008) has shown by his soil fertilizing experiments.

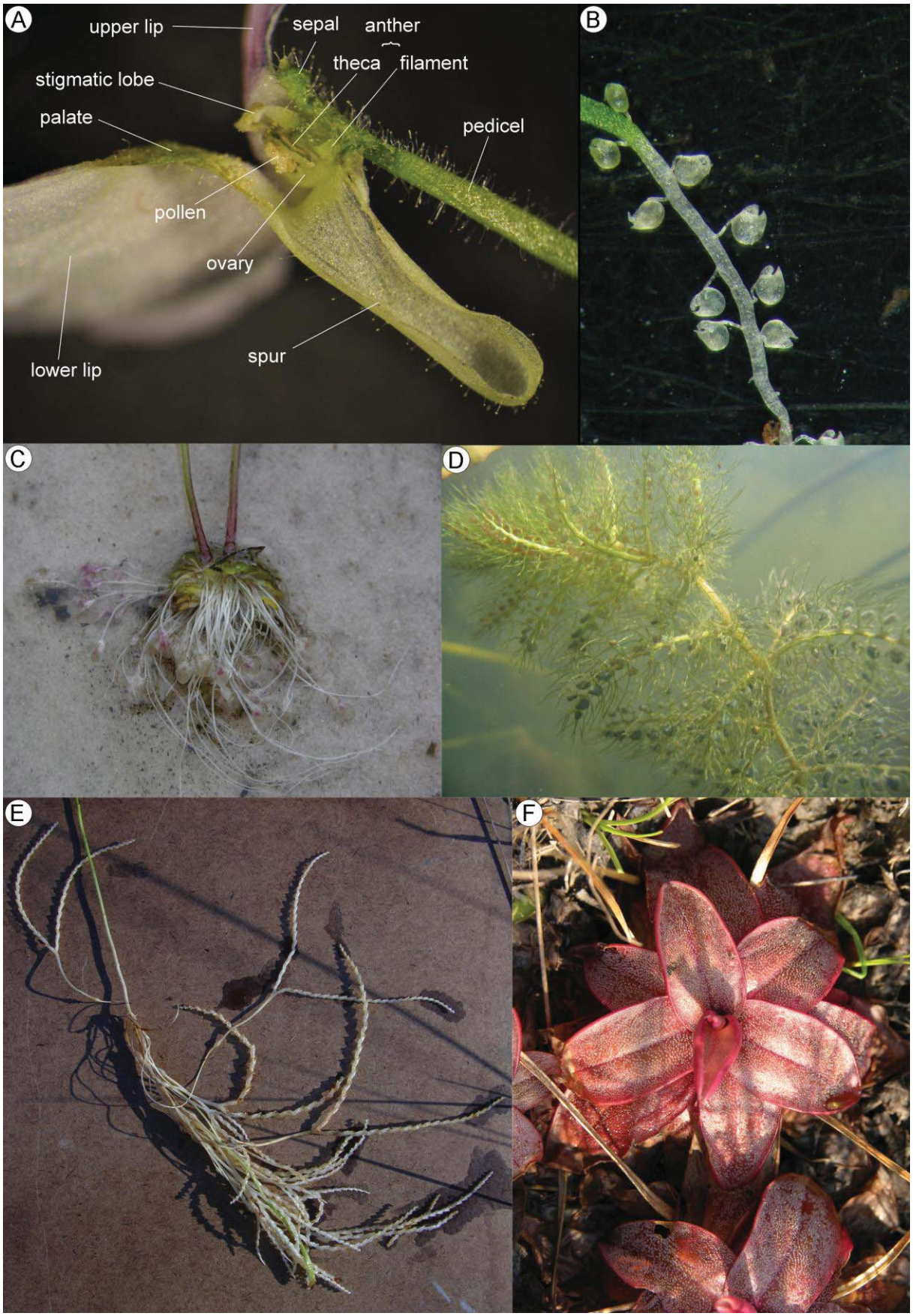


Fig. 1. Generative and vegetative morphology of Lentibulariaceae. A. Cross-section of a typical Lentibulariaceae flower (shown *Genlisea flexuosa*). B. Leaf-like stolon of *Utricularia rostrata* with traps. C. Rosetted growth of the heterophyllous *Utricularia multifida*. D. Stoloniferous growth of the aquatic *Utricularia australis*. E. Rosetted growth and traps of an excavated plant of *Genlisea guianensis*. G. Rosettes of glandular leaves of *Pinguicula planifolia*.

In the case of *Utricularia*, this is even more complicated, as some of the derived species are fully devoid of true leaves (Brugger & Rutishauser 1989; Sattler & Rutishauser 1990; Jobson et al. 2003; Müller & Borsch 2005; Albert et al. 2010). In all *Utricularia* species, the complex suction traps are verifiably derived from stolon-borne leaves (Goebel 1891, 1913; Llyod 1942; Juniper et al. 1989), although this has denied by some authors who proposed an anomalous bauplan of the *Utricularia* cormus. Some species of *Utricularia* still possess true leaves; they are heterophyllous and produce both photosynthetic foliage and trap leaves. However, in the majority of species the leaf-like photosynthetic organs represent modified stolons (Ridley 1888; Brugger & Rutishauser 1989; Sattler & Rutishauser 1990; Taylor 1989), which bear the actual trap leaves.
While the sticky flypaper leaves of *Pinguicula* trap small arthropods, the subterraneous traps of *Genlisea* and *Utricularia* have specialized in small soil organisms and microscopic aquatic prey (eg. Darwin 1875; Goebel 1891). The prey spectrum of *Genlisea* is explained in more detail in Chapter 1.3 below.

1.3. *Genlisea*

The genus *Genlisea* A.St.-Hil. was described from Brazil in 1833 by French botanist Auguste de Saint-Hilaire, named in honour of countess Stéphanie-Félicité du Crest de Saint-Aubin de Genlis, a famous contemporary authoress and owner of the renowned grand salon "Madame de Genlis" in Paris (Saint-Hilaire 1833). The English vernacular name for *Genlisea* is "corkscrew plant", based on the ends of the forked trap leaves, which are conspicuously spirally twisted, reminiscent of the end of a corkscrew (see below).

Vegetative morphology

All species of *Genlisea* are small rosetted herbs, which produce two kinds of leaves from a short vertical stem (stem stolon-like and horizontally spreading in *G. repens*). The epiterrestrial leaves are spatulate to linear and serve photosynthesic purposes. The achlorophyllous, subterraneous rhizophylls are tubular leaves, which are epiascidiate in ontogeny (Juniper 1986), and positively geotropic (Juniper et al. 1989). Their design is fundamentally similar in all species of the genus (Fig. 2A): a short trap stalk or "footstalk" on the distal end is followed by a widened, hollow bulb-like trap vesicle (also termed "stomach" or "digestion chamber" or "utricle"), that is narrowed down to a prolonged tubular part ("neck"). This tubular neck apically widens and branches into two helically twisted trap-arms. The overall shape of the rhizophylls thus resembles an inverted Y. The hollow rhizophyll has several entrances to its interior: a "trap mouth", which is situated in the branching zone between the two trap arms (Goebel 1891; Lloyd 1942; Reut 1993), as well as several slits that are gradually spaced along the suture of the twisted trap arms (Fig. 2E). The inner surface of the trap neck and arms is covered with rows of retrorse bristles (Fig. 2D, 2F) – termed "detentive hairs" by Reut (1993) – which are facing the trap bulb, and which therefore only allow an unidirectional movement of trapped animals towards the "stomach" of the trap. The interior of the vesicular bulb of the rhizophyll is lined with multicellular glandular hairs ("digestive glands"), consisting of three functional compartments, namely a basal cell, a middle cell and gland head formed by four to eight secretory cells (Goebel 1891; Lloyd 1942; Juniper et al. 1989; Reut 1993; Płachno et al. 2005a, 2007). The digestive glands are either distributed more or less equidistantly on the entire interior surface of the bulb (members of *G.* subgenus *Tayloria*), or they are predominantly concentrated in a row along the vascular bundle of the bulb (members of *G.* subgenus *Genlisea*, see Fig. 2B, 2C; Reut 1993; Płachno et al. 2007).

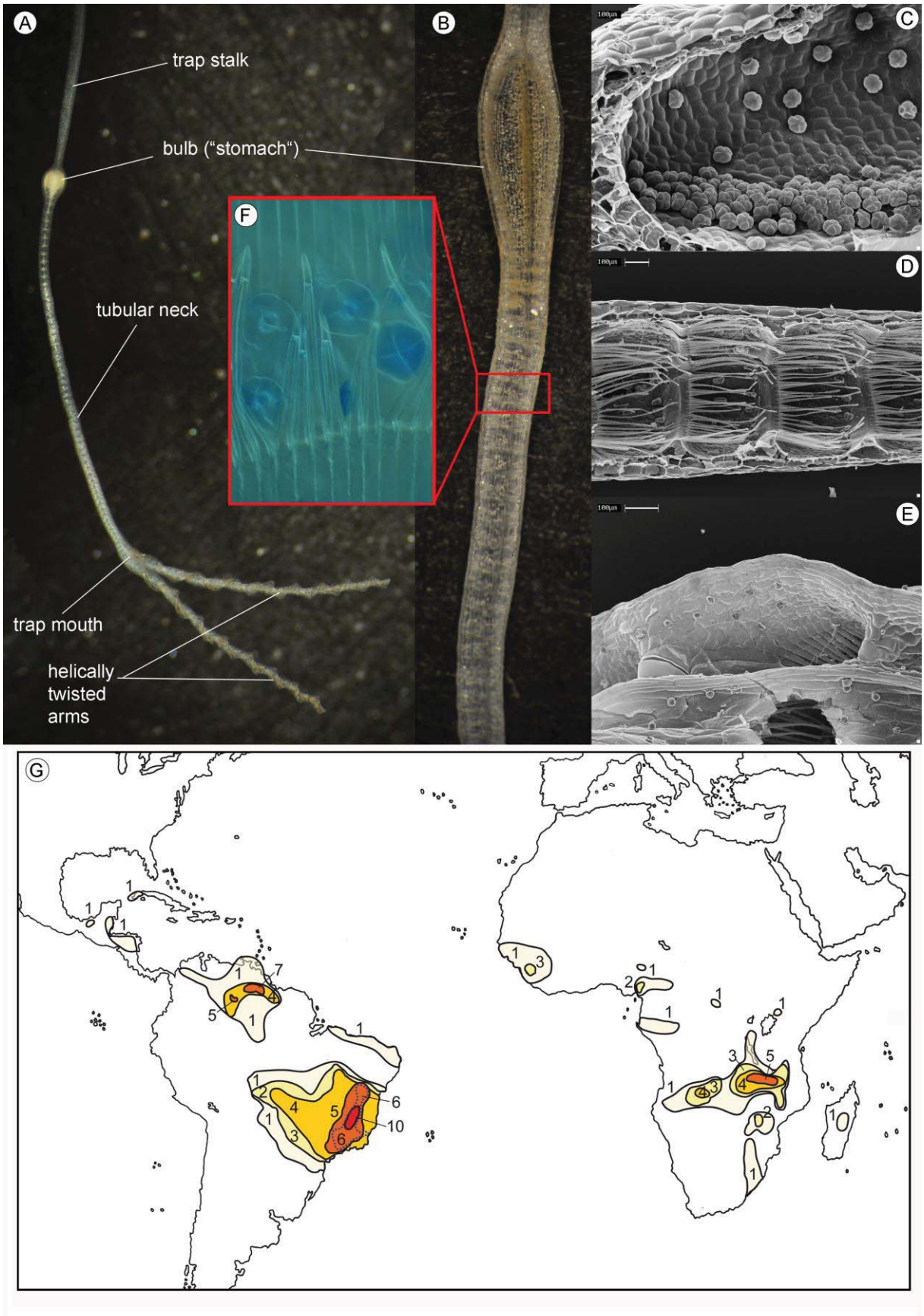


Fig. 2. Trap morphology and distribution of *Genlisea*. A. Rhizophyll of *G. aurea*. B. Longitudinal section of the upper part of a rhizophyll. C-E. SEM micrographs of the rhizophyll of *G. hispidula*. C. Longitudinal section of the trap vesicular bulb, showing the digestive glands. D. Longitudinal section of the tubular neck, showing the detentive hairs. E. Trap entrances along the helically twisted rhizophyll arm. F. Light microscope photograph of the detentive hairs and glands (stained with lactophenol blue) at the base of the tubular neck of *G. aurea*. G. Distribution of *Genlisea*, species numbers indicated. The dashed line marks the range of *G*. subgenus *Tayloria*.

Carnivory in *Genlisea*

The entire design of the *Genlisea* rhizophylls almost provokes to compare these curious foliar organs with the intestinal tract of higher animals, "just lacking peristaltic" (Heslop-Harrison 1975). Thus it is not surprising that the carnivorous nature of *Genlisea* was suspected soon after the odd rhizophylls were first described by Warming (1874). He was the first to recognize that the rhizophyll vesicles contained "soil debris and what probably constitutes animal remnants" (Warming 1874). Charles Darwin already included *Genlisea* as a carnivorous plant in his fundamental work "Insectivorous Plants" (Darwin 1875), based on Warming's description and own observations made on preserved specimens. The carnivorous traps were subsequently examined and illustrated in more detail by Goebel (1891), who was the first who also had access to living plant material for his morphological and anatomical studies, and his students (Merl 1915; Lloyd 1942). However it was not until the late 20th century that final evidence for carnivory in *Genlisea* was given with the detection of digestive enzymes that are secreted from the plant (Heslop-Harrison 1975), and the proof of direct uptake of nutrients originating from preyed animals by the plant with radio isotope tracing (Barthlott et al. 1998). Captured animals are probably killed by anoxia inside the vesicular "stomach" part of the trap (Adamec 2007), and then get digested by proteolytic enzymes that are released from the secretory cells of the glandular head of the digestive hairs, which also serve to take up the nutrients dissolved from the prey (Heslop-Harrison 1975; Barthlott et al. 1998; Płachno et al. 2005a, 2006, 2007).

Prey spectrum: What do they catch?

The prey found in *Genlisea* traps consists of various microscopic soil invertebrates, such as crustaceans (Goebel 1891; Lloyd 1942; Płachno et al. 2005b; Darnowski & Fritz 2010; Fleischmann, pers. obs.), soil mites (Goebel 1891; Lloyd 1942; Płachno et al. 2005b; Fleischmann, pers. obs.) and nematodes (Lloyd 1942; Studnička 1996, 2003b, c; Płachno et al. 2005b; Fleischmann, pers. obs.), but also protozoa (Heslop-Harrison 1975; Barthlott et al. 1998; Płachno et al. 2005b), and even small algae (Goebel 1891; Studnička 1996; Płachno et al. 2005b; Płachno & Wolowski 2008) as well as soil particles and debris can be found inside the traps (Darwin 1875; Juniper et al. 1989; Meyers-Rice 1994; Studnička 1996; Fleischmann, pers. obs.). The question whether prey is actively attracted to the traps, or rather unselectively enters the cavities and openings at the apices of the rhizophyll is not fully answered yet. Barthlott et al. (1998) postulate a chemotactic prey attraction, however were not able to detect any volatiles that are emitted from the traps. Studnička (2003a) assumes that the rhizophyll openings mimic soil interspaces that attract soil microfauna by creating oxygen-rich areas in the otherwise rather anaerobic conditions of the surrounding substrate. However this explanation is not satisfactory, as most species of *Genlisea* naturally grow in wet soils that are rinsed with swiftly seeping water (see Chapter 1.3.2), therefore it seems unlikely that their rhizophylls are indeed reaching into anaerobic soil.

Barthlott et al. (1998) concluded a prey specialisation of *Genlisea* to protozoa from their laboratory experiments and field observations on an African species *in situ*, despite the fact that previous analysis of trap contents (Darwin 1875; Goebel 1891; Lloyd 1942; Heslop-Harrison 1975) verifiably showed various metazoa trapped inside the rhizophylls. The rich and almost exclusive content of protozoa that was observed inside the traps of *G. stapfii* by Barthlott et al. (1998) might result from the inselberg habitats this species is confined to (see Chapter 1.3.2). The shallow soils of inselbergs host a rich protozoan microfauna (Steffens & Wilbert 2002), and thus the prey spectrum of this *Genlisea* species might rather reflect the soil microfauna composition of the habitat than a putative prey specialisation. Prey contents of several other species growing in different habitat types, as well as from cultivated plants (including *G. stapfii*) showed mainly metazoa (Studnička 1996, 2003c; Fleischmann, unpublished data). Studnička (1996) further noticed a trap size dimorphism in certain species

of *Genlisea*, which produce larger traps that are reaching deep into the substrate, but also smaller traps that are orientated more horizontally, close to the soil surface, and assumed a possible prey specialisation.
The traps of *Genlisea* probably show no specialisation to either protozoa or metazoa, but unselectively trap any kind of small soil organisms that are available to the plant, as it was shown by feeding experiments in the laboratory (Płachno et al. 2005b, 2008; Darnowski & Fritz 2010) and comparative field studies (Studnička 2003c; Fleischmann, unpublished data).

Trap mechanism: Aquatic hoover or passive shelter mimic?

Despite detailed research on the trap anatomy, the exact functioning of the eel traps of *Genlisea* is still not well understood. Contradictory evidence has been published whether the traps act as passive traps into which prey enters targeted for unknown reason (Darwin 1875; Lloyd 1942; Taylor 1991; Barthlott et al. 1998; Adamec 2003; Płachno et al. 2005b, 2008), or whether *Genlisea* rhizophylls constitute active traps which can inhaul their prey by creating a constant light water current resulting from a permanent exhaling of water from the trap interior (Juniper et al. 1989; Meyers-Rice 1994; Studnička 1996, 2003a, b). The presence of immobile prey items such as non-ciliate algae and soil debris inside the trap vesicle, however, as already observed by Goebel (1891), favours the theory of an active trapping system. This is also confirmed by own studies made on rhizophylls of cultivated *Genlisea* plants. In freshly excavated plants the rhizophylls will create a continuous water current if placed in Petri dishes filled with water (the water movement can be visualized by a few droplets of drawing ink), however this current rapidly declines in traps that have been detached from growing plants (Fleischmann, unpublished). This is probably due to physiological processes when the achlorophyllous rhizophyll is not supplied by photosynthetic energy from the green leaves anymore. The ageing process of the living traps segregated from the whole plant in the laboratory set-ups could be the reason why some studies showed no trap activity, while others did. None of the abovementioned experiments on *Genlisea* traps was conducted on entire plants, but all of them were performed on single detached traps.

1.3.1. Distribution

Data on distribution was extracted from literature (Fischer et al. 2000; Fleischmann et al. 2010, 2011; Fromm-Trinta 1979, 1981, 1984; Ritter & Crow 2000; Olvera & Martínez 2002; Taylor 1967, 1991, 1999), as well as based on own field observations and herbarium records. The first report of the Venezuelan *Genlisea sanariapoana* from adjacent Colombia was made recently by J.H. Madrid and documented photographically (Vieira 2004).

The genus *Genlisea* comprises 32 species (see Checklist in Chapter 2.2), occuring in the Neotropics and tropical Africa, including Madagascar. However not a single species of *Genlisea* occurs on both continents (Fleischmann et al., 2010; Fig. 2G). Several species are endemic to a narrow range. The distribution pattern of the genus matches some of the main centres of plant biodiversity in general (Barthlott et al. 1996a, 2007), but also the centres of global carnivorous plant diversity in particular (Barthlott et al. 2004).

13 species of Neotropical *Genlisea* are recognized in the taxonomic treatment presented in this thesis (see Checklist in Chapter 2.2). The centres of greatest species numbers and diversity in South America lie in the highlands of central Brazil and the Guiana Highlands, and one widespread short-lived species (*G. filiformis*) is reaching its southernmost limit in northern Brazil and Uruguay, and the northernmost range of the genus in isolated populations in Cuba, Belize, Guatemala, and southernmost Mexico (Olvera & Martínez 2002).
The entire subgenus *Tayloria*, comprising eight species (Fleischmann et al. 2011), is endemic to a small area in the highlands of eastern Brazil (Fig. 2G; Fromm-Trinta 1979, 1981, 1984; Taylor 1991). *Genlisea aurea*, as well as the closely related *G. minor*, which has been considered conspecific by most authors (Fromm-Trinta 1979; Taylor 1991), and a yet undescribed tuber-forming geophyte species from this alliance are also endemic to Brazil, however occupy a slightly wider range. Three species of *Genlisea* are endemic to the Guiana Shield: one of them, *G. sanariapoana*, is confined to the lowlands of the upper Orinoco, along the border of Venezuela and Colombia (Steyermark 1953; Taylor 1999); the narrowly endemic *G. glabra* is restricted to a few high tepui summits of the Chimantá Massif in central Bolívar state, Venezuela, whereas the more widespread *G. roraimensis* occurs on several high altitude plateaus of the Pantepui region of Venezuela, and adjacent Guiana (on Roraima tepui) and Brazil (on Roraima and the Sierra de la Neblina; Taylor 1999).

Eleven species of *Genlisea* occur in tropical West and East Africa, and one species (*G. margaretae*) is extending the range to Madagascar. The centres of diversity for the genus on the African continent are the large upland plateau of central tropical Africa (covering the vast range of Zambia, and bordering parts of Angola, Zimbabwe, the Republic of Congo and Mozambique). Parallel to the widespread annual *G. filiformis* from the Neotropics, the small annual *G. stapfii* reaches the widest range of distribution among the African species, extending from Senegal southwest (absent in the dry areas of the Ghana Dry Zone and Dahomey Gap) to the Central African Republic in the West and the Republic of the Congo (Congo-Brazzaville) in the South. *Genlisea stapfii* is also the African species occupying the widest spectrum of habitats (see below; Fischer et al. 2000).

1.3.2. Habitats

Like the majortity of carnivorous plants, all species of *Genlisea* are commonly confined to at least seasonally wet to waterlogged, nutrient poor, oligotrophic soils of exposed, open habitats with low vegetation cover. I have assigned the habitat preferences of the known species of *Genlisea* to five general ecological types of habitats here. Two species – interestingly one African (*G.stapfii*) and one Neotropical (*G. filiformis*), but both of them widespread annuals – show a broad ecological tolerance and are found in all of the below-mentioned nutrient poor habitats.
The five habitat types illustrated below have in common that they represent isolated patches of exposed habitats for a specialized vegetation, which are surrounded by a different, contrasting type of ecosystem that does not support growth of the elements of these plant communities ("functional islands", Macedo & Prance 1978; Prance 1996).

Granite rocks: inselbergs

Inselbergs are conspicuous, more or less dome-shaped granitic (rarely gneissic) rock outcrops, to which a specialized type of vegetation has adopted, which often sharply contrasts in structure and species composition with the surrounding vegetation (see Fig. 3B; Barthlott et al. 1993; Porembski & Barthlott 2000; Müller 2007). Most inselbergs are found in seasonally wet climates with a pronounced dry season, and are thus inhabited by a seasonal plant community, which can mainly be found on shallow slopes and depressions where water regularly drains to form small seepage areas during the rainy season. The term "ephemeral flush vegetation" has been established for these plant communities (Richards 1957; see Fig. 3D). As the thin layers of organic soil overlying bare rock are poor in nutrients, this is a suitable habitat for various carnivorous plant species (Bosser 1958; Klotz & Köck 1991; Seine et al. 1995; Dörrstock et al. 1996; Barthlott et al. 1996b, 2004). Five species of *Genlisea* occur on inselbergs in Africa, one of them (*G. barthlottii*) is exclusively found on inselbergs (Porembski et al. 1996; Fischer et al. 2000). In the Neotropics, granitic inselbergs can mainly be found in the coastal mountain ranges of Southeastern Brazil and on the Guiana Shield, especially in Guiana and southern Venezuela (Gröger 1995, 2000; Porembski & Barthlott 2000; Safford & Martinelli 2000; Gröger & Huber 2007). Three neotropical species of *Genlisea* can be found on granitic mountains and inselbergs (usually in shallow, wet depressions or seasonal seepages and wet flushes, Huber 1995; Safford & Martinelli 2000), but only one of them (the Brazilian *G. lobata*) is strictly confined to this type of habitat (Fromm-Trinta 1989; Rivadavia 2002; Fleischmann et al. 2011).

Laterite outcrops: ferricretes

Ferricretes ("ferriferous concretes"), "duricretes", or lateritic crusts, are hard backend iron-rich soil layers that got exposed from the surrounding soil surface by erosion (Gledhill 1970; Müller 2007; Beauvais 2009). Ferricrete habitats are common in tropical West and Central Africa, but also in northern South Africa (see Fig. 3H). In shallow depressions or on slopes with seeping water, seasonal rain-fed marshes or permanently wet peat bogs (see "*dambos*" below) can form over the laterite crust, the latter even allow the presence of specialized perennial plant communities and the occurence of peat mosses (*Sphagnum*) (Gledhill 1970; Müller 2007). Wet ferricretes are rich in carnivorous plant species, which often grow in carpets of *Sphagnum* mosses in seepage zones (pers. obs.). Ten of the eleven African species of *Genlisea* can be found on ferricretes (Fischer et al. 2000), however many of them can also be found in nutrient poor seasonal or permanent bogs. In contrast to the inselberg habitats, ferricretes can also host perennial species of *Genlisea*.

Sandstone rocks: *campos rupestres* and tepuis

The African ferricrete habitats can be compared in terms of carnivorous plant species richness with the Neotropical vegetation types of nutrient poor quartzitic soils overlying sandstone rocks. In Brazil, these habitats are predominantly covered by a typical sclerophyllous vegetation type called "*campos rupestres*" ("rocky fields", see Fig. 3C). This type of vegetation is usually found in higher elevated areas above 800-900 m elevation (Giulietti & Pirani 1988; Giulietti et al. 1997), and is characterized by a great species richness and high degrees of endemism, often localized to only a very small area (Giulietti & Pirani 1988; Alves & Kolbek 1994; Rapini et al. 2002; Alves et al. 2007; Echternacht et al. 2011). Like the African ferricretes, these sandstone rocks host the greatest number of Neotropical species of *Genlisea*, perennials as well as annuals (Rivadavia 2007; Silva et al. 2011; Fleischmann et al. 2011).

The sandstone plateaus of the table mountains of the Guiana Highlands, the tepuis, host a unique vegetation that is adapted to a harsh climate. On the rocky, open and windswept summits, specialized plant communities establish where shallow pockets of soil accumulate in cavities and shallow depressions (Huber 1995; McPherson 2010). They support a specialized, isolated, tepui summit ecosystem of sclerophyllous "high altitude tepui scrub" and "wet tepui meadows", which are dominated by herbaceous plants and small subshrubs (Huber 1988; Huber 1995), as well as many carnivorous plants, including *Genlisea*.

Wet savannah: *dambos* and *morichales*

The "*dambos*" of tropical and subtropical Africa are flat, grass-covered savannah wetlands along headwater drainage lines, which are seasonally inundated in the wet season, but usually become substantially dry during the dry season, however often retaining perennially wet areas along depressions (Mildbraed & Domke 1966; Boast 1990; von der Heyden 2004). These seepage peat bogs of tropical Africa host a plant communitiy rich in carnivorous plants, and several perennial and annual *Genlisea* species frequently occur in *dambos* in Angola, Zambia, Zimbabwe, Cameroon, and South Africa (Jessen 1936; Mildbraed & Domke 1966; Taylor 1988; Fischer et al. 2000; Fleischmann, pers. obs., Fig. 3F).

The wet lowland savannas of the Gran Sabana and the Amazon region of Venezuela and Brazil are characterized by sparse herbaceous cover on poor, acidic, sandy soils (Huber 1995). Extensive stands of "*moriche*" palms (*Mauritia flexuosa*) typically occur in the perennially wet, inundated areas of these savannas (so-called "*morichales*", see Fig. 3E; Brito & Ramirez 1988; Huber 1995). In permanently wet pools certain *Genlisea* species are growing as submerged aquatics, or freely floating in algae mats.

Fig. 3. Typical habitats of *Genlisea* (facing page). A. High altitude tepui meadow over sandstone rocks along shallow streams on Churí-tepui of the Chimantá Massif in Venezuela. Vegetation dominated by *Brocchinia reducta* (Bromeliaceae). Habitat of *Genlisea glabra* and *G. roraimensis*. B. Inselberg in northern Sierra Leone in the rainy season. The wet ephemeral flush vegetation on the upper slopes consists of mats of the perennial, poikilohydric „resurrection plant" *Afrotrilepis pilosa* (Cyperaceae), and annual Poaceae. Habitat of *Genlisea stapfii*. C. Perennially wet seepage site in scrubland over sandstone rock ("*campos rupestres*") in Minas Gerais, Brazil. In the cool, seeping water a sparse vegetation of Cyperaceae, Eriocaulaceae, *Drosera* (Droseraceae), lichens and algae grows in peaty soil. A typical habitat of *Genlisea aurea*, *G. repens*, *G. flexuosa* and *G. metallica*. D. Wet ephemeral flush vegetation on an inselberg in northern Sierra Leone. Habitat of *Genlisea barthlottii*, which grows in pockets of soil with Eriocaulaceae and Cyperaceae. E. "*Moriche*" palm swamp dominated by *Mauritia flexuosa* (Arecaceae) and Cyperaceae in the Gran Sabana, Venezuela. Typical habitat of *Genlisea repens*, *G. filiformis* and *G. guianensis*. F. Perennially wet spring-fed seepage site in a periodically dry swamp ("*dambo*") in northern Zambia. This site is rich in many carnivorous plants, such as *Drosera*, *Utricularia*, as well as *Genlisea glandulosissima*, *G. africana*, *G. margaretae* and *G. subglabra*. G. Periodically wet white silica sand plain in the Gran Sabana, Bolívar state, Venezuela. Habitat of the annual species *Genlisea filiformis*, *G. oxycentron* and *G. pygmaea*, as well as many species of *Utricularia*. In permanent wet ditches and pools, *G. guianensis*, *G. repens* and *G. nigrocaulis* occur. H. Perennial seepage habitat over ferricrete layer in northern South Africa. In the *Sphagnum* moss cover at the flush margins a rich community of *Xyris* and *Lobelia* sp., as well as the carnivorous plants *Drosera longiscapa*, *Utricularia welwitschii* and *Genlisea hispidula* were found.


A
B
C
D
E
F
G
H

White quartzitic sands: *campinas* and *muri*

This habitat type represents perennial or seasonal, extremely nutrient poor, acidic, infertile soils consisting of a thick layer of almost pure silica sand, with almost no vegetation cover, however rich in endemic plants specialized to these habitats (Anderson 1981; Splett 1997; Oliveira et al. 2001), White sands also represent a valuable habitat for many carnivorous plant species, provided that they are at least seasonally wet (Barthlott et al. 2004). The Brazilian "*campinas*" (lowland patches of white sand soils, eg. in the Amazon, central and south-eastern Brazil (Splett 1997) are floristically very similar to the white sand savannas of the Guianas (eg. "*muri*" bush, Macedo & Prance 1978; Huber 1995; see Fig. 3G), and both host similar species of *Genlisea*, usually annuals, but also a few perennial taxa.

1.3.3. History of *Genlisea* research in Munich

The first plant of *Genlisea* that entered cultivation was grown in Munich in the late 19th century – a *G. violacea* raised by Karl von Goebel in the Botanic Gardens from seed that was sent to him by Carl A.W. Schwacke from Diamantina, Brazil (Goebel 1893). Goebel was fascinated by the carnivorous nature, and especially the odd morphological modifications of the common bauplan of vascular plants he observed in *Genlisea* and *Utricularia*. The diverse anatomy and morphology of these two Lentibulariaceae genera, based on numerous preserved and living specimens he thoroughly studied, has been described and illustrated by him in detail in his fundamental works "Pflanzenbiologische Schilderungen" and "Organographie der Pflanzen" (Goebel 1891, 1913). In the third edition of his comprehensive three volume work "Organographie der Pflanzen", published in 1928, he even started the introductory chapter with an elaborate part about *Genlisea* as an example of a plant with unusual morphology ("§ 2. Genlisea als Beispiel einer Pflanze mit ungewöhnlicher Organbildung", pages 1-5). In all of his publications dealing with *Genlisea*, he kept pointing out his scientific interest and fascination in the genus, which he considered to be "one of the strangest among all carnivorous plants" (Goebel 1891).

Two of Goebel's students, Edmund Merl and Francis Ernest Lloyd, at the time in Munich, both continued and deepened his studies on *Utricularia* and *Genlisea* (eg. Merl 1915; Lloyd 1934), and the latter later summarized them in the probably most profound and detailed work on carnivorous plants (Lloyd 1942) since Darwin's "Insectivorous Plants" (1875).

Philipp von Luetzelburg, also a student of Goebel at the Botanical Institute in Munich (his doctoral thesis was about trap anatomy of *Utricularia*, Luetzelburg 1910), emigrated to Brazil and there contributed much to the knowledge of Brazilian Lentibulariaceae (Luetzelburg 1922). His herbarium collections, including many new species, are deposited in Munich herbarium, and – together with Martius' Brazilian collections – provide a valuable basis for the taxonomic work on Neotropical *Genlisea* species in Munich.

2. Discussion

The present thesis is based on six publications, which deal with the systematics, phylogeny, and evolution, as well as the biodiversity and taxonomy of Lentibulariaceae, with a special focus on the genus *Genlisea*. Two of the publications cover both topics. One publication has its focus on methodical problems of DNA extraction from carnivorous plants. Unpublished observations on flower biology of *Genlisea*, as well as a taxonomic checklist of the genus are additionally presented in this thesis.

2.1. Systematics, phylogeny and evolution

2.1.1. Evolution of carnivory in Lamiales: Article I

A robust phylogenetic backbone of Lamiales

Our largely resolved phylogenetic tree of the order Lamiales, based on three fast evolving choroplast markers (*trnK/matK*, *trnL-F*, and *rps16*) comprises major representatives from all lamialean families.

Within the order Lamiales, the basal branching families Plocospermataceae, Oleaceae and Carlemanniaceae (revealed as sister taxa), and Tetrachondraceae are indicated as consecutive sisters to the remaining lamialean families, which have been circumscribed previously as "core Lamiales" (Hilu et al. 2003). In the present topology, core Lamiales are arranged in four major clades, which are consecutive sisters (all with maximum support). The sister pair of Gesneriaceae and Calceolariaceae is sister to Plantaginaceae (sensu APGIII 2009), these are sister to Scrophulariaceae (in the circumscription of Oxelman et al. 2005), which are sister to a clade that was here referred to as "higher core Lamiales", comprising the families Byblidaceae, Linderniaceae, Stilbaceae, Lamiaceae, Phrymaceae (here revealed as paraphyletic), Paulowniaceae, the genus *Rehmannia* (formerly Scrophulariaceae s.l.), Orobanchaceae, as well as an unresolved polytomy of the crown-group, consisitng of Acanthaceae, Bignoniaceae, Lentibulariaceae, Martyniaceae, Pedaliaceae, Schlegeliaceae, Thomandersiaceae, and Verbenaceae.

Polyphyly of carnivory, and the path to carnivorous Lamiales

Lamiales host the majority of the carnivorous plant species diversity known today (Ellison & Gotelli 2009; Fleischmann 2010), and they are the single order among angiosperms in which carnivory evolved at least twice, in only distantly related families.

The independent origin of Byblidaceae, not in relation to Lentibulariaceae, is highly confirmed by our data. *Byblis* was indicated as closely related to Lentibulariaceae in several previous phylogenetic reconstructions (Albert et al. 1992; Bremer et al. 2002; Jobson et al. 2003), a fact that even led to the inclusion of this genus in the family Lentibulariaceae in APGII (2003). Morphological characters (flower symmetry, corolla morphology, gland anatomy) however underline the discreteness of Byblidaceae, and more recent phylogenies (including this one; but also Müller et al. 2004, 2006), which are based on a more comprehensive taxon sampling, and thus providing a solid phylogenetic backbone for Lamiales, clearly support *Byblis* as a distinct lineage at the base of the higher core Lamiales, but not closely related to Lentibulariaceae. The latter family, represented by at least two species from all three genera *Pinguicula*, *Genlisea*, and *Utricularia* in the present phylogenetic reconstructions, is shown in an unresolved polytomy of the crown-group of higher core Lamiales (in accordance with Müller et al. 2006). Unfortunately, the present topology still does not allow to identify the immediate sister of Lentibulariaceae.

The lack of resolutions further hampers to reveal the actual affinity of Lentibulariaceae to Martyniaceae, a familiy which comprises many strongly glandular genera, two of which (*Ibicella* and *Proboscidea*) have been suggested to be carnivorous (Beal 1875; Mameli 1916), or "proto-carnivorous" (Rice 1999). Indeed, both genera have very sticky glandular leaves of the flypaper trap type, and they catch numerous arthropods (Rice 1999), however are not able to absorb any nutrients from their "prey" (Płachno et al. 2009). The glandular hairs of Martyniaceae are also not of a specialized type as found in Lentibulariaceae (see below), but follow the general anatomy as found ubiquitously in glandular lamialean genera (Raman 1987; Müller et al. 2004). The presence of sticky mucilage-secreting glands is widespread among Lamiales (and other angiosperms), especially in floral parts, and small arthropdos have frequently been reported to be adhering to these glands, which led to speculation of carnivory in various plant genera since Darwin (1875; see Chapter 1.1). The main purpose of this "devensive killing" (Juniper et al. 1989) is probably to exclude non-pollinating insects from the flowers, and to protect the generative organs from herbivores (Kerner 1878). In several other densely glandular lamialean genera, especially the parasitic members of Orobanchaceae, the glandular hairs have been demonstrated to serve mainly for water excretion (Groom 1897). These secretory glands show a remarkably similarity in design and function to the digestive glands of carnivorous Lamiales. This trapping and secretion equipment could represent a "preadaptation" towards carnivory in Lamiales (Müller et al. 2004), in line with the postulated evolutionary steps that led to the carnivorous syndrome in Caryophyllales (Heubl et al. 2006).

There is given experimental evidence that the carnivorous glands in *Pinguicula* also play an additional defensive role against herbivores (Alcalá et al. 2010), and the sticky foliage might actually have evolved for defence purposes first case, and later was modified into a successful flypaper trap. In both Lentibulariaceae and Byblidaceae, we find a gland specialisation to either stalked secretory glands or sessile digestive glands. This parallels the gland dimorphism observed in the carnivorous Caryophyllales (Juniper et al. 1989; Heubl et al. 2006; Renner & Specht 2011), and represents a further specialisation towards carnivory, as discussed by Heubl et al. (2006).

In contrast to the superficially similar glands of the adhesive traps found in the carnivorous Caryophyllales (*Drosophyllum*, *Triphyophyllum*, *Drosera*), which are vascularized (Fenner 1904; Juniper et al. 1989; Heubl et al. 2006) to enable exchange of digestive fluid and nutirents, the unicellular or pluricellular stalks of the glands of Lamiales are not lined with vascular bundles – neither in the non-carnivorous members, nor in the highly specialized carnivorous taxa. Therefore, one key innovation towards carnivory in the gland anatomy of Lentibulariaceae must have been the attachment of the digestive glands to tracheid elements (Müller et al. 2004), which is accompolished by a single large basal cell that is embedded in the epidermis of the leaf. This prominent cell of the Lentibulariaceae glands, which has storage purposes for prey digestion and nutrient uptake ("reservoir cell"; Heslop-Harrison 1975), is physiologically connected to the subjacent tracheid cells by plasmodesmata (Heslop-Harrison 1975, 1976). The cuticule of the gland head cells also became modified in adaptation to the carnivorous syndrome, in that is bears several cavities, so-called "cuticular gaps", which serve for mucus secretion, enzyme release and nutrient uptake from the dissolved prey (Juniper et al. 1989).

Although the shape of the glandular hairs found in the three genera of Lentibulariaceae is very different (and even differs among members of the same genus in case of *Utricularia* (Taylor 1989)), the functional anatomy is identical in all three genera (Heslop-Harrison 1975, 1976; Juniper et al. 1989; Płachno et al. 2005a, 2007). The different structure rather represents an adaptation to different trap types and ecosystems, which is also mirrored by the analogous evolution of highly similar quadrifid glands in the aquatic traps of both Caryophyllales (*Aldrovanda*) and Lamiales (*Utricularia*).

A possible reconstruction of the evolution of digestive glands and other morphological traits of trap characters in the carnivorous Lamiales is illustrated in Fig. 4.

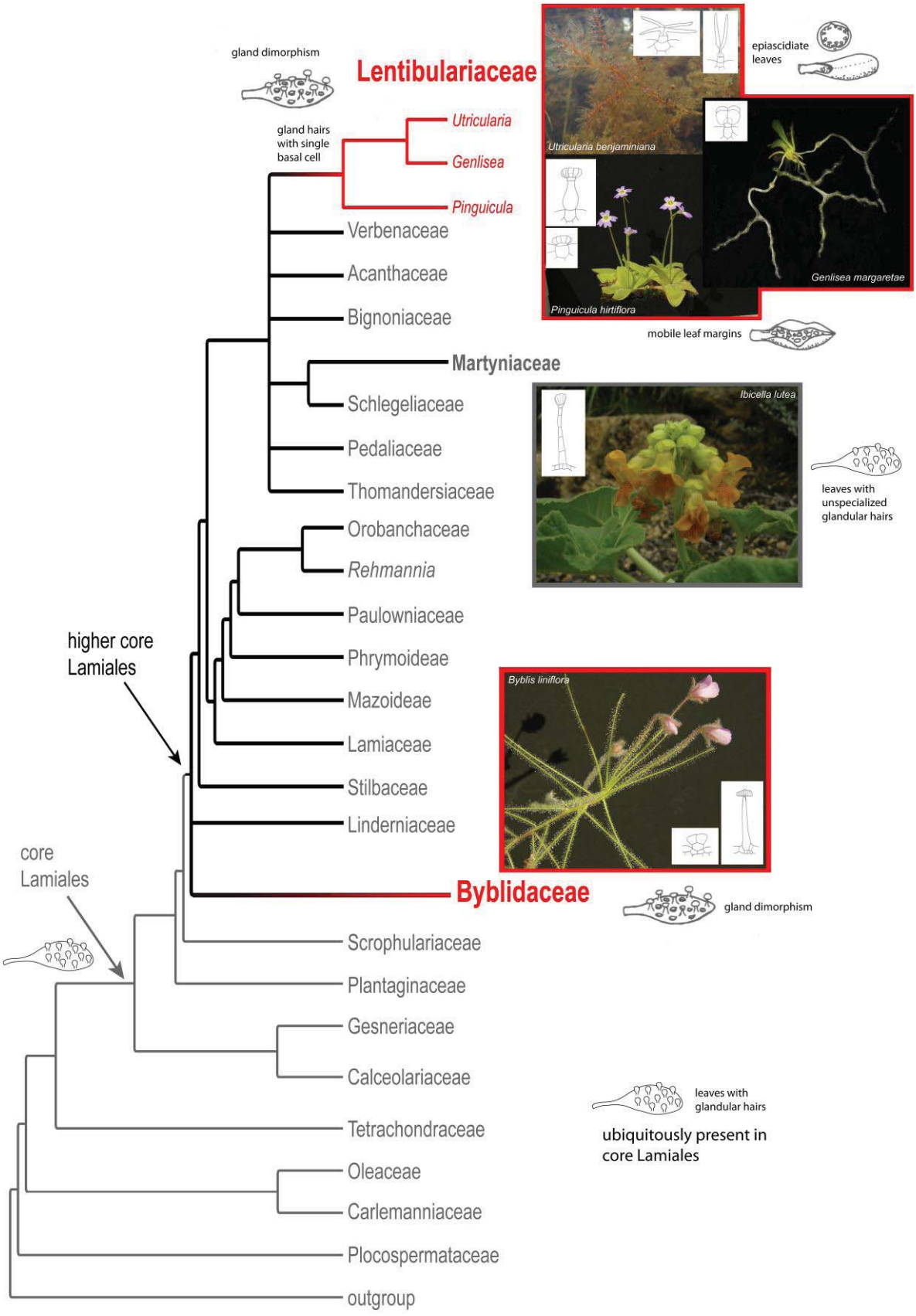


Fig. 4. Phylogeny of Lamiales and evolution of carnivorous traits. Tree topology based on Schäferhoff et al. 2010, drawings of glandular hairs modified from Fenner 1904, Lloyd 1942; Juniper et al. 1989, Ihlenfeldt 2004. Carnivorous lineages highlighted in red, the "proto-carnivorous" Martyniaceae in grey.

2.1.2. Phylogeny and phylogeography of *Genlisea*: Article II

The genus *Genlisea* was revealed as monophyletic, as it was already shown in preceding molecular reconstructions focusing on Lentibulariaceae (Jobson et al. 2003; Müller et al. 2004, 2006; Müller & Borsch 2005). In further accordance with these studies, our phylogeny -that is based on the most comprehensive sampling of taxa from the genus to date- also clearly revealed two major sister clades within *Genlisea*. These correspond to the two subgenera (*Tayloria* and *Genlisea*) that have been put up based on capsule dehiscence characters. Within *G.* subgenus *Tayloria*, the large, perennial species represent the basal branching lineages, whereas the derived species are annuals or short-lived polycarpic species. Three consecutive sister clades were obvious in *G.* subgenus *Genlisea*, which reflect morphology and biogeography of their members, and were thus taxonomically circumscribed as sections (see Chapter 2.2.1).

Genlisea is likely to have originated in the Neotropics, like its sister genus *Utricularia* (Jobson et al. 2003; Müller & Borsch 2005). We assume the origin in the south-eastern Brazilian highlands, where the highest number of extant species (the greatest α-diversity) can be found (Fromm-Trinta 1979; Taylor 1991; Fleischmann et al. 2011). Subgenus *Tayloria* is also confined to this area (comprising the Brazilian states of Bahia, Minas Gerais, Espírito Santo and São Paulo), which coincidentally is simultaneously remarkable for hosting the largest number of species of the sister genus *Utricularia* (Taylor 1989).

Genlisea subgenus *Genlisea* consists of three clades, two of them African (representing sections *Africanae* and *Recurvatae*) and one of exclusively Neotropical species (representing section *Genlisea*). The origin of this subgenus can be assumed in Africa: out of all extant species sampled of this subgenus, the two consecutive sister clades comprising all African species represent the basal lineages. One of them (section *Recurvatae*) gave rise to all extant Neotropical species. The African *Genlisea* species therefore are paraphyletic, and do not form a natural entity. The Neotropical species, in contrast, form a monophyletic group; therefore a single colonization event of subgenus *Genlisea* in South America is likely.

Ancient vicariance or recent long-distance dispersal?

Given the disjunct transatlantic distribution pattern of *Genlisea*, one might argue that the genus originated in the Western Gondwana area, while South America was still connected to the African continent (as put forward by Płachno & Swiatek 2009). However, this would imply an origin of the lamialean family Lentibulariaceae not later than the Late Cretaceous, because from the Mid-Late Cretaceous, 110-100 million years ago (mya), northern South America and the African continent started to drift away along a transform fault between Brazil and Guinea, which opened the central South Atlantic (Scotese et al. 1988). Connections between the two newly formed continents, which could have served as stepping stones for biota exchange, could have persisted via volcanic islands on mid-ocean ridges until about 95 mya (Raven & Axelrod 1972). Still, this geologically too old, reckoning that Lentibulariaceae belong to one of the most derived lineages of the crown group of the phylogenetically young angiosperm order Lamiales ("higher core Lamiales", Schäferhoff et al. 2010). The minimum age of the crown group of Lamiales is estimated ca. 95-97 mya, that of the stem group ca. 104-106 mya (Bremer et al. 2002; Janssens et al. 2009). Unfortunately, reliable relaxed-clock estimates for the age of Lentibulariaceae have thus far been hampered by both the absence of useful fossil calibration points and the still unresolved phylogenetic position of the family within Lamiales (Schäferhoff et al. 2010). Nevertheless, it cannot be denied from the evidence given above that the entire affinity of crown Lamiales is verifiably too young as that the disjunct trans-Atlantic distribution pattern observed in *Genlisea* could be explained by vicariance hypothesis.

Long-distance dispersal nowadays is widely accepted to explain disjunct ranges of many plant taxa (Nathan 2001, 2006; Renner 2004). To explain the extant distribution of *Genlisea*, two subsequent colonization events by long-distance dispersal in opposite directions across the Atlantic have to be assumed. Presumably the common ancestral lineage for all African species (leading to subgenus *Genlisea*) has colonized the African continent by long-distance dispersal events from South America (assuming the Neotropics as centre of origin for the genus). After a radiation of subgenus *Genlisea* in Africa (extending to Madagascar) that gave rise to the extant sections *Africanae* and *Recurvatae*, an ancestral member of this lineage re-colonized South America, there leading to a second radiation of Neotropical *Genlisea*. The paraphyly of the African *Genlisea* species (giving rise to all Neotropical members of subgenus *Genlisea*) contradicts a boreotropics migration, but favors transatlantic long-distance dispersal to explain the extant disjunction in the range of *Genlisea*, like it had already been proposed by Thorne (1973).

Transatlantic long-distance dispersal by water seems to be much more common than dispersal by birds or wind, and even bidirectional transatlantic dispersal of diaspores of several angiosperm taxa can parsimoniously be linked to existing or past sea currents between South America and Africa (Renner 2004). Seeds of *Genlisea* are minute (Kamienski 1890; Fromm-Trinta 1979; Taylor 1991; Fleischmann et al. 2011), rather short-lived and not able to float for a prolonged time (Fleischmann, pers. obs.), and therefore not likely to be drifted in sea water directly. However small seeded plant taxa could have possibly crossed the Atlantic in packets of soil attached to "floating islands" of drifting vegetation (Renner 2004).

Interestingly, species of plant communities adapted to spatially scattered habitats ("functional islands", Prance 1996), such as white sands and granitic inselbergs –typical habitats of *Genlisea* (see Chapter 1.3.2) – show a higher dispersal ability than florisitc elements of the surrounding vegetation, in order to colonize new suitable habitats (Macedo & Prance 1978; Gröger 2000; Arbeláez & Duivenvoorden 2004). However this does not mean that they feature a special "predestination" to long-distance dispersal, as these rare and occasional events that constitute the tail-end of the distribution kernel are neither purposive nor predictable, therefore there is no possibility for organisms to (pre-)adopt to it (S. Renner, pers. com.). Moreover, there is no verifiable correlation between seed size or morphology and long-distance dispersal events (Higgins et al. 2003).

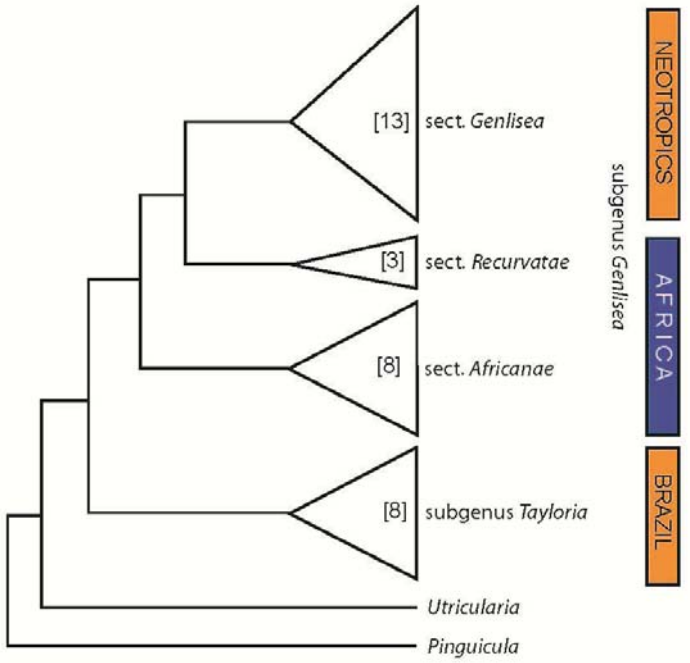


Fig. 5. Simplified phylogram of *Genlisea*, based on the topology published in Fleischmann et al. 2010. All branches shown got maximal statistical support. Size of the triangles corresponds to the [species number] of each clade. Species numbers according to the checklist published in this thesis.

2.1.3. Radiation of the Andean *Pinguicula*: Article IV

The South American species of *Pinguicula* were considered to represent a closely related natural entity by Casper (1966), based on biogeography, growth type and corolla characters, and classified by him as *Pinguicula* section *Ampullipalatum*.

However we revealed that Casper's section *Ampullipalatum* is polyphyletic. The two south Andean rain forest species from Antarctic to Subantarctic latitudes (*P. antarctica* and *P. chilensis* - the latter was not sampled for Beck et al. 2008, but ITS sequence data (Kondo & Shimai, unpublished; accession number AB212101, available at NCBI GenBank) confirm a sister relationship) are sister to the central Andean species (*P. calyptrata*, *P. involuta* and *P. chuquisacensis*). The northern Andean *P. elongata* from Venezuela and Colombia is not related to this alliance, but sister to a clade comprising the Mexican and Carribean species. This group presents the majority of species of the genus, and resulted from a young radiation of *Pinguicula* in Central America (Cieslack et al. 2005; Shimai & Kondo 2007; Shimai et al. 2007).

The phylogenetic connection of the northern Andean *P. elongata* to the Central American species is corroborated by the growth type of this species, which is tropical-heterophyllous (Fernández-Pérez 1964; Casper 1966; Hernandez-Melan 2009). The plant forms two types of leaves during a vegetation period, namely long thread-like carnivorous glandular leaves during the period of active growth, and small, succulent, scale-like eglandular, non-carnivorous leaves arranged in a bulb-like resting bud ("hibernaculum") for draught and winter dormancy. A transitional intermediate type of short succulent leaves is formed at the beginning of the short dry sommer dormany (Hernandez-Melan 2009). This growth type connects to the majority of Central American species, which show a tropical-heterophyllous growth type that is adapted to a seasonal climate with pronounced dry summer dormancy, during which they form bulb-like subterraneous resting buds or rosettes of small succulent non-carnivorous leaves (Casper 1966; Legendre 2000). The remaining Andean species, in contrast, display a homophyllous growth type (Casper 1966), this means they do not respond to the seasonal climate of their habitats by forming a special type of leaves, but produce carnivorous foliage throughout the year.

The homophyllous Andean species belong to a well-supported clade at the base of the *Pinguicula* phylogenetic tree. They are sister to a group of species confined to coastal regions of the eastern United States, and common sister to both is a sister pair of two Mediterranean taxa. All species of this affinity are of homophyllous growth type, which thus can be considered to represent the ancestral state in the genus (Cieslack et al. 2005; Müller et al. 2006; Degtjareva 2006). This clade is sister to four consecutive sister clades, the first three branching comprising the European and East Asian species of heterophyllous growth type. The most derived group consists of a clade formed by the Central American species and their sister *P. elongata*, which are also primary heterophyllous. However some annual species of this clade, as well as the species of the Carribean which occur in a perennial tropical climate display a tropical-homophyllous growth (Casper 1966; Legendre 2000), which however can be considered convergent evolution of this growth type (see also Cieslack et al. 2005; Shimai & Kondo 2007; Shimai et al. 2007).

Casper (1966) already postulated the diversification of South American *Pinguicula* connected to the raise of the Andes, based on the "Nordic Invasion" theory. This hypothesis assumes that most of the high Andean flora elements which belong to genera of largely north temperate distribution (including *Pinguicula*, according to Diels (1937)) migrated from North America via the Central American isthmus to South America during the cooling climates of the Mid-Pliocene. Evidence for this theory arises from paleobotanical data (Burnham & Graham 1999), and molecular phylogeographic reconstructions of other plant genera (eg. Hughes & Eastwood 2006). The fact that the closest extant sister group of the Andean *Pinguicula* are the species from the eastern United States favours a colonization of the Andes by a southward migrating lineage of *Pinguicula*, probably from the northern hemisphere.

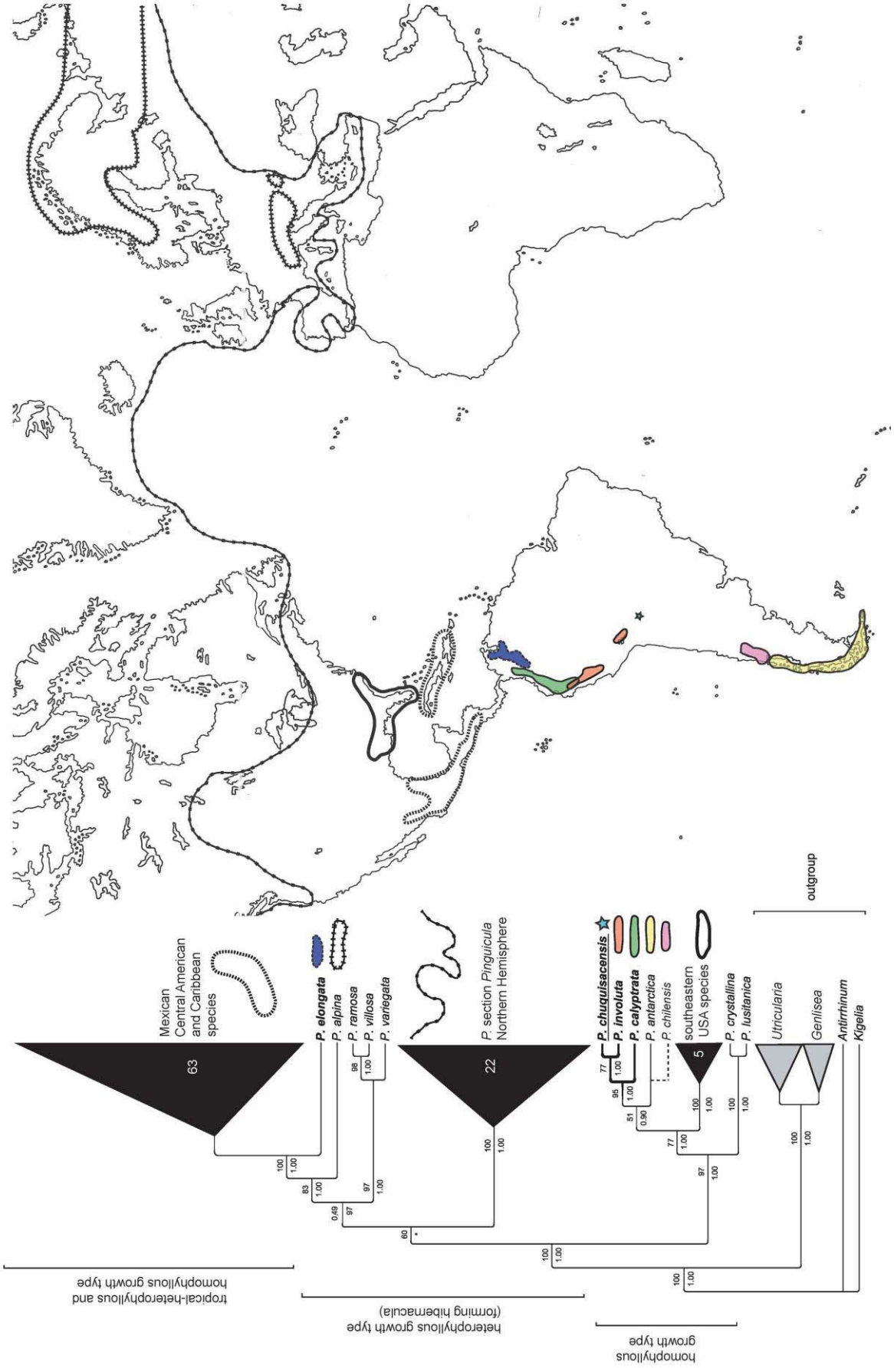


Fig. 6. Distribution and phylogeny of the Andean *Pinguicula*. Tree topology based on Beck et al. 2008. The position of *P. chilensis*, which was not sampled, is indicated with a dashed line, based on ITS sequence data (Kondo & Shimai, unpublished). Distribution data from Casper 1966, Legendre 2000, Beck et al. 2008, and herbarium records.

2.2. Biodiversity and taxonomy

2.2.1. Infrageneric classification of *Genlisea*: Article II

Three modes of capsule dehiscence are known in *Genlisea* fruits. Longitudinally bivalvate capsules were first recognized by Benjamin (1847) in his *G. reflexa*, but he erroneously assumed this character for the whole genus. Stopp (1958) described and illustrated the unique spiral circumscissile dehiscence he observed in an African member of the genus. Fromm-Trinta was the first to recognize that longitudinally valvate capsules were only found in a single species known at the time, *G. violacea* (which also differs in other characters from the remaining species known at the time), and proposed a new section, *G.* section *Tayloria* Fromm, for that species (Fromm-Trinta 1977). Two further species were described later and added to that section (Taylor & Fromm-Trinta 1983; Fromm-Trinta 1989).

Fischer et al. (2000) weighted the taxonomic value of the capsule dehiscence even higher (capsule dehiscence characters are considered taxonomically important in several other genera of Lamiales, see Fischer et al. 2004), and raised Fromm's section *Tayloria* to subgenus rank. This classification is supported by further morphological characters which distinguish members of both subgenera, namely corolla shape and spur orientation (Fleischmann et al. 2010), the hairs covering the inflorescence parts (which are pluricellular ("septate") in *G.* subgenus *Genlisea*, but bicellular or unicellular ("non-septate") in *G.* subgenus *Tayloria*; Fromm-Trinta 1979; Fleischmann et al. 2011), pollen morphology (Fromm-Trinta 1981; Taylor 1989), ovule development (Płachno & Swiatek 2009), micromorphological characters of the rhizophyll detentive hairs (Fromm-Trinta 1979, 1981; Reut 1993; Fleischmann et al. 2010), and distribution pattern and anatomy of the digestive glands inside the traps (Reut 1993; Płachno et al. 2005a, 2007). Further evidence arises from molecular phylogenetic data (Jobson et al. 2003; Müller et al. 2004, 2006; Fleischmann et al. 2010), which show both subgenera to represent monophyletic sister groups.

Based on the three clades which were evident for *G.* subgenus *Genlisea*, we proposed a further classification in three sections to comprise all members of that subgenus. Fischer et al. (2000) arranged the African species of *Genlisea* in three different informal groups based on the morphological characters of inflorescence and capsule indumentum, life history, and position of the pedicel in fruit. They distinguished a group I of perennial plants with glandular inflorescence and ovaries, and with pedicels that are reflexed in fruit, a group II that comprises annual species with glandular inflorescence and ovaries and pedicels not reflexed in fruit, and a group III for the perennial species with glabrous scapes and ovaries covered only with eglandular hairs, as wells as pedicels not reflexed in fruit.

Our sectional classification agrees with group I of Fischer et al. (2000), which represents *G.* section *Recurvatae* A. Fleischm. Kai Muell., Barthlott & Eb. Fisch., comprising the three African species which have a pedicel that is strongly recurved (to circinate) in fruit, namely *G. margaretae*, *G. glandulosissima* and *G. pallida*. All three are perennial plants that have densely glandular inflorescence scapes and ovaries. The African species that were separated to groups II and III respectively by Fischer et al. (2000) on the base of life history and the presence or absence of glandular hairs on scapes and ovaries were revealed as monophyletic entity (however representing sister subclades), and thus were collectively grouped in *G.* section *Africanae* A. Fleischm., Kai Muell., Barthlott & Eb. Fisch. All members of this section are characterized by a pedicel that remains erect in fruit, in combination with an African distribution.

The remaining species are the Neotropical members of *G.* subgenus *Genlisea*, which are found united in a clade comprising also the generic type, *G. aurea*, and thus automatically became the type section, *Genlisea* A. St.-Hil. section *Genlisea*. The members of this group are the Neotropical species that have a pedicel that is erect in fruit – or simply speaking those New World species of *Genlisea* which do not belong to *G.* subgenus *Tayloria*.

Therefore any *Genlisea* species can be clearly assigned to one of the four infrageneric categories based on the two characters "distribution" (Neotropical *versus* African) and "fruiting pedicel pattern" (erect *versus* reflexed to circinate) alone:

distribution	pedicel in fuit	taxonomic category		capsule dehiscence
Neotropical	**reflexed/circinate**	***G.* subgenus *Tayloria***	— subgen. *Tayloria*	bivalvate
Neotropical	**erect**	***G.* section *Genlisea***	} subgen. *Genlisea*	circumscissile/ spiral
African	**reflexed/circinate**	***G.* section *Recurvatae***		
African	**erect**	***G.* section *Africanae***		

Of course further morphological characters aid this classification (see Fleischmann et al. 2010), if biogeography or fruiting characters are not known for a given specimen. In addition, Neotropical species of *Genlisea* can readily be assigned to either subgenus by the capsule dehiscence alone (read above diagram from the right).

2.2.2. Five new species and a revision of *Genlisea* subgenus *Tayloria*: Article III

Subgenus *Tayloria* is endemic to the highlands of eastern Brazil. All but one species are confined to at least seasonally wet habitats in *campos rupestres* vegetation of the Espinhaço Range (and few djacent mountain ranges) that lies in the states of Minas Gerais and Bahia, whereas a single member, *G. lobata*, only occurs on granitic mountains and inselbergs at the border between Minas Gerais and Espírito Santo states. Detailed distribution maps for all species, based on comprehensive herbarium and field studies, are presented in the revision. The flora of the Epsinhaço Highlands shows a remarkable degree of endemism, often on a very small local scale (Giulietti & Pirani 1988; Rapini et al. 2002; Echternacht et al. 2011), and indeed, three of the five newly described species (*G. metallica*, *G. nebulicola* and *G. oligophylla*) are microendemic to a narrowly restricted area, and are currently known only from a single, or two locations. As these locations, like the majority of ecologically vulnerable carnivorous plant habitats in the Espinhaço Range, are threatened by human development – mainly by mining and agriculture – we assigned these three new species to the Red List category "critically endangered" according to the IUCN (2001) criteria.

The pattern of the fruiting pedicel reflexion was found to be a taxonomically useful morphological character for species delimitation in *G.* subgenus *Tayloria*. A single species, the phylogenetically basal branching *G. uncinata* (Fleischmann et al. 2010), has pedicels that are strongly circinate in fruit, whereas in all other species, the pedicel becomes reflexed or recurved in a distinctive, species-specific way after the flower has been pollinated successfully.
The species of *G.* subgenus *Tayloria* can further be distinguished by seed shape and testa ornamentation (characters which are also useful for species delimitation in the sister genus *Utricularia* (Taylor 1989)), and seeds are illustrated by line drawings and scan electron microscope (SEM) micrographs for all eight species of the subgenus. The basalmost branching member of the subgenus, *G. uncinata*, is characterized by a unique seed shape and papillate testa surface, which is distinct from all other *Genlisea* species. This species also differs from all other members of *G.* subgenus *Tayloria* by its entire corolla upper lip, and a

(slightly) expressed gibbous palate, two characters shared with species of *G*. subgenus *Genlisea*. The overall corolla shape, however, with a spur that is divergent from the corolla lower lip, but paralleling the pedicel, forming salverform blossom (Erbar & Leins 2010), is in accordance with the remaining members of *G*. subgenus *Tayloria*.

When put in phylogenetic context (*G. flexuosa* and *G. metallica* correspond to *G*. aff. *violacea* 'giant' and *G*. sp. 'Itacambira' respectively in Fleischmann et al. 2010; unpublished sequence data for *G. oligophylla* reveal it as consecutive sister to *G. metallica*; no sequence data available for the two new annual species yet), it becomes evident that the large, perennial species (*G. uncinata*, *G. oligophylla*, *G. flexuosa*, and *G. metallica*) represent the early branching taxa in *G.* subgenus *Tayloria*, which are consecutive sisters to the annual species. The origin of *G*. subgenus *Tayloria* can be assumed in the northern part of its range, as the extant most basally branching species, *G. uncinata*, is confined to the high mountain tops of the Chapada Diamantina, Bahia. From there, a phylogeographic southward migration is observed in the subsequently branching species: *G. flexuosa* occurs further south, but still represents the northernmost outpost of *G*. subgenus *Tayloria* in the Espinhaço Range of Minas Gerais, followed by the slightly more southerly occurring *G. metallica*, and finally the derived group, comprising the annual species *G. violacea* and *G. lobata*, which greatly extend the range far to the south and south-west, and east respectively. The polyphyly of *G. violacea* observed in the phylogenetic reconstructions of Fleischmann et al. (2010) – a single accession of *G. violacea* did not group with the remaining specimens sampled of this species but showed up in an unresolved trichotomy comprising *G. metallica* and the clade of annual species – can additionally be explained after the taxonomic revision of the subgenus. This "outlier" most likely resulted from material of an intermediate plant between *G. violacea* and *G. flexuosa*, which either represents a natural hybrid, or a fertile lineage of hybrid origin. These plants are found in certain regions, where the ranges of both putative parent species border.

2.2.3. Preliminary notes on *Genlisea* flower biology and pollination

Pollinator observations in Lentibulariaceae are rare, and only a few publications are dealing with their floral biology, mainly focussing on aquatic species of *Utricularia* (Kondo 1972; Jérémie 1989; Khosla et al. 1998; Yamamoto & Kadono 1990; Araki & Kadono 2003; Hobbhahn et al. 2006). No reports about floral visitors or mating system of *Genlisea* have been made thus far, and therefore some notes on the flower biology of the genus, based on my own observations made on plants in cultivation and *in situ* in Western African and Brazil are presented here.

Flower types and pollinators

Both subgenera further display a slightly different corolla morphology, which corresponds to two distinct flower types (Fleischmann et al. 2010).

Members of *G*. subgenus *Genlisea* are characterized by masked flowers ("snap-dragon blossoms"). In this type of personate flowers, a gibbous mask formed by the palate of the lower lip is firmly appressed to the upper lip, and therefore hiding the entrance to the corolla tube and nectar spur. The way to the flower interior has to be pushed open, either by the weight of the pollinator that is landing on the lower lip, or the pollinator is forcing its way by pulling the mask down. In the snap-dargon type flowers, the corolla tube is usually entered by the pollinator (see Fig. 7E). An unidentified member of the bee family Halictidae ("sweat bees"; bee taxa identified to the best of my knowledge) was observed by myself acting as pollinator of *G. stapfii* in a mass population of the plant on a small inselberg in northern Sierra Leone, where the small bees were frequently visiting the abundantly present small blue

flowers. The bees were pushing open the two corolla lobes, entering the corolla tube with their front body and forelegs (see Fig. 7E), and the white pollen of the *Genlisea* got deposited on the dorsal thorax of the bee, while it was feeding on the nectar from the spur. The bees stayed at each individual flower for a few seconds. As the bees were usually visiting several flowers of the *Genlisea* (almost the exclusive flowers present in the wet sward community on this inselberg among many Poaceae and Cyperaceae) subsequently, and pollen transfer from one flower to another was recorded. Therefore, melittophily can be reported for flowers of certain *Genlisea*. A larger bee, probably a male Megachilidae was observed and pictured as floral visitor of the large-flowered *G. aurea* in Brazil by F. Rivadavia (Fig. 7F). Megachilid bees are common generalist visitors of the majority of plants of the *campos rupestres* vegetation in Brazil (Freitas & Sazima 2006).

The members of *G*. subgenus *Tayloria* (perhaps except *G. uncinata*) display a different corolla design, with a slender, long corolla tube with narrowed entrance and disc-like spreading corolla lobes (creating a landing platform). They are typical "salverform blossoms" (hypocrateriform flowers), which are generally pollinated by insects with a long proboscis, like butterflies, moths, or dipterans like bombyliid flies (bee flies). In salverform flowers, the pollinator does not enter the corolla tube with its head, and pollen is likely to be deposited on the proboscis of the pollinator (Leins & Erbar 2010). Unfortunately, no flower visitors of any members of this subgenus could be observed during a field-trip to Brazil in 2010. The overall corolla shape of *G*. subgenus *Tayloria* closely resembles the flowers of the basal Lentibulariaceae genus *Pinguicula*, whereas the corolla design of *G*. subgenus *Genlisea* is identical to that of the sister genus *Utricularia*.

A possible specialization of members of both subgenera of *Genlisea* to different pollinators might be the reason for the different corolla morphology, and perhaps this is also linked to the observed differences in pollen morphology (Fromm-Trinta 1979, 1981; Taylor 1991). However comparative palynological data and pollinator observations are still lacking for the genus.

Breeding system

The zygomorphic flowers of *Genlisea* are chasmogamous and entomophilous (Taylor 1991; Fleischmann pers. obs.), however little is known about the breeding system yet. Autogamy was suspected by Taylor (1991), based on his observation and reports that certain species of *Utricularia* - which display a similar flower design - are facultative autogamous (Jérémie 1989; Taylor, 1989). My own hand-pollination and crossing experiments on plants grown in cultivation[2], and based on a broad sampling of species, showed that some members of *G*. subgenus *Genlisea* are indeed facultative autogamous and will self readily (especially in species with a small corolla, eg. *G. margaretae*, *G. glandulosissima*, *G. pygmaea*, *G. filiformis*, *G. oxycentron*, *G. repens* and *G. nigrocaulis*, almost every flower will develop into a ripe capsule even without pollination), while others (especially those with larger sized corollae) do not autonomously self. Non of the eight species of *G*. subgenus *Tayloria* was observed to be selfing in cultivation. And there is strong evidence that all species of this subgenus are allogamous when growing in their natural habitats, too: in all members of this subgenus, the pedicels reflex or bend down after the flower has successfully been pollinated (Fleischmann et al. 2011), whereas the pedicel of a flower with no fruit set will remain erect after anthesis. During my field studies I noticed that on ripe infructescences of any species, usually a certain percentage of pedicels is not reflexed and has not set ripe capsules. This is confirmed from herbarium specimens of fruiting plants of all taxa, which frequently show several pedicels on a scape that are not reflexed, and which do not bear a developing capsule.

Therefore several species of *Genlisea* seem to need insect visitation and allogamy for successful fruit set, however apparently not necessarily xenogamy ("outcrossing"), because in

[2] plants were kept indoors in a closed terrarium setup under artificial lights, in order to exclude possible pollinators.

cultivation all species of *Genlisea* (from both subgenera) can be selfed manually with their own pollen, which leads to normally developing capsules containing fertile seed (the seed produced from manual selfing did not show less germination ability than seed produced from artificial cross-pollination). Therefore no pollen self-incompatibility seems to be given. Parts of my results got supported independently by the crossing experiments of F. Rivadavia and M. Welge made with greenhouse-grown plants (pers. com.). Interspecific hybrids between closely related species can be made artificially (Fleischmann et al. 2011; K. Pasek, pers. com.), and do also rarely occur naturally (Fischer et al. 2000; Fleischmann et al. 2010), however no successful crossings between members of both subgenera, or different sections could be artificially created (Fleischmann, pers. obs.).

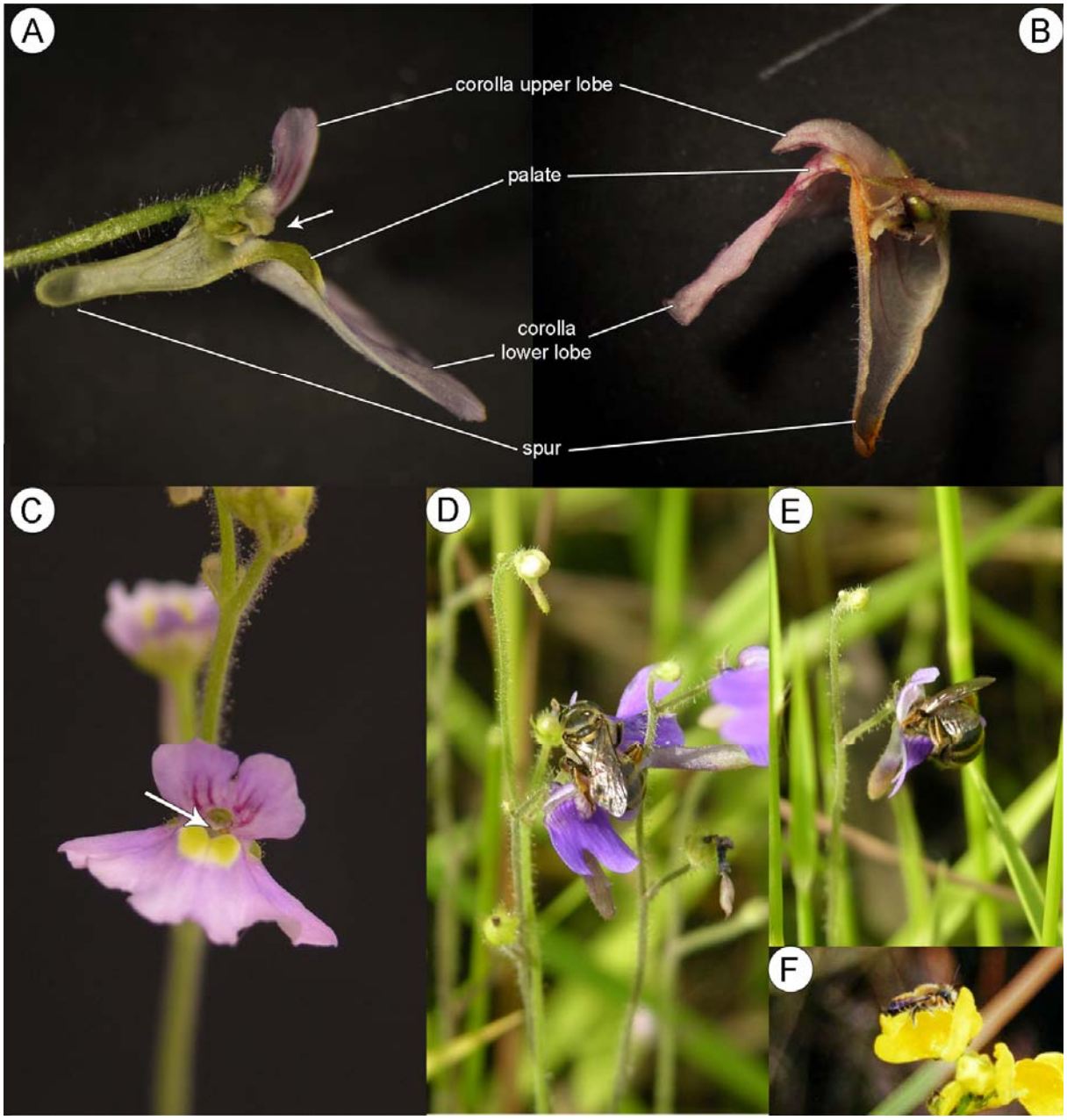


Fig. 7. Flower types and pollinators of *Genlisea*. Comparison of corolla design in both subgenera, longitudinal sections of flowers. A. *G.* subgenus *Tayloria* (shown *G. flexuosa*). B. *G.* subgenus *Genlisea* (shown *G. hispidula*). The orientation of the spur differs, forming a long tubular throat in *Tayloria*. In subgenus *Genlisea*, the entrance to the corolla tube is masked by the upwardly arching gibbous palate. C. Front view of a typical *Tayloria* flower (shown *G. flexuosa*), depicting the small slit between upper corolla lip and the yellow guide-mark of the lower lip, where the proboscis of the pollinator is likely to be entered (arrow). D, E. Pollinator of *G. stapfii* in Sierra Leone (Halictidae indet.). F. Flower visitor of *G. aurea* in Brazil (pollination not observed), probably Megachilidae (photo by F. Rivadavia with kind permission).

2.2.4. A new Andean species of *Pinguicula*: Article IV

Pinguicula chuquisacensis S. Beck, A. Fleischm. & Borsch was described and illustrated as a new species. The plant is narrowly endemic to a single vertical sandstone dripping wall in the valley of Nuevo Mundo in the department of Chuquisaca of south-eastern Bolivia, where it occurs at submontane to montane altitudes from ca. 2100 to 2500 m. Because of its narrow distribution to a single site, it is assigned to the Red List category "Critically Endangered" according to the IUCN (2001) criteria.

At about the same time, *P. jarmilae* Halda & Malina (Halda et al. 2007) was described based on unauthorized collected material of the same taxon from idem location in Bolivia. The respective authors published their description in a journal edited and released by the first author himself, with the publication dated to late 2007. Although the publication was only sent electronically as a PDF file, which does not constitute an effective publication according to the nomenclature rules of the ICBN, the publication date was accepted, as the authors claimed that the print version could not be distributed in time. It seems likely that this publication has to be given priority, and thus *P. chuquisacensis* actually represents a nomenclatural synonym of *P. jarmilae*.

Morphologically regarded, the new species appears most closely related to *P. calyptrata*, especially in terms of corolla morphology (Beck et al. 2008), and growth habit (see below). However molecular data (Beck et al. 2008) clearly show that *P. chuquisacensis* is sister to *P. involuta*, and it has already become evident from previous phylogenetic studies (Cieslack et al. 2005; Müller et al. 2006; Kondo & Shimai 2006; Shimai & Kondo 2007; Shimai et al. 2007) that vegetative characters and especially the corolla morphology are in fact useful for species delimitation in *Pinguicula*, but not for a solid infrageneric classification of the genus.

Pinguicula chuquisacensis differs from all Andean species by its large oblong leaves, in which the leaf margins are only slightly incurved. Like *P. calyptrata* it forms stolons that are originating from the leaf axils (Fleischmann, pers. obs. from cultivated specimens). Interestingly, stolons are absent in its sister species *P. involuta*, but are prolifically produced by the common sister of both, *P. calyptrata* (Casper 1966). This could represent an adaptation to different habitats, as *P. chuquisacensis* and *P. calyptrata* are colonizing wet open soil and steep dripping rock surfaces with shallow soil cover (Casper 1966; Beck et al. 2008), whereas *P. involuta* predominantly grows in carpets of moss, bogs, and in deep peaty soils that are rich in organic matter (Casper 1966; Brako & Zarucchi 1993; Rivadavia pers. com.), but it is found only very rarely on bare clayey soils (Casper 1966). Therefore, the stolon-forming habit of the two former species could represent an adaptation to quickly colonize exposed soil. Stolon runners are also known from two European *Pinguicula* species (*P. vallisneriifolia* and *P. longifolia*; Casper 1962, 1966; Blanca et al. 1999; Legendre 2000), as well as few Mexican and Central American species (*P. stolonifera*, *P. gigantea*; Luhrs 1995; pers. obs.), all of which are matt-forming lithophytes on vertical cliffs or exposed rock. On the other hand, a great number of lithophytic *Pincuicula* species do not form runners, thus this mode of vegetative propagation, which evolved several times in the genus, sees not to be necessarily connected with certain habitat preferences.
The stolon-forming habit of *P. chuquisacensis* was first noticed by Halda et al. (2007), but not obvious from the herbarium material I studied for Beck et al. 2008, and apparently not noticed by Hibert Huaylla, who studied *P. chuquisacensis* at the *locus classicus* in Bolivia.

2.2.5. A new Brazilian species of *Utricularia*: Article V

Utricularia rostrata A.Fleischm. & Rivadavia has been discovered by Fernando Rivadavia in the Chapada Diamantina mountain range of Bahia state, north-eastern Brazil in 1992. It is quite frequent in the high altitude sandstone mountains of this area, and it was personally studied by myself *in situ* in April 2010.

The highlands of the sandstone mountains of the Chapada Diamantina, which represent the northern section of the Espinhaço Range, are well-known for their species diversity and high levels of endemism (Stannard 1995; Funch et al. 2009). 20 species of *Utricularia* occur in this area (Taylor 1989), four of which are endemic, including the newly described *U. rostrata*. The area is quite well-explored botanically, nevertheless this delicate species has been overlooked in Flora treatments thus far (eg. Harley & Mayo 1980; Harley & Simmons 1986; Zappi et al. 2003), which, considering its widespread range and common occurance in the Chapada Diamantina, can only be explained by its diminuitive size.

We explain the unique seed dispersal mechanism of *U. rostrata*, in which the prolonged lower calyx lobe (which is persistant in fruit, like in all members of the genus), onto which the seed is shed from the ripe capsule, will act like a catapult to spread the seeds when hit by a rain dropplet. This "splash cup" mechanism of rain dispersal (ombrochory) might also apply to a few other species of *Utricularia* which have a similar lower calyx lobe that is greatly enlarged in fruit), and was postulated by us for the genus for the first time. Many plant species of open soil habitats in the Neotropics have either wind or water-dispersed seed (Macedo & Prance 1978; Gröger 2000; Arbeláez & Duivenvoorden 2004), and this is also true for several carnivorous plants from these habitats (Fleischmann et al. 2007; Rivadavia et al. 2009).

Utricularia rostrata was assigned to *U.* section *Aranella* (Barnhart) P.Taylor based on morphological characters (trap morphology, corolla characters and shape and sculpture of the seed). The species was meanwhile included in phylogenetic reconstructions of the genus *Utricularia* (Schäferhoff 2011), and its placement within this section is confirmed by molecular data as well. Trap morphology and seed characters have been shown to be useful taxonomic tools for the infrageneric classification of *Utricularia* (Taylor 1989), and the morphology-based sectional concept proposed by Taylor (1989) has been shown largely congruent with the phylogenetic reconstructions of the genus (Jobson et al. 2003; Müller & Borsch 2005; Müller et al. 2004, 2006; Schäferhoff 2011).
U. catolesensis G.L.Campos, M.Cheek & Giul. (Campos et al. 2010), which has been described later from the same area, is conspecific and represents a nomenclatural synonym of *U. rostrata*.

2.2.6. Checklist of *Genlisea*

In treatments previous to those of this thesis, 21 species of *Genlisea* were recognized (Taylor 1991; Fromm-Trinta 1984). However several species that were sunken in synonymy are considered as distinct species based on distinctive morphological characters (from herbarium studies, including the type specimens, and from field observations made on plants in situ). This species concept is also confirmed by molecular data (Fleischmann et al. 2010).
Furthermore, five species were newly described in Article III.

Following the current species concept, the genus comprises 32 taxa (31 accepted species and one yet undescribed species), 21 (including the undescribed species from section *Genlisea*) occurring in South America, and 11 from Africa (including Madagascar).

Distribution ranges were determined from herbarium records, and from literature (location data that seemed doubtful were verified by personal examination of the respective herbarium specimens: Fernández-Pérez 1964; Fischer et al. 2000; Fleischmann et al. 2010, 2011; Fromm-Trinta 1979, 1984; Olvera & Martínez 2002; Ritter & Crow 2000; Taylor 1967, 1991, 1999). The countries of the distribution range are arranged from North to South, the country of the type collection is underlined.

Genlisea A.St.-Hil. – Voy. Distr. Diam. 2: 428 (1833).

Genlisea* subgen. *Tayloria (Fromm) Eb.Fisch., S.Porembski & Barthlott - Nordic J. Bot. 20: 293 (2000), basionym: *Genlisea* sect. *Tayloria* Fromm – Bol. Mus. Nac. Rio de Janeiro, Bot. 44: 1(1977).

G. biloba Benj. – Fl. Bras. (Martius) 10: 254 (1847). = *violacea* A. St.-Hil.
G. cylindrica Sylvén – Ark. Bot. 8(6): 4 (1909). = *violacea* A. St.-Hil.
G. exhibitionista Rivadavia & A.Fleischm. – Phytotaxa 32: 19 (2011). Brazil.
G. flexuosa Rivadavia, A.Fleischm. & Gonella – Phytotaxa 32: 15 (2011). Brazil.
G. lobata Fromm – Bradea 5(14): 152 (1989). Brazil.
G. metallica Rivadavia & A.Fleischm. – Phytotaxa 32: 5 (2011). Brazil.
G. nebulicola Rivadavia, Gonella & A.Fleischm. – Phytotaxa 32: 21 (2011). Brazil.
G. oligophylla Rivadavia & A.Fleischm. – Phytotaxa 32: 10 (2011). Brazil.
G. reflexa Benj. – Fl. Bras. (Martius) 10: 254 (1847). = (?) *violacea* A. St.-Hil.
G. uncinata P.Taylor & Fromm – Bradea 3(41): 365 (1983). Brazil.
G. violacea A.St.-Hil. – Voy. Distr. Diam. 2: 429 (1833). Brazil.

Genlisea* subgen. *Genlisea* sect. *Africanae A.Fleischm., Kai Muell., Barthlott & Eb.Fisch. – Mol. Phylogenet. Evol. 56: 781 (2010).

G. africana Oliv. – J. Linn. Soc., Bot. 9: 145 (1865) [1867 publ. 1865]. Angola, Zambia, Zimbabwe, DR Congo (Congo-Kinshasa).
G. africana Oliv. subsp. *stapfii* (A.Chev.) P.Taylor – in Fl. Afr. Centr. Spermatophyt. Lentibulariac.: 58 (1972). = *stapfii* A. Chev.
G. africana Oliv. f. *pallida* R.E.Fries – Schwed. Rhod.-Kongo Exp.1: 301 (1916) = *africana* Oliv.
G. angolensis R.D.Good – J. Bot. 62: 165 (1924). Angola, DR Congo (Congo-Kinshasa)
G. barthlottii Porembski, Eb.Fisch. & B.Gemmel – Bull. Mus. Natl. Hist. Nat., B, Adansonia Sér. 4, 18: 152 (1996). Guinea, Sierra Leone.
G. hispidula Stapf – Fl. Cap. (Harvey) 4, II: 437 (1904). Nigeria, Cameroun, Central African Republic, DR Congo (Congo-Kinshasa), Kenya, Tanzania, Zambia, Zimbabwe, Malawi, Mozambique, Angola, South Africa.
G. hispidula Stapf subsp. *subglabra* (Stapf) P.Taylor – Kew Bull. 26 (3): 444 (1972). = *subglabra* Stapf
G. stapfii A.Chev. – Bull. Soc. Bot. France 58(Mém. 8d): 188. 1912 [1911 publ. 1912]. Senegal, Mali, Guinea-Bissau, Guinea, Sierra Leone, Liberia, Ivory Coast, Burkina Faso, Cameroun, Central African Republic, Gabon, Rep. Congo (Congo-Brazzaville), DR Congo (Congo-Kinshasa).

G. subglabra Stapf – Fl. Trop. Afr. [Oliver et al.] 4(2.3): 498. 1906 [Jun 1906]. Zambia, DR Congo (Congo-Kinshasa), Burundi, Tanzania, Malawi.
G. subviridis Hutch. – Botanist S. Afr. 528 (1946), in adnot. Zambia.
G. taylorii Eb.Fisch., Porembski & Barthlott – Nordic J. Bot. 20(3): 311 (2000), as '*taylori*'. Angola.

Genlisea* subgen. *Genlisea* sect. *Recurvatae A. Fleischm., Kai Muell., Barthlott & Eb. Fisch. – Mol. Phylogenet. Evol. 56: 781 (2010).

G. glandulossisima R.E.Fr. – Wiss. Erg. Schwed. Rhodesia-Kongo-Exp. 1911-1912 i: 301 (1916). Zambia, Tanzania.
G. margaretae Hutch. – Botanist S. Afr.: 529 (1946), in adnot. Zambia, Tanzania, Madagascar.
G. pallida Fromm & P.Taylor – Bradea 4(27): 177 (1985). Zambia, Angola.
G. recurva Bosser – Naturaliste Malgache X: 23 (1959). = *margaretae* Hutch.

Genlisea* subgen. *Genlisea* sect. *Genlisea

G. aurea A.St.-Hil. – Voy. Distr. Diam. 2: 429 (1833). Brazil.
G. anfractuosa Tutin – J. Bot. 72: 310. (1934). = *filiformis* A. St.-Hil.
G. esmeraldae Steyerm. – Fieldiana, Bot. 28: 534 (1953). = *pygmaea* A. St.-Hil.
G. filiformis A.St.-Hil. – Voy. Distr. Diam. 2: 430 (1833). Brazil, Venezuela, Guiana, Colombia, Bolivia, Cuba, Belize, Honduras, Nicaragua, Mexico.
G. glabra P.Taylor – Mem. New York Bot. Gard. 17(1): 203 (1967). Venezuela.
G. guianensis N.E.Br. – Hooker's Icon. Pl. 27: t. 2629. 1900 [1901 publ. May 1900]. Venezuela, Guiana, Brazil, Bolivia.
G. luetzelburgii Merl – ex Luetzelb. Estud. Bot. Nordeste Braz. 3: 223 (1923) [Insp. Fed. Obras Secc.Publ. 57] nomen, = *guianensis* N.E. Br.
G. luteoviridis C.Wright – Anales Acad. Ci. Med. Habana 6: 319. 1869 in Sauv. Fl. Cub. 90. = *filiformis* A.St.-Hil.
G. minor A.St.-Hil. – Voy. Distr. Diam. 2: 430. Brazil.
G. nigrocaulis Steyerm. – Bull. Torrey Bot. Club 75: 657 (1948). Brazil, Venezuela, Guiana.
G. ornata Mart. ex Benj. – Fl. Bras. (Martius) 10: 252, t. 21, f. 2 (1847). = *aurea* A. St.-Hil.
G. ornata Mart. ex Benj. var. *gracilis* Merl – ex Luetzelb. Estud. Bot. Nordeste Braz. 3: 223 (1923) [Insp. Fed. Obras Secc.Publ. 57] nomen, = *aurea* A.St.-Hil.
G. oxycentron P.Taylor – Fl. Trinidad & Tobago 2: 288 (1954). Trinidad-Tobago, Brazil, Venezuela.
G. pulchella Tutin – J. Bot. 72: 309 (1934). Guiana, Venezuela, Brazil.
G. pusilla Warm. – Vidensk. Meddel. Dansk Naturhist. Foren. Kjobenhavn (1874) 11 = *repens* Benj.
G. pygmaea A.St.-Hil. – Voy. Distr. Diam. 2: 431 (1833). Brazil, Venezuela.
G. repens Benj. – Fl. Bras. (Martius) 10: 253 (1847). Brazil, Venezuela, Guiana, Suriname, Paraguay.
G. roraimensis N.E.Br. – Trans. Linn. Soc. London, Bot. 6: 56. 1901 [1901-05 publ. Jan 1901]. Venezuela, Brazil, Guiana.
G. sanariapoana Steyerm. – Fieldiana, Bot. 28: 534 (1953). Venezuela, Colombia.
***G.* sp. nov.** 'tuberous' (in press.). Brazil.

2.3. Methodology

2.3.1. DNA extraction from problematic carnivorous plant tissue: Article VI

DNA extractions preceding molecular phylogenetic constructions of Lentibulariaceae (this thesis), Sarraceniaceae, and Droseraceae proved to be difficult from both fresh plant tissues, silica-dried material, and herbarium specimens. The main reason for the difficulty to obtain high-quality DNA that is suitable for PCR ampification and sequencing from these plants are secondary plant compounds (mainly polyphenols like flavonoids and tannins) and a high content of polysaccharides (especially present in the mucilage secreted from the glands of taxa with sticky flypaper traps). The polyphenolic compounds of many carnivorous plant taxa are found in the bright coloured foliage, as colourfull visual guides to attract insect prey (Juniper et al. 1989), but also as complex secondary metabolites that probably serve herbivore repellants (eg. complex quinones like plumbagin of carnivorous Caryophyllales; Hegnauer 1989).

Both polyphenols and polysaccharides are well-known to impede DNA extractions. During the homogenization of the plant tissue, polyphenols can become oxidized, and then will covalently bind to DNA molecules (Loomis 1974). This inferes with subsequent molecular standard techniques for DNA amplification, restriction digest, and cloning (Katterman & Shattuck 1983; Porebski et al. 1997; Stange et al. 1998). On the other hand, polysaccharides eluated with the genomic DNA will inhibit the activity of enzymes such as Taq polymerase, restriction endonucleases and ligases (Shioda & Marakami-Muofshi 1987; Richards 1988). Commercially available kits for standard DNA extraction, such as the ones used in the laboratories of the Heubl group, do not consider isolation from problematic plant tissues. Several modified protocols have been published for DNA extraction specifically from plants with high contents of polyphenols and sugars (eg. Porebski et al. 1997; Stange et al. 1998) have been published, some even designed for carnivorous *Drosera* leaf tissue (Bekesiova et al. 1999). However none of them tested did yield in good quality DNA for our purposes. Genomic DNA extraction in carnivorous plants therefore often had to be acquired from corolla material, which contains less inhibiting compounds (eg. Müller et al. 2004).

For our long-term phylogenetic research on a wide range of carnivorous plant taxa, we established a new modified standard protocol for quick, easy and reliable DNA extraction from any carnivorous plant tissue, that overcomes the usual problems that arise from the high contents of secondary plant compounds and polysaccharides. Our protocol is based on a commercially available kit, with additional steps to fully remove the two main inhibiting compounds. Polysaccharides get dissolved during the cell lysis step in a high salt concentration buffer of our modified DNA extraction procedure. Polyphenols are bound during the lysis step by added PVP and N-laurolyl sarcosine, and effectively separated from the DNA by centrifugation. Total genomic DNA extracted with our protocol showed no degradation, and could be used successfully for standard molecular analysis.

3. Outlook and Perspectives: genome sizes and chromosomes

Although *Genlisea* is a rather elusive genus of the carnivorous Lentibulariaceae, with restricted distribution, rather inconspicuous habit, confined to certain nutrient poor habitats, and rarely grown in botanical gardens, it nevertheless gained an increased interest of the scientific community recently, with discovery that some species of *Genlisea* posses ultra-small nuclear genomes, which are the smallest known among angiosperms (Greilhuber et al. 2006). Within the genus *Genlisea*, genome sizes range from 63.4 to 1510 Mbp (Million basepairs, Greilhuber et al. 2006), and in different populations of certain species (most notably *G. aurea*), the genome size can range about the two-fold (Albert et al. 2010).

Both the smallest and the largest genomes known for Lentibulariaceae are found within the genus *Genlisea*, with the ultrasmall genomes reported for *G. aurea* and *G. margaretae* representing one end of the scale, and the about 24-fold larger genomes of members of subgenus *Tayloria* and *G. hispidula* on the other side (Greilhuber et al. 2006; Albert et al. 2010). The genome sizes of those members of the sister genera *Utricularia* and *Pinguicula* that have been recorded so far, fall in a gap of about 931 Mbp that lies between the ultrasmall and the larger genomes.

High substitution rates have been observed in *Genlisea* and *Utricularia* across all three genomic compartiments (Jobson & Albert 2002), those are the highest substitution rates currently known among angiosperms (Müller et al. 2004, 2006). The fast molecular evolution can possible linked to speciation and diversitifcation (Jobson & Albert 2002), but also to genome size minituarisation (Müller et al. 2006; Greilhuber et al. 2006).

Moreover, the chromosomes of some members of *Genlisea* are diminutive in size, reaching those of bacteria (Greilhuber et al. 2006) – one reason why karyotypes have not previously been reported for the genus yet. Only very few cytological studies have been carried out for Lentibulariaceae thus far, and these have been predominantly focusing on *Pinguicula* (summarized in Casper & Stimper 2009) or aquatic species of *Utricularia* section *Utricularia* (summarized in Rahman et al. 2001 and Casper & Manitz 1975). In the case of *Genlisea*, chromosome numbers have been reported for three species only thus far (Greilhuber et al. 2006), however two of them represent just approximations. The general lack of karyotype data available for Lentibulariaceae (excluding *Pinguicula*) is mainly due to the very small size of the metaphase chromosomes found in *Utricularia* and *Genlisea* (Rahman et al. 2001, Greilhuber et al. 2006), because of problems with chromosome staining using standard dyes (Rahman et al. 2001), but also because of the difficulty to obtain suitable living material of most taxa for chromosome counts.

One focus of the ongoing research is a forthcoming comprehensive study on the evolution of genome sizes and chromosome numbers in phylogenetic context. In a joint work with Prof. J. Greilhuber and Dr. E. Temsch from Vienna University, T. Michaels and W. Wang from Ruetgers University, Jersey, and F. Rivadavia, we have evaluated the genome from cultivated plant material for the majority of species of *Genlisea*, using both flow-cytometry and Feulgen densitometry, and chromosome counts were performed for the majority of taxa.

All data on taxonomy, ecology, chorology and phylobiography presented in this thesis, a generic revision, as well as additional anatomical and cytological data, will be compiled in a detailed monograph on the genus *Genlisea*, which is going to be published in due time (Fleischmann, in prep.).

4. Zusammenfassung

In der vorliegenden Arbeit werden Ergebnisse zur Systematik, Taxonomie, Evolution und Biologie der Familie Lentibulariaceae (Wasserschlauchgewächse) präsentiert, mit besonderem Schwerpunkt auf der Gattung *Genlisea* (Reusenpflanze).
Dabei wurden sowohl molekularbiologische Methoden der phylogenetischen Rekonstruktion, als auch Methoden der systematisch-taxonomischen Verwandtschaftsanalyse angewandt. Darüber hinaus werden einige blütenökologische Beobachtungen zu *Genlisea* erstmals geschildert, die an Naturstandorten in Afrika (Sierra Leone, Zambia und Südafrika) und Südamerika (Brasilien und Venezuela), sowie an Pflanzen in Kultur gemacht wurden.

Die anfänglichen, und meist hinreichend bekannten Probleme bei der DNA-Extraktion aus Material von Pflanzen mit einem hohen Gehalt an Polysacchariden und sekundären Inhaltstoffen, vor allem polyphenolischen Substanzen, – beide zahlreich in den Blättern von karnivoren Pflanzen vorhanden – wurden gelöst, indem empirisch und durch Vergleich und Kombination verschiedener bekannter Extraktionmethoden ein modifiziertes Verfahren zur DNA-Isolation aus karnivoren Pflanzen erstellt wurde. Dies ermöglichte im weiteren Verlauf die problemlose Gewinnung von hochqualitativer DNA verschiedenster karnivorer Pflanzenfamilien zur Analyse mit molekulargenetischen Standardmethoden (PCR, Klonierung, Sequenzierung).
Basierend auf einem umfangreichen Sampling von Gattungen aus der Angiospermenordung Lamiales (Lippenblütlerartige), zu der auch die Lentibulariaceae gehören, wurde anhand der schnell-evolvierenden Chloroplastenmarker *trnK/matK*, *trnL-F* und *rps16* erstmals eine umfassende Phylogeniehypothese zur dieser Ordnung aufgestellt. Die Baumtopologie ist in großen Teilen statistisch gut abgesichert, und wird weiterhin auch durch morphologische Merkmale gestützt. Die Evolution von Karnivorie und wesentlicher morphologischer Merkmale innerhalb der Lamiales wurde rekonstruiert, und taxonomische Konsequenzen bezüglich der Umschreibung einiger Familien aus der Baumtopologie diskutiert.
In einem weiteren Teilaspekt der Arbeit wurde eine robuste Phylogenie der Gattung *Genlisea* postuliert, die nahzu alle Arten der Gattung repräsentiert. Die Evolution vegetativer und generativer Merkmale, sowie die raum-zeitliche Differenzierung wurden rekonstruiert, wobei sich zeigt, dass die Gattung wahrscheinlich ihren Ursprung in den Tropen der Neuen Welt hat, und von dort aus die Besiedelung Afrika erfolgte, wobei aus einer abgeleiteten Gruppe afrikanischer Arten wiederum der Vorläufer einer zweiten Besiedelung Südamerikas durch Fernverbreitung entstammt. Die Baumtopologie wird durch zahlreiche morphologische Merkmale gestützt, und auch die bisherige Einteilung der Gattung in zwei Untergattungen wird durch die molekular-phylogenetische Analyse bestätigt. Die drei monophyletischen Gruppen (Clades) von Arten der Untergattung *Genlisea* lassen sich auch morphologisch gut abgrenzen, und wurden daher auf taxonomischem Rang als Sektionen formell beschrieben.
Eine eingehende Revision von *Genlisea* Untergattung *Tayloria*, durch morphologisch-vergleichende Untersuchung von Herbarmaterial, sowie Feldstudien und Beobachtungen der Pflanzen *in situ*, führten im Rahmen dieser Arbeit zur Beschreibung von fünf neuen Arten von *Genlisea* aus Brasilien. Dabei wurden die neuen Taxa, sowie die bisher bekannten drei Arten der Untergattung detailliert morphometrisch analysiert und beschrieben, sowie mit Zeichungen, Photos von Pflanzen am Naturstandort und rasterelektronenmikroskopischen Aufnahmen illustriert. Daten zur Verbreitung, Ökologie und Gefährdung für alle acht Arten wurden ermittelt und ein Bestimmungsschlüssel erstellt, der eine eindeutige Identifizierung aller Arten der Untergattung anhand von Herbarmaterial oder lebenden Pflanzen ermöglicht.
Aufsammlungen einer unbestimmten *Pinguicula* (Fettkraut) aus Bolivien konnten als neue Art identifiziert werden. Diese wurde morphologisch detailliert beschrieben und illustriert. In diesem Zusammenhang wurde die neue Art *P. chuquisacensis*, mit anderen Vertretern

Andiner *Pinguicula*, auch erstmals in phylogenetische Stammbaumberechungen mit einbezogen. Die Merkmalsevolution der Wuchsform, und die Besiedelung und Ausbreitungsgeschichte der Gattung *Pinguicula* in den Anden wurde rekonstruiert, wobei die Andinen Taxa als paraphyletische Gruppe aufgedeckt wurden.
Die Wasserschlauch-Art *Utricularia rostrata* wurde aus Brasilien neu beschrieben und erstmals wissenschaftlich dokumentiert. Ergänzende Bemerkungen zur Taxonomie, Morphologie und Biologie der neuen Art wurden gemacht, und ein erweiterter Bestimmungsschlüssel für die zugehörige Sektion erstellt.

Summary

In the present doctoral thesis the results of my studies on the systematics, taxonomy, evolutionary history and biology of the family Lentibulariaceae (Bladderwort Family) are provided, with special emphasis on the genus *Genlisea* (corkscrew plants). Methods of molecular phylogenetic reconstruction were used in combination with morphological analysis and descriptive taxonomy. Own observations on the pollination biology of *Genlisea* are reported for the first time, which have been made during field excursions in Africa (Sierra Leone, Zambia and South Africa) and South America (Brazil and Venezuela), as well as on cultivated plants.

Initial problems of DNA extraction from carnivorous plant material, which is rich in polysaccharids and secondary metabolites, especially polyphenols, were solved by developing a modified protocol, which resulted from experimental comparison and modifications of standard extraction methods. Based on this protocol, the extraction of highly pure genomic DNA was possible from various carnivorous plant taxa studied.
Based on the analysis of the rapidly evolving chloroplast regions *trnK/matK*, *trnL-F* and *rps16* from a comprehensive sampling of genera of the angiosperm order Lamiales, to which also many carnivorous genera belong to, a robust phylogeny of the entire order was postulated. The resulting topology gets high support from both statistical analysis and morphological characters. Evolution of carnivory and several morphological traits within the Lamiales were reconstructed, and taxonomic conclusions on the circumscription of certain families were discussed.
Another focus was to present a well-supported phylogeny of *Genlisea*, which included the majority of species of the genus. The evolution of vegetative and generative characters was discussed, and phylogeographic reconstructions revealed an origin in the Neotropics, from which Africa was colonized by long-distance dispersal. A lineage derived from the African grade re-colonized South America by a second trans-Atlantic long-distance dispersal event. Based on the tree topology and morphological character sets, a new infrageneric classification of *Genlisea* was proposed.
A taxonomic revision of *Genlisea* subgenus *Tayloria* was conducted by comparative studies of herbarium specimens and field studies. During this revision, five new species were described from Brazil, and all eight known taxa of the subgenus were described and illustrated in detail, including an identification key, photographs of plants *in situ*, scan electron micrographs of seeds, as well as data on distribution, ecology and conservation.
Herbarium collections of a hitherto unidentified *Pinguicula* taxon from Bolivia were identified as new species, which was formally described and illustrated. The new species was included in a first phylogenetic reconstruction of the Andean *Pinguicula* species, which were revealed to be paraphyletic, and the evolution of growth type as well as the radiation and phylogeography of the Andean taxa of the genus were discussed.
Finally, a new species of *Utricularia* was described and documented from Brazil, including remarks on taxonomy, morphology and biology, and an identification key was presented for the respective section of the genus.

5. Acknowlegements

First, I have to thank my supervisor Prof. Günther Heubl, who enabled and encouraged me to work about various topics of carnivorous plant systematics and biology, and who greatly supported my research from the very beginning. He introduced me to the techniques of molecular systematic biology, and readily helped solving emerging problems during the work leading to the present thesis. I am also grateful to him for involving me in his lab group.
Further thanks are due to Prof. Kai Müller, Bonn and Münster, who co-supervised most parts of my work, and who involved me in his DFG-funded Lamiales research project. Kai also adviced me a lot regarding methods of phylogenetic reconstructions and data processing.
I thank Prof. Susanne Renner for the numerous helpful discussions and information on plant pollination ecology, and long-distance dispersal theory and mechanisms, as well as for instantly providing and digging up supporting literature at all hours.
I am indebted to Prof. Wilhelm Barthlott, Bonn, who provided me with useful information on *Genlisea*, and supported my work in its initial phase. Prof. Eberhard Fischer, Koblenz, is thanked for his helpful contributions to African Lentibulariaceae. Prof. Thomas Borsch, Berlin, enabled me to join the phylogenetic work on Andean *Pinguicula*.

The graduate funding of the Universität Bayern e.V. is thanked for providing a research grant for this thesis.

I am much obliged to Tanja Ernst for her never-ending technical support in the laboratory, for having me patiently tought molecular work techniques, and for generous help all time.
Bastian Schäferhoff, Bonn and Münster, generated much of the phylogenetic data for the Lamiales project, and contributed to all studies dealing with this subject.

The present work would not have been possible without the help of many colleagues and friends, with whom I have been working on carnivorous plant systematics and biology for many years now. The first to thank is Fernando Rivadavia, for sharing his general carnivorous plant knowledge, but especially for generously providing essential information and herbarium material for the present studies, and for our common travels to Lentibulariaceae habitats in Zambia and South Africa. My good friend and travel buddy Stewart McPherson did not only enable me to see so many rare carnivorous plant taxa in their natural habitats all over the world in the past years, by initiating and organizing expeditions to even the most remote places, but he also kindly offered me the possibilty to join some of his carnivorous book and research projects, which I really appreciate a lot. I have to thank Paulo Gonella, who kindly supported me with herbarium studies and fieldwork in Brazil. Special thanks are also to Allen Lowrie, Alastair Robinson, François Mey, Andy Smith, Marcello Catalano, Jan Schlauer, and Otto Huber for discussions about carnivorous plant systematics and biology, and to Klaus Keller, Johannes Betz, Stefan Ippenberger, Kamil Pasek, Jan Flisek, Matthias Teichert, and Markus Welge for providing cultivated plant material for my studies. Special thanks are to my early mentor Paul Debbert, who deepend my interest in carnivorous plant systematics and cultivation, and who always willingly shared both plants and his secrets how to grow them. The late Jochen Schottler, Munich Botanical Gardens, will be remembered for kindly providing plant material and helpful hints and tips on carnivorous plant cultivation to me.

I am obliged to all my fellow colleages at the Institute of Systematic Botany, Munich Herbarium, and the Botanic Gardens for their help, support and the enjoyable working environment during my studies of biology.

Special thanks are addressed to Agnes Scheunert, Sylvia Söhner, Catalina Olano, Carmen Zinsmeister, Sebastian Gardt, Stefan Kattari, Florian Turini, and Christian Bräuchler for their friendship, discussions and professional advices all the time. Stefan, I enjoyed the numerous botanizing excursions we made, during which I learned a lot from your intimate botanical knowledge. Agnes and Flo, thank you for sharing your technical expertise with me, especially regarding statistics and analysis methods. Basti: Thanks for almost everything, the discussions, and the beer in the garden. Hanno Schäfer is acknowledged for our talks about plant pollination ecology, and for his travel company in Sierra Leone, Günter Gerlach, Christoph Heibl, and Boris Schlumpberger for sharing their knowlegde about pollination biology, and Andreas Gröger for helpful discussions about South American plant biology. The help of Eva Facher with SEM microscopic analysis is greatly acknowledged.

Finally, but most importantly, I thank my family and friends for their long-time support, and for more or less understanding and tolerating my everlasting obsession in plants. I am deeply grateful to my parents, for giving me the opportunity and support for my studies, and for always encouraging my interest in biology. I am much beholden to my beloved girlfriend Tamara.

6. References

Adamec, L., 1997. Mineral nutrition of carnivorous plants: a review. Botanical Revue 63: 273-299.

Adamec, L., 2003. Zero water flows in the carnivorous genus *Genlisea*. Carnivorous Plant Newsletter 32: 46-48.

Adamec, L., 2007. Oxygen concentrations inside the traps of the carnivorous plants *Utricularia* and *Genlisea* (Lentibulariaceae). Annals of Botany 100: 849-856.

Adamec, L., 2008. Soil fertilization enhances growth of the carnivorous plant *Genlisea violacea*. Biologia 63: 201-203.

Albert, V.A., Williams, S.E., Chase, M.W., 1992. Carnivorous plants: phylogeny and structural evolution. Science 257: 1491-1495.

Albert, V.A., Jobson, R.W., Michael, T.P., Taylor, D.J., 2010. The carnivorous bladderwort (*Utricularia*, Lentibulariaceae): a system inflates. Journal of Experimental Botany 61: 5-9.

Alcalá, R.E., Mariano, N.A., Osuna, F., Abarca, C.A., 2010. An experimental test of the defensive role of sticky traps in the carnivorous plant *Pinguicula moranensis* (Lentibulariaceae). Oikos 119: 891-895.

Alves, R.J.V., Kolbek, J., 1994. Plant species endemism in savanna vegetation on table mountains (*Campo Rupestre*) in Brazil. Vegetatio 113: 125-139.

Alves, R.J.V., Cardin, L., Kropf, M.S., 2007. Angiosperm disjunction "*Campos rupestres - restinga*": a re-evaluation. Acta Botanica Brasilica 21: 675-685.

Anderberg, A.A., Rydin, C., Källersjö, M., 2002. Phylogenetic relationships in the order Ericales s.l.: analysis of molecular data from five genes from the plastid and mitochondrial genomes. American Journal of Botany 89: 677-687.

Anderson, A.B., 1981. White sand vegetation of Brazilian Amazonia. Biotropica 13: 199-210.

Angiosperm Phylogeny Group [A.P.G. II], 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society 141: 399-436.

Angiosperm Phylogeny Group [A.P.G. III], 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161: 105-121.

Araki, S., Kadono, Y., 2003. Restricted seed contribution and clonal dominance in a free-floating aquatic plant *Utricularia australis* R.Br. in southwestern Japan. Ecological Research 18: 599-609.

Arbeláez, M.V., Duivenvoorden, J.F., 2004. Patterns of plant species composition on Amazonian sandstone outcrops in Colombia. Journal of Vegetation Science 15: 181-188.

Barthlott, W., Gröger, A., Porembski, S., 1993. Some remarks on the vegetation of tropical inselbergs: diversity and ecological differentiation. Biogéographica 69: 105-124.

Barthlott, W., Lauer, W., Placke, A., 1996a. Global distribution of species diversity in vascular plants: towards a world map of phytodiversity. Erdkunde 50: 317-327.

Barthlott, W., Porembski, S., Szarzynski, J., Mund, J.-P., 1996b. Phytogeography and vegetation of tropical inselbergs. In: Guillaumet, J.-L., Belin, M., Puig, H. (eds.) Phytogéographie tropicale. Réalités et perspectives. Colloques & Seminaires ORSTOM, Paris: 15-24.

Barthlott, W., Porembski, S., Fischer, E., Gemmel, B., 1998. First protozoa-trapping plant found. Nature 392: 447.

Barthlott, W., Porembski, S., Seine, R., Theisen, I., 2004. Karnivoren. Biologie und Kultur fleischfressender Pflanzen. Eugen Ulmer, Stuttgart.

Barthlott, W., Hostert, A., Kier, G., Küper, W., Kreft, H., Mutke, J., Rafiqpoor, M.D., Sommer, J. H., 2007. Geographic patterns of vascular plant diversity at continental to global scale. Erdkunde 61: 305-315.

Bayer, R.J., Hufford, L., Soltis, D.E., 1996. Phylogenetic relationships in Sarraceniaceae based on *rbcL* and ITS sequences. Systematic Botany 21: 121-134.

Beal, W.J., 1875. Carnivorous plants. Proceedings of the American Association for the Advancement of Science 1875B: 251-253.

Beauvais, A., 2009. Ferricrete biochemical degradation on the rainforest–savannas boundary of Central African Republic. Geoderma 150: 379-388.

Beck, S. G., Fleischmann, A., Huaylla, H., Müller, K. F., Borsch, T., 2008. *Pinguicula chuquisacensis* (Lentibulariaceae), a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula*. Willdenowia 38: 201-212.

Bekesiova, I., Nap, J.P., Mlynarova, L., 1999. Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. Plant Molecular Biology Reporter 17: 269-277.

Benjamin, L., 1847. Utricularieae. In: Martius, C.F.P. von (ed.), Flora Brasiliensis, 10. R. Oldenbourg, Munich and Leipzig: 233-256.

Benzing, D.H., 1986, Foliar specialization for animal-assisted nutrition in Bromeliaceae. In Juniper, B.E., Southwood, T.R.E. (eds.), Insects and the plant surface. Edward Arnold, London: 235-256.

Benzing, D.H., Givnish, T.J., Bermudes, D., 1985. Absorptive trichomes in *Brocchinia reducta* (Bromeliaceae) and their evolutionary and systematic significance. Systematic Botany 10: 81-91.

Blanca, G., Ruíz-Rejón, M., Zamora, R., 1999. Taxonomic revision of the genus *Pinguicula* L. in the Iberian Peninsula. Folia Geobotanica 34: 337-361.

Boast, R., 1990: Dambos: a review. Progress in Physical Geography 14: 153-177.

Bosser, J., 1958. Sur deux nouvelles Lentibulariacées de Madagascar. Naturaliste Malgache 10: 21-23.

Brako, L., Zarucchi, J.L., 1993. Catalogue of the flowering plants and gymnosperms of Peru. Monographs in Systematic Botany from the Missouri Botanical Garden 45, Missouri.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Källersjö, M., Barkhordarian, E., 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Molecular Phylogenetics and Evolution 24: 274-301.

Brito, Y., Ramirez, N., 1988. Algunas características estructurales y especificidad de la vegetación en una comunidad pantanosa de los Altos Llanos Centrales Venezolanos. Boletin de la Sociedad Venezolana de Ciencias Naturales 145: 11-31.

Brugger, J., Rutishauer, R., 1989. Bau und Entwicklung landbewohnender *Utricularia*-Arten. Botanica Helvetica 99: 91-146.

Burnham, R.J., Graham, A., 1999. The history of neotropical vegetation: new developments and status. Annals of the Missouri Botanical Garden 86: 546-589.

Cameron, K.M., Wurdack, K.J., Jobson, R.W., 2002. Molecular evidence for the common origin of snap-traps among carnivorous plants. American Journal of Botany 89: 1503-1509.

Campos, G.L., Cheek, M., Giulietti, A.M., 2010. Uma nova espécie de *Utricularia* L. (Lentibulariaceae) da Chapada Diamantina, Bahia, Brasil. Sitientibus Série Ciêcias Biologicas 10: 233-235.

Casper, S.J., 1966. Monographie der Gattung *Pinguicula* L. Bibliotheca Botanica 127/128: 1-209.

Casper, S.J., Manitz, H., 1975. Beiträge zur Taxonomie und Chorologie der mitteleuropäischen *Utricularia*-Arten. Feddes Repertorium 86: 211-232.

Casper, S.J., Stimper, R., 2009. Chromosome numbers in *Pinguicula* (Lentibulariaceae): survey, atlas, and taxonomic conclusions. Plant Systematics and Evolution 277: 21-60.

Chase, M.W., Christenhusz, M.J.M., Sanders, D., Fay, M.F., 2009. Murderous plants: Victorian Gothic, Darwin and modern insights into vegetable carnivory. Botanical Journal of the Linnean Society 161: 329-356.

Cieslack, T., Polepalli, J.S., White, A., Müller, K., Borsch, T., Barthlott, W., Steiger, J., Marchand, A., Legendre, L., 2005. Phylogenetic Analysis of *Pinguicula* (Lentibulariaceae): Chloroplast DNA Sequences and Morphology Support Several Geographically Distinct Radiations. American Journal of Botany 92: 1723-1736.

Conran, J.G., Dowd, J.M., 1993. The phylogenetic relationship of *Byblis* and *Roridula* (Byblidaceae-Roridulaceae) inferred from partial 18S ribosomal RNA sequences. Plant Systematics and Evolution 188: 73-86.

Croizat, L., 1960. Principia botanica, or beginnings of botany (with sketches by the author). Léon Croizat, Caracas.

Cronquist, A., 1981. An integrated system of flowering plants. Columbia University Press, New York.

Darnowski, D.W., Fritz, S., 2010. Prey preference in *Genlisea* small crustaceans, not protozoa. Carnivorous Plant Newsletter 39: 114-116.

Darwin, C., 1875. Insectivorous plants. John Murray, London.

Diels, L., 1937. Beiträge zur Kenntnis der Vegetation und Flora von Ecuador. Bibliotheca Botanica 116, Schweizerbart, Stuttgart.

Dörrstock, S., Porembski, S., Barthlott, W., 1996. Ephemeral flush vegetation on inselbergs in the Ivory Coast (West Africa). Candollea 51: 407-419.

Echternacht, L., Trovó, M., Oliveira, C.T., Pirani, J.R., 2011. Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. Flora 206: 782-791.

Ellison, A.M., Gotelli, N.J., 2009. Energetics and the evolution of carnivorous plants: Darwin's 'most wonderful plants in the world'. Journal of Experimental Botany 60: 19-42.

Fenner, C.A., 1904. Beiträge zur Kenntnis der Anatomie, Entwicklungsgeschichte und Biologie der Laubblätter und Drüsen einiger Insektivoren. Flora 23: 335-434.

Fernández-Pérez, A., 1964. Plantas insectívoras, I: Lentibulariáceas de Colombia y Perú. Caldasia 9: 5-84.

Fischer, E., Barthlott, W., Seine, R., Theisen, I., 2004. Lentibulariaceae. In: Kubitzki, K. (ed.), The Families and Genera of Vascular Plants. Springer, Berlin.

Fischer, E., Porembski, S., Barthlott, W., 2000. Revision of the genus *Genlisea* (Lentibulariaceae) in Africa and Madagascar with notes on ecology and phytogeography. Nordic Journal of Botany 20: 291-318.

Fish, D., 1976, Structure and composition of the aquatic invertebrate community inhabiting epiphytic bromeliads in south Florida and the discovery of an insectivorous bromeliad. PhD-Thesis, University of Florida, Gainesville.

Fleischmann, A., Wistuba, A., McPherson, S., 2007. *Drosera solaris* (Droseraceae), a new sundew from the Guayana Highlands. Willdenowia 37: 551-555.

Fleischmann, A., Schäferhoff, B., Heubl, G., Rivadavia, F., Barthlott, W., Müller, K.F., 2010. Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae). Molecular Phylogenetics and Evolution 56: 768-783.

Fleischmann, A., 2010. Evolution of carnivorous plants. In: McPherson, S., Carnivorous plants and their habitats. Redfern, Dorset: 68-123.

Fleischmann, A., Rivadavia, F., Gonella, P.M., Heubl, G., 2011. A revision of *Genlisea* subgenus Tayloria (Lentibulariaceae). Phytotaxa 33: 1-40.

Frank, J.H., O'Meara, G.F., 1984. The bromeliad *Catopsis berteroniana* traps terrestrial arthropods but harbors *Wyeomyia* larvae (Diptera: Culicidae). Florida Entomologist 67: 418-424.

Freitas, L., Sazima, M., 2006. Pollination biology in a tropical high-altitude grassland in Brazil: interactions at the communitiy level. Annals of the Missouri Botanical Garden 93: 465-516.

Fritsch, P.W., Almeda, F., Martins, A.B., Cruz, B.C., Estes, D., 2007. Rediscovery and phylogenetic placement of *Philcoxia minensis* (Plantaginaceae), with a test of carnivory. Proceedings of the California Academy of Sciences 58: 447-467.

Fromm-Trinta, E., 1977. *Tayloria* Fromm-Trinta - Nova seção do gênero *Genlisea* St.-Hil. (Lentibulariaceae). Boletim do Museu Nacional Rio de Janeiro, Botanica 44: 1-4.

Fromm-Trinta, E. 1979. Revisão das espécies do gênero *Genlisea* St.-Hil (Lentibulariaceae) das regiões sudeste e sul do Brasil. Rodriguésia 31(49): 17-139.

Fromm-Trinta, E., 1981. Revisão do gênero *Genlisea* St.-Hil. (Lentibulariaceae) no Brasil. Boletim do Museu Nacional Rio de Janeiro, Botanica 61: 1-21.

Fromm-Trinta, E., 1984. Genliseas americanas. Sellowia 36: 55-62.

Fromm-Trinta, E., 1989. *Genlisea lobata* Fromm-Trinta – Uma nova espécie para o gênero *Genlisea* St.-Hil. Sect. *Tayloria* (Lentibulariaceae). Bradea 4: 152-155.

Funch, R.R., Harley, R.M., Funch, L.S., 2009. Mapping and evaluation of the state of conservation of the vegetation in and surrounding the Chapada Diamantina National Park, NE Brazil. Biota Neotropica 9: 2130.

Giulietti, A.M., Pirani, J.R., 1988. Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. In: Vanzolini, P.E.. Heyer, W.R. (eds.), Proceedings of a workshop on neotropical distribution patterns. Academia Brasileira de Ciências, Rio de Janeiro: 39-69.

Giulietti, A.M., Pirani, J.R., Harley, R.M., 1997. Espinhaço Range region, Eastern Brazil. In: Davis, S.D., Heywood, V.H., Herrera-MacBryde, O., Villa-Lobos, J., Hamilton, A.C. (eds.), Centres of plant diversity: a guide and strategy for their conservation, 3: The Americas. WWF/IUCN Publications Unit, Cambridge: 397-404.

Givnish, T.J., Burkhardt, E.L., Happel, R.E., Weintraub, J.D., 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. The American Naturalist 124: 479-497.

Givnish, T.J., 1989. Ecology and evolution of carnivorous plants. In: Abrahamson, W.G. (ed.), Plant–animal interactions. McGraw-Hill, New York: 243-290

Gledhill, D., 1970. The vegetation of superficial ironstone hardpans in Sierra Leone. Journal of Ecology 58: 265-274.

Goebel, K., 1891. Pflanzenbiologische Schilderungen II. N.G. Elwert, Marburg.

Goebel, K., 1893. Zur Biologie von *Genlisea*. Flora 77: 208-212.

Goebel, K., 1913. Organographie der Pflanzen I. Gustav Fischer, Jena.

Greilhuber, J., Borsch, T., Müller, K., Worberg, A., Porembski, S., Barthlott, W., 2006. Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. Plant Biology 8: 770-777.

Gröger, A., 1995. Die Vegetation der Granit-Inselberge Südvenezuelas: Ökologische und biogeographische Untersuchungen. PhD-Thesis, Universität Bonn.

Gröger, A., 2000. Flora and vegetation of inselbergs of Venezuelan Guayana. In: Porembski, S., Barthlott, W. (eds.), Inselbergs: biotic biversity of isolated outcrops in tropical and temperate regions. Ecological Studies 146. Springer, Berlin: 291-314.

Gröger, A., Huber, O., 2007. Rock outcrop habitats in the Venzuelan Guayana lowlands: Main vegetation types and floristic components. Revista Brasileira Botânica 30: 599-609.

Groom, P., 1897. On the leaves of *Lathraea squamaria* and of some allied Scrophulariaceae. Annals of Botany 11: 385-398.

Halda, J.J., Heřtus, P., Malina, M., 2007. Několik nových bolívijských rostlin - Some new Bolivian plants. Acta Musei Richnoviensis, Sect. Nat. 14: 105-126.

Harley, R.M., Mayo, S.M., 1980. Towards a checklist of the flora of Bahia. Royal Botanic Gardens, Kew.

Harley, R.M., Simmons, N.A., 1986. Florula of Mucugê, Chapada Diamantina, Bahia, Brazil. Royal Botanic Gardens, Kew.

Hegnauer, R., 1989. Chemotaxonomie der Pflanzen, 8. Birkhäuser, Basel.

Hernandez-Melan, J.H., 2009. *Pinguicula elongata* of Colombia. Canrivorous Plant Newsletter 38: 83-85.

Heslop-Harrison, Y., 1975. Enzyme release in carnivorous plants. In: Dingle, J.T., Dean, R.T. (eds.), Lysozymes in biology and pathology. North Holland Publishing Company, Amsterdam: 525-578.

Heslop-Harrison, Y., 1976. Carnivorous plants a century after Darwin. Endeavor 35: 114-122.

Heubl, G., Bringmann, G., Meimberg, H., 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales – revisited. Plant Biology 8: 821-830.

Higgins, S.I., Nathan, R., Cain, M.L., 2003. Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? Ecology 84: 1945-1956.

Hilu, K.W., Borsch, T., Müller, K.F., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M., Powell, M., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A., Rohwer, J.G., Campbell, C.S., Chatrou, L., 2003. Angiosperm phylogeny based on *matK* sequence information. American Journal of Botany 90: 1758-1776.

Hobbhahn, N., Küchmeister, H., Porembski, S., 2006. Pollination biology of mass flowering terrestrial *Utricularia* species (Lentibulariaceae) in the Indian Western Ghats. Plant Biology 8: 791-804.

Holst, B.K., 1997. Bromeliaceae. In: Steyermark, J.A., Berry, P.E., Holst, B.K. (eds.), Flora of the Venezuelan Guayana, 3. Missouri Botanical Garden Press, St. Louis: 548-676.

Huber, O., 1988. Vegetación y flora de Pantepui. Acta Botanica Brasilica. 1: 41-52.

Huber, O., 1995. Vegetation. In: Steyermark, J.A., Berry, P.E., Holst, B.K. (eds.), Flora of the Venezuelan Guayana, 1. Missouri Botanical Garden Press, St. Louis: 97-160.

Hughes, C., Eastwood, R., 2006. Island radiations on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Acadademy of Science USA 103: 10334-10339.

Ihlenfeldt, H.-D., 2004. Martyniaceae. In: Kubitzki, K., (ed.), The families and genera of vascular plants, 7. Springer, Berlin. : 283-288.

IUCN, 2001. IUCN Red List categories and criteria, version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, United Kingdom.

Janssens, S.B., Knox, E.B., Huysmans, S., Smets, E.F., Merckx, V.S.F.T., 2009. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. Molecular Phylogenetics and Evolution 52: 806-824.

Jérémie, J., 1989. Autogamie dans le genre *Utricularia* L. (Lentibulariaceae). Bulletin du Museum National d'Histoire Naturelle, section B, Adansonia 11: 17-28.

Jessen, O., 1936. Reisen und Forschungen in Angola. Dietrich Reimer, Berlin.

Jobson, R.W., Albert, V.A., 2002. Molecular rates parallel diversification contrasts between carnivorous plant sister lineages. Cladistics 18: 127-136.

Jobson, R.W., Playford, J., Cameron, K.M., Albert, V.A., 2003. Molecular phylogenetics of Lentibulariaceae inferred from plastid *rps16* intron and *trnL-F* DNA sequences: implications for character evolution and biogeography. Systematic Botany 28: 157-171.

Juniper, B.E., 1986. The Path to Plant Carnivory. In: Juniper, B.E., Southwood, R. (eds.), Insects and the Plant Surface. Echvard Annald, London

Juniper, B.E., Robins, R.J., Joel, D.M., 1989. The carnivorous plants. Academic Press, London.

Kamienski, F., 1890. Recherches sur la famille des Lentibulariées (Utriculariées). Zapiski Novorossijskogo Oscestva Estestvoispytatelej 12: 179-210.

Katterman, F.R.H., Shattuck, V.I., 1983. An effective method of DNA isolation from the mature leaves of *Gossypium* species that contain large amounts of phenolic terpenoids and tannins. Preparative Biochemistry 13: 347-359.

Kerner, A., 1878. Flowers and their unbidden guests. C. Kegan Paul & Co., London.

Khosla, C., Shivanna, K.R., Mohan Ram, H.Y., 1998. Pollination in the aquatic insectivore *Utricularia inflexa* var. *stellaris*. Phytomorphology 48: 417-425.

Klotz, S., Köck, U.V., 1991. Neufund von *Genlisea recurva* (Lentibulariaceae) auf Madagaskar. Willdenowia 20: 131-133.

Kondo, K., 1972. A comparison of variability in *Utricularia cornuta* and *Utricularia juncea*. American Journal of Botany 59: 23-37.

Kondo, K., Shimai, H., 2006. Phylogenetic analysis of the northern *Pinguicula* (Lentibulariaceae) based on Internal Transcribed Spacer (ITS) sequence. Acta Phytotaxonomica et Geobotanica 57: 155-164.

Legendre, L., 2000. The genus *Pinguicula* L. (Lentibulariaceae): an overview. Acta Botanica Gallica 147: 77-95.

Leins, P., Erbar, C., 2010. Flower and fruit. Morphology, ontogeny, phylogeny, function and ecology. Schweizerbarth Science Publishers, Stuttgart.

Lloyd, F.E., 1934. Additional observations on some Utriculariaceae. Canadian Journ. Research 10: 557-562.

Lloyd, F.E., 1942. The carnivorous plants. Chronica Botanica Company, Waltham, Massachusetts.

Loomis, W.D., 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. Methods in Enzymology 31(Pt A): 528-544.

Luetzelburg, P. v., 1910. Beiträge zur Kenntnis der *Utricularia*. Flora 100: 145-212.

Luetzelburg, P. v., 1922. Estudo Botanico do Nordéste, 3. Inspectoria Federal de Obras Contra as Seccas 57 (Série 1,A), Rio de Janeiro.

Luhrs, H., 1995. New additions to the genus *Pinguicula* (Lentibulariaceae) of Mexico. Phytologia 79:114-122.

Macedo, M., Prance, G.T., 1978. Notes on the vegetation of Amazonia II. The dispersal of plants in Amazonian white sand campinas: the campinas as functional islands. Brittonia 30: 203-215.

Mameli, E., 1916. Ricerche anatomiche, fisiologiche e biologiche sulla *Martynia lutea* Lindl. Atti dell'Istituto Botanico e del Laboratorio Crittogamico dell'Universita di Pavia, Serie II, 16: 137-188.

Mannagetta, G.R. Beck von, 1897. Orobanchaceae. In: Engler, A., Prantl, K. (eds.), Die natürlichen Pflanzenfamilien, 4, Abt. 3b. W. Engelmann, Leipzig: 123-132.

McPherson, S., 2010. Carnivorous plants and their habitats. Redfern, Dorset.

McPherson, S., Wistuba, A., Fleischmann, A., Nerz. J., 2011. Sarraceniaceae of South America. Redfern, Dorset.

Meimberg, H., Dittrich, P., Bringmann, G., Schlauer, J., Heubl, G., 2000. Molecular phylogeny of Caryophyllidae s.l. based on *matK* sequences with special emphasis on carnivorous taxa. Plant Biology 2: 218-228.

Meimberg, H., Wistuba, A., Dittrich, P., Heubl, G., 2001. Molecular phylogeny of Nepenthaceae based on cladistic analysis of plastid *trnK* intron sequence data. Plant Biology 3: 164-175.

Meimberg, H., Heubl, G., 2006. Introduction of a nuclear marker for phylogenetic analysis of Nepenthaceae. Plant Biology 8: 831-840.

Merl, E.M., 1915. Beiträge zur Kenntnis der Utricularien und Genliseen. Flora 108: 127-200.

Meyers-Rice, B.A., 1994. Are *Genlisea* traps active? A crude calculation. Carnivorous Plant Newsletter 23: 40-42.

Mildbraed, J., Domke, W., 1966. Grundzüge der Vegetation des tropischen Kontinental-Afrikas. Willdenowia Beihefte 2: 1-254.

Müller, J.V., 2007. Herbaceous vegetation of seasonally wet habitats on inselbergs and lateritic crusts in West and Central Africa. Folia Geobotanica 42: 29-61.

Müller, K., Borsch, T., Legendre, L., Porembski, S., Theisen, I., Barthlott, W., 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. Plant Biology 6: 477-490.

Müller, K., Borsch, T., 2005. Phylogenetics of *Utricularia* (Lentibulariaceae) and molecular evolution of the *trnK* intron in a lineage with high substitutional rates. Plant Systematics and Evolution 250: 39-67.

Müller, K., Borsch, T., Legendre, L., Porembski, S., Barthlott, W., 2006. Recent progress in understanding the evolution of Lentibulariaceae. Plant Biology 8: 748-757.

Nathan, R., 2001. Dispersal biogeography. In: Levin, S.A. (ed.), Encyclopedia of biodiversity. Academic Press, San Diego: 127-152.

Nathan, R., 2006. Long-distance dispersal of plants. Science 313: 786-788.

Neyland, R., Merchant, M., 2006. Systematic relationships of Sarraceniaceae inferred from nuclear ribosomal DNA sequences. Madroño 53: 223-232.

Oliveira, A.A., Daly, D., Vicentini, A., Cohnhaft, M., 2001. Vegetação sobre solos arenosos. In: Oliveira, A.A., Daly, D. (eds.), As florestas do Rio Negro. Companhia das Letras, São Paulo: 179-219.

Olvera, G.M., Martínez, S.E., 2002. Primer registro de *Genlisea* (Lentibulariaceae) para México. Acta Botanica Mexicana 59: 71-73.

Oxelman, B., Kornhall, P., Olmstead, R.G., Bremer, B., 2005. Further disintegration of Scrophulariaceae. Taxon 54: 411-425.

Płachno, B.J., Faber, J., Jankun, A., 2005a. Cuticular discontinuities in glandular hairs of *Genlisea* A.St.-Hil. in relation to their functions. Acta Botanica Gallica 152: 125-130.

Płachno, B.J., Adamus, K., Faber, J., Kozlowski, J., 2005b. Feeding behaviour of carnivorous *Genlisea* plants in the laboratory. Acta Botanica Gallica 152: 159-164.

Płachno, B.J., Adamec, L., Lichtscheidl, I.K., Peroutka, M., Adlassnig, W., Vrba, J., 2006. Fluorescence labelling of phosphatase activity in digestive glands of carnivorous plants. Plant Biology 8: 813-820.

Płachno, B.J., Kozieradzka-Kiszkurno, M., Swiatek, P., 2007. Functional utrastructure of *Genlisea* (Lentibulariaceae) digestive hairs. Annals of Botany 100: 195-203.

Płachno, B.J., Kozieradzka-Kiszkurno, M., Swiatek, P., Darnowski, D.W. 2008. Prey attraction in carnivorous *Genlisea* (Lentibulariaceae). Acta Biologica Cracoviensia, series Botanica 50: 87-94.

Płachno, B.J., Wolowski, K., 2008. Algae commensal community in *Genlisea* traps. Acta Societatis Botanicorum Poloniae 77: 77-86.

Płachno, B.J., Adamec, L., Huet, H., 2009. Mineral nutrient uptake from prey and glandular phosphatase activity as a dual test of carnivory in semi-dessert plants with glandular leaves suspected of carnivory. Annals of Botany 104: 649-654.

Płachno, B.J., Swiątek, P. 2009. Functional anatomy of the ovule in *Genlisea* with remarks on ovule evolution in Lentibulariaceae. Protoplasma 236: 39-48.

Porebski, S., Bailey, L.G., Baum, B.R., 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Molecular Biology Reporter 15: 8-15.

Porembski, S., Fischer, E., Gemmel, B., 1996. *Genlisea barthlottii* (Lentibulariaceae), a new species from Guinean inselbergs. Bulletin du Museum National d'Histoire Naturelle, section B, Adansonia 18: 151-154.

Porembski, S., Barthlott, W., 1997. Seasonal dynamics of plant diversity on inselbergs in the Ivory Coast (West Africa). Botanica Acta 110: 466-472.

Porembski, S., Barthlott, W., 2000 (eds.). Inselbergs: biotic biversity of isolated outcrops in tropical and temperate regions. Ecological Studies 146. Springer, Berlin.

Prance, G.T., 1996. Islands in Amazonia. Philosophical Transactions of the Royal Society London, Series B, Biological Sciences 351: 823-833.

Rahman, M.O., Adamec, L., Kondo, K., 2001. Chromosome numbers of *Utricularia bremii* and *Utricularia dimorphantha* (Lentibulariaceae). Chromosome Science 5: 105-108.

Raman, S., 1987. A code proposed for the classification of the trichomes as applied to the Scrophulariaceae. Beiträge zur Biologie der Pflanzen 62: 349-367.

Rapini, A., Mello-Silva, R.D., Kawasaki, M.L., 2002. Richness and endemism in Asclepiadoideae (Apocynaceae) from the Espinhaço Range of Minas Gerais, Brazil – a conservationist view. Biodiversity and Conservation 11: 1733-1746.

Raven, P.H., Axelrod, D.I., 1972. Plate tectonics and Australasian paleogeography. Science 176: 1379-1386.

Renner, S.S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. In. J. Plant Science 165 (Supplement 4): S23-S33.

Reut, M.S., 1993. Trap structure of the carnivorous plant *Genlisea* (Lentibulariaceae). Botanica Helvetica 103: 101-111.

Rice, B.A., 1999. Testing the appetites of *Ibicella lutea* and *Drosophyllum*. Carnivorous Plant Newsletter 28: 40-43.

Richards, E. 1988. Preparation of genomic DNA from plant tissue. In: Ausubel, F.M. et al. (eds.), Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience, New York: 2.3.2-2.3.3

Richards, P.W., 1957. Ecological notes on West African vegetation. I. The plant communities of the Idanre Hills, Nigeria. Journal of Ecology 45: 563-577.

Ridley, H.N., 1888. On the foliar organs of a new species of *Utricularia* from St. Thomas, West Africa. Ann. Bot. (London) 2: 305-307.

Ritter, N.P., Crow, G.E., 2000. Notes on the Lentibulariaceae in Bolivia: a new genus record (*Genlisea*) for the country, with two additional species records in the genus *Utricularia*. Rhodora 102: 217-224.

Rivadavia, F., 2002. *Genlisea lobata* Fromm-Trinta. Carnivorous Plant Newsletter 31: 116-118.

Rivadavia, F., 2007. A *Genlisea* myth is confirmed. Carnivorous Plant Newsletter 36: 122-125.

Rivadavia, F., Kondo, K., Kato, M., Hasebe, M., 2003. Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast *rbcL* and nuclear 18S ribosomal DNA sequences. American Journal of Botany 90: 123-130.

Rivadavia, F., Vicentini, A., Fleischmann, A., 2009. A new species of sundew (*Drosera*, Droseraceae), with water-dispersed seed, from the floodplains of the northern Amazon basin, Brazil. Ecotropica 15: 13-21.

Rutishauser, R., 1999. Polymerous leaf whorls in vascular plants: developmental morphology and fuzziness of organ identities. International Journal of Plant Sciences 160, Supplement 6: S81-S103.

Rutishauser, R., Isler, B., 2001. Developmental genetics and morphological evolution of flowering plants, especially bladderworts (Utricularia): fuzzy arberian morphology complements classical morphology. Annals of Botany 88: 1173-1202.

Saint-Hilaire, A., 1833. Voyage dans le district des Diamans du Brésil. Paris: Librairie-Gide.

Safford, H.D., Martinelli, G., 2000. Southeast Brazil. In: Porembski, S., Barthlott, W. (eds.), Inselbergs: biotic biversity of isolated outcrops in tropical and temperate regions. Ecological Studies 146. Springer, Berlin: 339-389.

Sattler, R., Rutishauser, R., 1990. Structural and dynamic descriptions of the development of *Utricularia foliosa* and *U. australis*. Canadian Journal of Botany 68: 1989-2003.

Schäferhoff, B., 2011. Carnivory in Lamiales: phylogeny, taxonomy, and chloroplast genome evolution. PhD-Thesis, Universität Münster.

Schäferhoff, B., Fleischmann, A., Fischer, E., Albach, D.C., Borsch, T., Heubl, G., Müller, K.F., 2010. Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. BMC Evolutionary Biology 10: 352-374.

Scotese, C.R., Gahagan, L.M., Larson, R.L., 1988. Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. Tectonophysics 155: 27-48.

Seine, R., Porembski, S., Barthlott, W., 1995. A neglected habitat of carnivorous plants: inselbergs. Feddes Repertorium 106: 555-562.

Shimai, H., Kondo, K., 2007. Phylogenetic analysis of Mexican and Central American *Pinguicula* (Lentibulariaceae) based on internal transcribed spacer (ITS) sequence. Chromosome Botany 2: 67-77.

Shimai, H., Masuda, Y., Panfet Valdés, C.M., Kondo, K., 2007. Phylogenetic analysis of Cuban *Pinguicula* (Lentibulariaceae) based on internal transcribed spacer (ITS) region. Chromosome Botany 2: 151-158.

Shioda, M., Marakami-Muofushi, K., 1987. Selective inhibition of DNA polymerase by a polysaccharide purified from slime of *Physarum polycephalum*. Biochemical and Biophysical Research Communications 146: 61-66.

Silva, N.G. da, Alves, R.J.V., Pereira, J.F., Rivadavia, F., 2011. Lentibulariaceae, Serra de São José, Minas Gerais, Brazil. Checklist 7: 120-127.

Splett, S., 1997. Pflanzenökologische Untersuchungen auf Weißsandfeldern in Zentral- und Südostbrasilien. Struktur und Diversität einer tropischen Lebensgemeinschaft. Martina Galunder-Verlag, Wiehl.

Stange,C., Prehn, D., Arce-Johnson, P. 1998. Isolation of *Pinus radiata* genomic DNA suitable for RAPD analysis. Plant Molecular Biology Reporter 16: 1-8.

Stannard, B.L., (ed.) 1995. Flora of Pico das Almas. Royal Botanic Gardens, Kew.

Steffens, S., Wilbert, N., 2002. Ciliates (Protozoa: Ciliophora) of selected ephemeral flushs on tropical inselbergs. International Review of Hydrobiology 87: 401-410.

Steyermark, J.A., 1953. Lentibulariaceae. In: Botanical exploration of Venezuela – III. Fieldiana, Botany 28: 534-553.

Stopp, K. 1958. Notiz über die Dehiszenzweise der Kapselfrüchte von *Genlisea hispidula* Stapf. Beiträge zur Biologie der Pflanzen 34: 126-142.

Studnička, M., 1982. The problem of carnivory in the common toothwort (*Lathraea squamaria* L.). Carnivorous Plant Newsletter 11: 16-20.

Studnička, M., 1996. Several ecophysiological observations in *Genlisea*. Carnivorous Plant Newsletter 25: 14-16.

Studnička, M., 2003a. *Genlisea* traps – a new piece of knowledge. Carnivorous Plant Newsletter 32: 36-39.

Studnička, M., 2003b. Further problem in *Genlisea* trap untangled? Carnivorous Plant Newsletter 32: 40-45.

Studnička, M., 2003c. Observations on life strategies of *Genlisea*, *Heliamphora* and *Utricularia* in natural habitats. Carnivorous Plant Newsletter 32: 57-61.

Taylor, P., 1967. Lentibulariaceae. In: Maguire, B., et al. (eds.), Botany of the Guayana Highland, 7. Memoirs of the New York Botanical Garden 17: 201-228.

Taylor, P., 1988. Lentibulariaceae. In: Launert, E. (ed.), Flora Zambesica, 8(3). London: 9-42.

Taylor, P., 1989. The genus *Utricularia*: a taxonomic monograph. Royal Botanic Gardens, Kew, London.

Taylor, P., 1991. The genus *Genlisea*. Carnivorous Plant Newsletter 20: 20-26.

Taylor, P., 1999. Lentibulariaceae. In: Berry, P.E., Yatskievych, K., Holst, B.K. (eds.), Flora of the Venezuelan Guayana, 5. Missouri Botanical Garden Press, St. Louis: 782-803.

Taylor, P., Fromm-Trinta, E., 1983. Uma nova espécie para o gênero *Genlisea* St.-Hil. sect. *Tayloria* (Lentibulariaceae): *Genlisea uncinata* P.Taylor & Fromm-Trinta. Bradea 3: 365-368.

Taylor, P., Souza, V.C., Giulietti, A.M., Harley, R.M., 2000. *Philcoxia*: A new genus of Scrophulariaceae with three new species from eastern Brazil. Kew Bulletin 55: 155-163.

Thorne, R.F., 1973. Floristic relationships between tropical Africa and tropical America. In: Meggers, B.J., Duckworth, W.D. (eds.), Tropical forest ecosystems in Africa and South America: a comparative review. Smithsonian Institution, Washington: 27-47.

Tutin, T.G., 1934. New species from British Guiana, Cambridge University Expedition, 1933. The Journal of Botany, British and foreign 72: 306-314 + 333-341.

Vieira, S., 2004. Some carnivorous plants from "Parque Nacional Natural el Tuparro" in Colombia. Carnivorous plants of Latinamerica: http://www.carnivoras-la.org/new/en/fieldtrip/Tuparro-JMadrid.html [last accessed October 2011].

von der Heyden, C.J., 2004. The hydrology and hydrogeology of dambos: a review. Progress in Physical Geography 28: 544-564.

Warming, E., 1874. Bidrag til Kundskaben om Lentibulariaceae. Videnskabelige Meddelelser fraden naturhist. Forening i Kjöbenhavn, Nr. 3-7.

Williams, S.E., Albert, V.A., Chase, M.W., 1994. Relationships of Droseraceae: a cladistic analysis of rbcL sequence and morphological data. American Journal of Botany 81: 1027-1037.

Yamamoto, I., Kadono, Y., 1990. A study on the reproductive biology of aquatic *Utricularia* species in southwestern Japan. Acta Phytotaxonomica et Geobotanica 41: 189-200.

Zappi, D.C., Lucas, E., Stannard, B.L., Lughadha, E.N., Pirani, J.R., de Queiroz, L.P., Atkins, S., Hind, D.J.N., Giulietti, A.M., Harley, R.M., de Carvalho, A.M., 2003. Lista das plantas vasculares de Catolés, Chapada Diamantina, Bahia, Brasil. Boletim de Botânica da Universidade de São Paulo 21: 345-398.

7. Appendix

The present dissertation is based on the following publications:

Schäferhoff, B., **Fleischmann, A.**, Fischer, E., Albach, D.C., Borsch, T., Heubl, G., Müller, K.F., 2010. Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. BMC Evolutionary Biology 10: 352–374. ..... Article I

Fleischmann, A., Schäferhoff, B., Heubl, G., Rivadavia, F., Barthlott, W., Müller, K.F., 2010. Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae). Molecular Phylogenetics and Evolution 56: 768–783. ..... Article II

Fleischmann, A., Rivadavia, F., Gonella, P.M., Heubl, G., 2011. A revision of *Genlisea* subgenus *Tayloria* (Lentibulariaceae). Phytotaxa 33: 1-40. ..... Article III

Beck, S.G., **Fleischmann, A.**, Huaylla, H., Müller, K.F., Borsch, T., 2008. *Pinguicula chuquisacensis* (Lentibulariaceae), a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula*. Willdenowia 38: 201–212. ..... Article IV

Fleischmann, A., Rivadavia, F., 2009. *Utricularia rostrata* (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil. Kew Bulletin 64: 155–159. ..... Article V

Fleischmann, A., Heubl, G., 2009. Overcoming DNA extraction problems from carnivorous plants. Anales del Jardín Botánico de Madrid 66: 209–215. ..... Article VI

Article I

Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences

Schäferhoff, B., Fleischmann, A., Fischer, E., Albach, D.C., Borsch, T., Heubl, G., Müller, K.F., 2010. *BMC Evolutionary Biology* 10: 352-374.

Schäferhoff et al. BMC Evolutionary Biology 2010, 10:352
http://www.biomedcentral.com/1471-2148/10/352

BMC Evolutionary Biology

RESEARCH ARTICLE Open Access

Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences

Bastian Schäferhoff[1*], Andreas Fleischmann[2], Eberhard Fischer[3], Dirk C Albach[4], Thomas Borsch[5], Günther Heubl[2], Kai F Müller[1]

Abstract

Background: In the large angiosperm order Lamiales, a diverse array of highly specialized life strategies such as carnivory, parasitism, epiphytism, and desiccation tolerance occur, and some lineages possess drastically accelerated DNA substitutional rates or miniaturized genomes. However, understanding the evolution of these phenomena in the order, and clarifying borders of and relationships among lamialean families, has been hindered by largely unresolved trees in the past.

Results: Our analysis of the rapidly evolving *trnK/matK*, *trnL-F* and *rps16* chloroplast regions enabled us to infer more precise phylogenetic hypotheses for the Lamiales. Relationships among the nine first-branching families in the Lamiales tree are now resolved with very strong support. Subsequent to Plocospermataceae, a clade consisting of Carlemanniaceae plus Oleaceae branches, followed by Tetrachondraceae and a newly inferred clade composed of Gesneriaceae plus Calceolariaceae, which is also supported by morphological characters. Plantaginaceae (incl. Gratioleae) and Scrophulariaceae are well separated in the backbone grade; Lamiaceae and Verbenaceae appear in distant clades, while the recently described Linderniaceae are confirmed to be monophyletic and in an isolated position.

Conclusions: Confidence about deep nodes of the Lamiales tree is an important step towards understanding the evolutionary diversification of a major clade of flowering plants. The degree of resolution obtained here now provides a first opportunity to discuss the evolution of morphological and biochemical traits in Lamiales. The multiple independent evolution of the carnivorous syndrome, once in Lentibulariaceae and a second time in Byblidaceae, is strongly supported by all analyses and topological tests. The evolution of selected morphological characters such as flower symmetry is discussed. The addition of further sequence data from introns and spacers holds promise to eventually obtain a fully resolved plastid tree of Lamiales.

Background

With more than 23,000 species in at least 23 families [1], Lamiales (eudicots/asterids) are one of the largest orders of flowering plants, with representatives found all over the world. The highest diversity is contributed by herbaceous plants with mono-symmetric flowers. Some members are economically important, such as Lamiaceae (pot-herbs like mint, sage, oregano or basil), Oleaceae (olives), Pedaliaceae (sesame), Verbenaceae (timber, medicinal) Plantaginaceae (drugs like digitalis, ornamentals) and Scrophulariaceae (ornamentals). The order contains lineages with highly specialized life forms and traits of particular scientific interest. So far, their comparative study has been limited by the lack of a robust phylogenetic framework for Lamiales. Desiccation-tolerant members (so-called "resurrection plants", see Figure 1a) of the recently described family Linderniaceae [2] are a focus of molecular and evolutionary studies [3,2]. Extreme metabolic and genomic shifts are exhibited by parasitic plants. With Orobanchaceae, Lamiales harbor the largest number of parasitic angiosperms (Figure 1b). The family comprises both hemi- and holoparasites [4], with some species causing serious damage in agriculture [5]. Chloroplast genomes of members of Orobanchaceae show gene order rearrangements, high evolutionary rates and gene losses, potentially as a

* Correspondence: schaeferhoff@uni-muenster.de
[1]Institute for Evolution and Biodiversity, University of Muenster, Hüfferstraße 1, 48149 Münster, Germany
Full list of author information is available at the end of the article

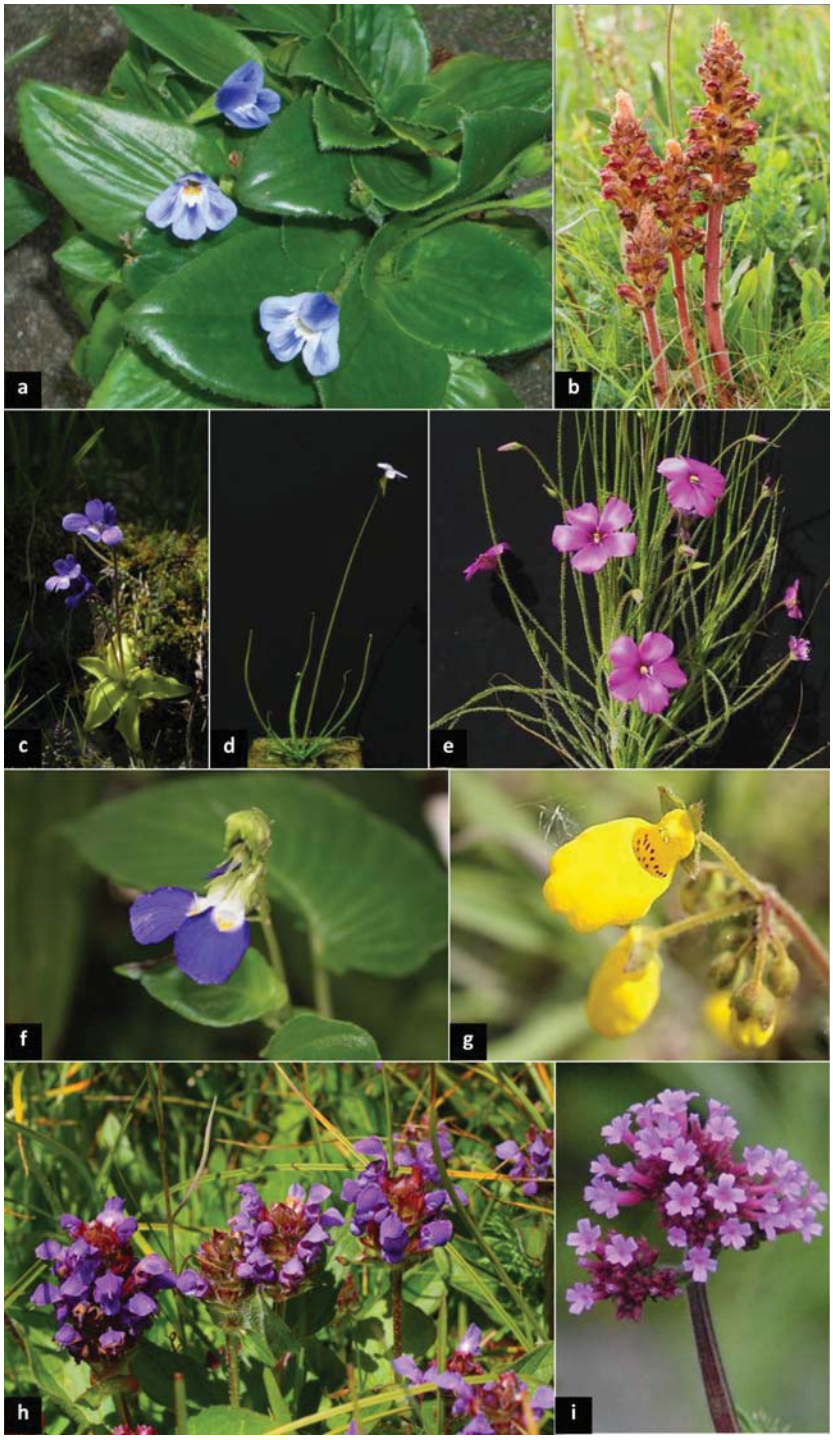


Figure 1 Example taxa from Lamiales, showing representatives of desiccation-tolerant, parasitic, and carnivorous lineages, as well as members from families frequently referred to in the text. **a**: the desiccation-tolerant *Craterostigma pumilum* from Linderniaceae; **b**: the holoparasitic *Orobanche gracilis* from Orobanchaceae, a family that contains all hemi- and holoparasites from Lamiales; **c**: *Pinguicula leptoceras* from Lentibulariaceae, the largest family of carnivorous plants in angiosperms; **d**: *Pinguicula filifolia*, with a habit resembling *Byblis;* **e**: *Byblis gigantea* from Byblidaceae, another carnivorous lineage previously suspected to be the closest relative of Lentibulariaceae; **f**: *Rhynchoglossum gardneri* from Gesneriaceae and **g** *Calceolaria andina* from Calceolariaceae, two families inferred here as sister groups based on molecular data, alveolated seeds and pair-flowered cymes; **h** *Prunella grandiflora* (Lamiaceae)**, i**: *Verbena bonariensis* (Verbenaceae)*;* both families were long regarded as close relatives but are inferred as only distantly related (Figure 2). Photos: a: E.F.; c, d, e: A.F.; f: Nadja Korotkova; g: D.C.A.; b, h, i: K.F.M.

consequence of parasitism in this family. One line of current research in the family concentrates on gradual plastid evolution under increasingly relaxed functional constraints [Wicke et al., in prep].

Carnivory in Lamiales

Lentibulariaceae, the most species-rich family of carnivorous plants (ca. 350 spp.) belongs to Lamiales (Figure 1c, d). This family is unique for a variety of reasons: traps of *Utricularia* (bladderworts) are regarded as a complex modification of leaves [6,7], and the typical angiosperm body plan is strongly relaxed in members of this genus [8-10]. *Utricularia* and its sister genus, *Genlisea* (the corkscrew plants), are the only carnivorous angiosperms known to feed on protozoa [11]. They have the smallest holoploid genome sizes among angiosperms, with some nuclear genomes as small as 63 Mbp or less [12], and exhibit the highest relative DNA substitution rates for some of the investigated chloroplast genome regions [13,14]. *Pinguicula* (butterworts), the third genus of Lentibulariaceae, is far less extreme in genome size, substitution rate and morphology, and exhibits glandular leaves that function as adhesive ("flypaper") traps (Figure 1c, d).

Apart from Lentibulariaceae, the monogeneric Australian family Byblidaceae (Figure 1e) also attracts and catches insects with simple flypaper traps comparable in function to those of *Pinguicula*. The carnivorous syndrome of *Byblis* was questioned by some authors, as the plants were considered to lack their own digestive enzymes and have not been demonstrated to be able to take up released nutrients, thus being ranked as merely "protocarnivorous" [15]. However, a recent study [16] detected phosphatase activity, thereby restoring the rank of carnivory to *Byblis*. Morphological links - flypaper trap leaves that are densely covered with multicellular, non-vascularized epidermal glands, as well as embryology [17,18] - and early phylogenetic studies suggested a sister relationship of Byblidaceae and Lentibulariaceae [19], thus hypothesizing a single origin of carnivory in the order, which was questioned later [14]. With the recently described genus *Philcoxia* [20], a further supposedly "protocarnivorous" lineage emerged and was placed in Lamiales [21]. Although a first test of enzymatic activity was negative [21], this might have been an artifact caused by the minuteness of the leaves, and further experiments to test its status as potentially fully carnivorous are underway.

Understanding the evolution of the morphological, ecological, and genomic peculiarities in the order heavily relies on having robust hypotheses on organismal relationships. For example, knowledge of the closest relatives of resurrection plants, parasites, and carnivores, respectively, would enable us to infer (pre-) adaptations and genomic changes on the evolutionary path leading to each of these specialized groups.

Phylogeny and systematics of Lamiales: current state of knowledge

While the monophyly of many of the currently accepted families has been inferred with confidence by a number of molecular phylogenetic studies [22,23], there has been only little progress on understanding the relationships among families. Nearly all phylogenetic trees produced so far lacked resolution and support for interfamiliar relationships of Lamiales [24-26]. This has earned Lamiales the reputation of being among the most difficult angiosperm clades to resolve [27].

Circumscription of Lamiales and the inclusion of Hydrostachys

The current concept of Lamiales [28] expands the earlier order Lamiales from pre-cladistic classification systems [29,30] to also include former Scrophulariales and Oleales. While there is overwhelming evidence for the monophyly of Lamiales circumscribed like this [28], the surprising inclusion of *Hydrostachys* as an early branch in Lamiales was recently proposed [31]. *Hydrostachys* is a rheophyte from Africa and Madagascar suggested to be related to Cornales in most previous analyses of DNA sequence data, albeit without consistent placement in this order [32-34].

Most studies converged on a set of most likely candidates for the first branches of the Lamiales tree. Oleaceae have been consistently identified as being among the first branches [2,14,24,35]. Whenever the monotypic Plocospermataceae from Central America had been included in the sampling [26,35], they were found to be sister to the remaining Lamiales. In contrast, the Carlemanniaceae-suspected to have affinities of some kind to early branching Lamiales - have never been analyzed in the context of a broad Lamiales sampling. Tetrachondraceae have been resolved as a branch following Oleaceae [36,26].

No clear picture in more derived parts of tree

In contrast, there has not been any consistent hypothesis on the "backbone" of the remainder of the Lamiales tree [37,31]. Conflicting hypotheses have been put forward with regard to the relationships of Gesneriaceae and Calceolariaceae (Figure 1f, g) to each other and to remaining Lamiales. A successive branching order of Oleaceae, Calceolariaceae, Gesneriaceae, and remaining Lamiales was originally suggested [38,39], but support for the placement of Gesneriaceae and for the monophyly of the more derived remaining Lamiales was always negligible. On the other hand, a clade including Gesneriaceae and Calceolariaceae was hypothesized [2,40,41]. Consequently, relationships of Calceolariaceae remained indistinct, and until now there has been no

study sampling all families from early branching Lamiales with a sufficient amount of sequence data to provide a clear picture.

The situation is even worse for the more derived, remaining lineages of the Lamiales tree - as far as the backbone and relationship among families is concerned, almost no resolution could be obtained by previous studies [42,31,43].

The new circumscription of many traditional families

Lamiales are also known for the decomposition of previously widely accepted families due to phylogenetic insights.

Scrophulariaceae and Plantaginaceae

The most prominent case for a family that turned out to be polyphyletic are the Scrophulariaceae. In their traditional circumscription they used to be the largest family (more than 5000 spp. [44]) among Lamiales. In the first report on the polyphyly of Scrophulariaceae [45], members of the "old" Scrophulariaceae sensu lato were found in two different clades, named "scroph I" (including *Scrophularia*) and "scroph II" (containing *Plantago, Antirrhinum*, *Digitalis*, *Veronica*, *Hippuris* and *Callitriche*). The first clade was later [38] referred to as Scrophulariaceae sensu stricto (s. str.), while the "scroph II" clade was called Veronicaceae. However, since *Plantago* is contained in that clade, Plantaginaceae as the older name should be given priority and meanwhile became accepted for this clade [46,28]. Plantaginaceae experienced an enormous inflation since these early studies, when more and more genera from former Scrophulariaceae s. l. were included in phylogenetic studies and identified as members of this newly circumscribed family [22,37-39]. Some genera from tribe Gratioleae, including *Gratiola* itself, have been found in a well supported clade. Based on the unknown relationships to the the other lamialean families, it has been suggested to separate this part of the inflated Plantaginaceae by restoring family rank to former tribe Gratioleae from Scrophulariaceae as traditionally circumscribed [2].

Orobanchaceae

Initial molecular phylogenetic studies [47,48] showed that all hemi-parasitic members of the former Scrophulariaceae s. l. should be included in a newly circumscribed Orobanchaceae while the non-parasitic genus *Lindenbergia* was found sister to all hemi- and holoparasites and also included in Orobanchaceae. In this expanded circumscription [4,49], the monophyly of Orobanchaceae is strongly supported by all studies, and the family now comprises 89 genera with about 2000 species [49] and unites phototrophic, hemi- and holoparasitic plants. As next relatives to Orobanchaceae, a clade consisting of the East Asian genera *Rehmannia* (six species) and *Triaenophora* (one or two species) was identified recently [43,50].

Phrymaceae

Shortly after the first reports on the polyphyly of Scrophulariaceae [45], it was noticed that *Mimulus* (tribe Mimuleae) neither clustered with the "scroph I" nor the "scroph II" clade, but instead was found in a group together with Lamiaceae, *Paulownia* and Orobanchaceae [38]. Sampling the taxonomically isolated *Phryma* (Phrymaceae), but not *Mimulus*, *Phryma* appeared as sister to Orobanchaceae plus *Paulownia* [26]. In an attempt to redefine the Phrymaceae, their circumscription was expanded to include *Mimulus*, *Hemichaena*, *Berendtiella*, *Leucocarpus*, *Glossostigma*, *Peplidium*, *Elacholomia*, *Lancea*, and *Mazus* [51]. However, relationships to other families of Lamiales remained unclear. Sampling six genera from Phrymaceae [39], two clades emerged: one comprising *Mimulus*, *Phryma*, *Hemichaena* and *Berendita*, the other including *Mazus* and *Lancea* being sister to *Rehmannia*. Thus, the monophyly of Phrymaceae was put into question.

Linderniaceae

Linderniaceae were described as a new family independent from Scrophulariaceae, comprising genera formerly classified in the tribe Lindernieae of Scrophulariaceae s. l. and are characterized by stamens in which the abaxial filaments are conspicuously geniculate, zigzag shaped or spurred [2,52,53]. The original recognition as a distinct clade was based upon a taxon set including the genera *Artanema*, *Craterostigma*, *Crepidorhopalon*, *Torenia* and *Lindernia*. The existence of a Linderniaceae clade was confirmed by other studies comprising *Craterostigma*, *Lindernia*, *Torenia* and *Micranthemum* [22] or *Stemodiopsis*, *Micranthemum*, *Torenia* and *Picria* [39].

Calceolariaceae

Jovellana and *Calceolaria* (formerly Calceolarieae/Scrophulariaceae) were identified as another lineage separate from Scrophulariaceae, which led to recognizing them at family level (Calceolariaceae) [38]. The authors of this study initially also listed *Porodittia* as genus of this new family, but a subsequent study [41] showed *Porodittia* to be nested in *Calceolaria*.

Schlegeliaceae, Paulowniaceae, and Stilbaceae

The genera *Paulownia* and *Schlegelia*, which had been traditionally included either in Bignoniaceae or Scrophulariaceae, were not found to be related to any of these families based on molecular data [54] and therefore treated as families of their own [55,56]. In addition, *Halleria* was transferred from Scrophulariaceae to Stilbaceae [38]. Molecular phylogenetic studies later expanded the circumscription of Stilbaceae to a total of 11 genera [37,39].

Aims of this study

Using a dataset representing all major lineages from Lamiales, the goal of the present study was to investigate

inter-familial relationships within Lamiales, in the hope to come up with a better resolved tree that provides the basis for an interpretation of the evolution of the above-mentioned morphological, ecological, and molecular peculiarities observed in the order.

Since the protein-coding genes usually applied to the inference problem in Lamiales have not provided satisfactory resolution in the past, the approach in the current study was to employ non-coding and rapidly evolving chloroplast DNA. Introns and spacers have been demonstrated to be a valuable source of phylogenetic signal even on deeper taxonomic levels than they used to be applied to [57-59]. Mutational dynamics of non-coding regions also include microstructural changes in addition to substitutions, and generally are less constrained than coding genes [60]. Non-coding markers have been shown to be significantly more informative than coding regions [57]. Even more, non-coding markers have been successfully applied to disentangle deep nodes in angiosperm evolution [58].

Methods

Taxon sampling and plant material

Sequences from the plastid markers *trnK*/*matK*, *trnL-F* and *rps*16 were newly generated or downloaded from GenBank for 98 taxa from Lamiales, two outgroup taxa from Solanaceae, and one from Rubiaceae. All 23 families currently accepted for Lamiales [28] were sampled. Since one of the specific questions in our study was the relationship between Lentibulariaceae and Byblidaceae, which might have been blurred by long branch attraction (LBA) problems in previous studies, we slightly enhanced sampling for both families in one set of analyses and included two to three species for each genus. The complete material sampled is shown in Table 1. Using fewer representatives for either family did not change results. We also used a somewhat denser taxon sampling for Gratioleae (Plantaginaceae) in order to (i) examine whether the distinctness of this tribe [2] can be confirmed after taxan sampling enhancement and (ii) doublecheck the position of the apparently "protocarnivorous" genus *Philcoxia*.

Amplification and sequencing

Total genomic DNA was isolated using the AVE Gene Plant Genomics DNA Mini Kit (AVE Gene, Korea), according to the manufacturer's protocol. As phylogenetic markers, the *trnK* intron including the coding *matK*, the *trnL-F* region, and the *rps*16 intron were amplified using standard PCR protocols. Primers used for amplification and sequencing are given in Table 2. Reactions were performed in 50 μl volumes containing 2 μl template DNA (10 ng/μl), 10 μl dNTP mix (1.25 mM each), 2 μl of each forward and reverse primer (20 pm/μl), and 0.25 μl Taq polymerase (5 U/μl, Peqlab). Thermal cycling was performed on an Biometra T3 thermocycler using the following PCR profiles: 1:30 min at 96°C, 1 min at 50°C, 1:30 min at 72°C, 35 cycles of 30 sec at 96°C, 1 min at 50°C, 1:30 min at 72°C, and a final extension time of 10 min at 72°C for the *trnK* intron; 35 cycles of 1 min at 94°C, 1 min at 52°C and 2 min at 72°C, followed by a final extension time of 15 min at 72°C for the *trnL-F* region; 1:30 min at 94°C, 30 cycles of 30 sec at 94°C, 30 sec at 56°C and 1 min at 72°C, and a final extension time of 15 min at 72°C for the *rps16* intron. Fragments were gel-purified on a 1.2% agarose gel (Neeo-agarose, Roth), extracted with the Gel/PCR DNA Fragments Extraction Kit (AVE Gene, Korea) and sequenced on an ABI3730XL automated sequencer using the Macrogen sequencing service (Macrogen Inc., Seoul, Korea). Pherogram editing and contig assembly was done manually.

Addition and analysis of GenBank sequence data

We additionally took *rbcL* and *ndhF* sequences (see Additional file 1, Table S1) for relevant taxa from GenBank, and in a separate set of analyses combined them with our three marker dataset. Taxon sampling of these four- and five-region datasets was adapted to include only taxa with all regions present.

Because the position of *Hydrostachys* remained inconsistent in previous studies, all sequences from that genus existing in GenBank were blasted against the entire data of GenBank via blastn [61]. Additionally, *trnK*/*matK*, *rps16* and *trnL-F* sequences for *Hydrostachys* from a collection independent from those previously used [31,33,62,63] were generated; all sequences used, including voucher information, are given in Table 1. The newly generated *Hydrostachys matK* sequence was aligned to an existing angiosperm *matK* alignment [35] and subjected to parsimony analysis.

Alignment and indel coding

DNA sequences were manually aligned in PhyDE [64], taking microstructural changes into account as outlined elsewhere [58,65]. Regions of uncertain homology were excluded from phylogenetic analyses. For maximum parsimony (MP) analyses and Bayesian Inference of Phylogeny (BI), indels were coded according to simple indel coding (SIC) [66] using the program SeqState [67].

Parsimony analyses

Searches for the shortest tree were performed using the parsimony ratchet approach implemented in PRAP2 [68] using the following settings: 10 random addition cycles with 200 ratchet replicates, setting the weight for 25% of the characters to 2. The files generated were executed in PAUP* v4.0b10 [69]. Bootstrapping was

Table 1 Taxa, specimens and GenBank acession numbers for sequences used in the present study

Genus	Family	*trnK/matK*	*trnLF*	*rps16*
Acanthus	Acanthaceae	*Acanthus longifolius* Poir.; [GenBank:AJ429326.1]	*Acanthus sennii* Chiov.; [GenBank:DQ054856.1]	*Acanthus sennii* Chiov.; [GenBank:DQ059148.1]
Anastrabe	Stilbaceae	*Anastrabe integerrima* E. Mey. Ex Benth.; H. Joffe 171; (M); [EMBL:FN773529]	*Anastrabe integerrima* E. Mey. Ex Benth.; H. Joffe 171; (M); [EMBL:FN794042]	*Anastrabe integerrima* E. Mey. Ex Benth.; [GenBank: AJ609216]
Angelonia	Plantaginaceae	*Angelonia sp.*; Löhne; BG Bonn; [EMBL:FN773530]	*Angelonia sp.*; Löhne; BG Bonn; [EMBL:FN794043]	*Angelonia sp.*; Löhne; BG Bonn; [EMBL:FN794079]
Antirrhinum	Plantaginaceae	*Antirrhinum majus* L.; [GenBank:AF051978]	*Antirrhinum majus* L.; [GenBank:AY316707]	*Antirrhinum majus* L.; [GenBank:AJ431054]
Avicennia	Acanthaceae	Avicennia germinans L.; [GenBank:AF531771]	Avicennia germinans L.; [GenBank:AY008819]	Avicennia marina (Forssk.) Vierh.; [GenBank: AJ431038]
Bacopa	Plantaginaceae	*Bacopa monnieri* (L.) Pennell; [GenBank:AY667458]	*Bacopa monnieri* (L.) Pennell; [GenBank:AY492170]	*Bacopa monnieri* (L.) Pennell; [GenBank:AY492196]
Barthlottia	Scrophulariaceae	*Barthlottia madagascariensis* Eb.Fisch.; A. Erpenbach s.n. (BONN); [EMBL:FN773531]	*Barthlottia madagascariensis* Eb.Fisch.; A. Erpenbach s.n. (BONN); [EMBL:FN794044]	*Barthlottia madagascariensis* Eb.Fisch.; A. Erpenbach s.n. (BONN); [EMBL:FN794080]
Bryodes	Linderniaceae	*Bryodes micrantha* Benth.; E. Fischer 10258; (BONN); [EMBL:FN773532]	*Bryodes micrantha* Benth.; E. Fischer 10258; Madagascar; (BONN); [EMBL:FN794045]	*Bryodes micrantha* Benth.; E. Fischer 10258; Madagascar; (BONN); [EMBL:FN794081]
Buchnera	Orobanchaceae	*Buchnera hispida* D. Don; E. Fischer 10230; (BONN); [EMBL:FN773533]	*Buchnera hispida* D. Don; E. Fischer 10230; (BONN); [EMBL:FN79046]	*Buchnera hispida* D. Don; E. Fischer 10230; (BONN); [EMBL:FN794082]
Buddleja	Scrophulariaceae	*Buddleja alternifolia* Maxim.; [GenBank:AF531772]	*Buddleja alternifolia* Maxim.; [GenBank:AF380857]	*Buddleja asiatica* Lour.; [GenBank:AJ431058]
Byblis	Byblidaceae	*Byblis gigantea* Lindl.; [GenBank:AF531774]	*Byblis gigantea* Lindl.; Kai Müller KM 733; (BONN); [EMBL:FN794047]	*Byblis gigantea* Lindl.; Kai Müller KM 733; (BONN); [EMBL:FN794083]
Byblis	Byblidaceae	*Byblis lamellata* Conran & Lowrie; Schäferhoff 49; (BONN); [EMBL:FN773534]	*Byblis lamellata* Conran & Lowrie; Schäferhoff 49; (BONN); [EMBL:FN794048]	*Byblis lamellata* Conrad & Lowrie; Schäferhoff 49; (BONN); [EMBL:FN794084]
Byblis	Byblidaceae	*Byblis liniflora* Salisb.; Schäferhoff 44; (BONN); [EMBL: FN773535]	*Byblis liniflora* Salisb.; Schäferhoff 44; (BONN); [EMBL: FN794049]	*Byblis liniflora* Salisb.; [GenBank:AJ431070]
Calceolaria	Calceolariaceae	*Calceolaria falklandica* Kraenzl.; [GenBank: AY667457.1]	*Calceolaria arachnoidea* Graham; [GenBank: AY423126]	*Calceolaria mexicana* Benth.; [GenBank:AJ609202]
Callicarpa	Lamiaceae	*Callicarpa bodinieri* H.Lév.; Schäferhoff 57; (BONN)	*Callicarpa japonica* Thunb.; [GenBank:AJ505536.1]	*Callicarpa japonica* Thunb.; [GenBank:AJ505413.1]
Campsis	Bignoniaceae	*Campsis radicans* Seem.; [GenBank:AF531775]	*Campsis radicans* Seem.; Kai Müller KM701; (BONN); [EMBL:FN794050]	*Campsis radicans* Seem.; Kai Müller KM701; (BONN); [EMBL:FN794085]
Carlemannia	Carlemanniaceae	*Carlemannia griffithii* Benth.; Grierson, A.J.C. & Long, D.D. 3027; (K); [EMBL:FN773536]	*Carlemannia griffithii* Benth.; Grierson, A.J.C. & Long, D.D. 3027; (K); [EMBL:FN794051]	*Carlemannia griffithii* Benth.; Grierson, A.J.C. & Long, D.D. 3027; (K); [EMBL:FN794086]
Castilleja	Orobanchaceae	*Castilleja linariifolia* Benth.; [GenBank:AF051981.1]	*Castilleja linariifolia* Benth.; [GenBank:EF103866.1]	*Castilleja integrifolia* L.f.; [GenBank:EF103789.1]
Clerodendrum	Lamiaceae	*Clerodendrum thomsoniae* Balf.; [GenBank:AY840129]	*Clerodendrum thomsoniae* Balf.; Schäferhoff 39; (BONN); [EMBL:FN794052]	*Clerodendrum thomsoniae* Balf.; Schäferhoff 39; (BONN); [EMBL:FN794087]
Conobea	Plantaginaceae	*Conobea multifida* (Michx.) Benth.; V. Mühlenbach 278; (M); [EMBL:FN773563]	*Conobea multifida* (Michx.) Benth.; V. Mühlenbach 278; (M); [EMBL:FN794053]	*Conobea multifida* (Michx.) Benth.; V. Mühlenbach 278; (M); [EMBL:FN794088]
Craterostigma	Linderniaceae	*Craterostigma hirsutum* S.Moore; [GenBank: AF531776]	*Craterostigma hirsutum* S.Moore; N. Peine 2; (BONN); [EMBL:FN794054]	*Craterostigma hirsutum* S.Moore; N. Peine 2; (BONN); [EMBL:FN794089]
Dermatobotrys	Scrophulariaceae	*Dermatobotrys saundersii* Bolus; B. Schäferhoff 64 (BONN); [EMBL:FN773537]	*Dermatobotrys saundersii* Bolus; [GenBank:AJ608596]	*Dermatobotrys saundersii* Bolus; [GenBank:AJ609191]
Diascia	Scrophulariaceae	*Diascia barbarae* Hook.f.; [GenBank:AY667464]	*Diascia capsularis* Benth.; [GenBank:AJ608595]	*Diascia capsularis* Benth.; [GenBank:AJ609190]
Diclis	Scrophulariaceae	*Diclis ovata* Benth.; E. Fischer 10255; (BONN); [EMBL: FN773538]	*Diclis ovata* Benth.; E. Fischer 10255; (BONN); [EMBL: FN794055]	*Diclis reptans* Benth.; [GenBank:AJ609188]
Dipteracanthus	Acanthaceae	*Dipteracanthus portellae* (Hook.f.) Boom; [GenBank: AF531773]	*Dipteracanthus portellae* (Hook.f.) Boom; Kai Müller KM734; (BONN); [EMBL:FN794056]	*Dipteracanthus portellae* (Hook.f.) Boom; Kai Müller KM734; (BONN); [EMBL:FN794090]

Table 1 Taxa, specimens and GenBank acession numbers for sequences used in the present study *(Continued)*

Dodartia	Phrymaceae	*Dodartia orientalis* L.; N. Hölzl M34434; (M); [EMBL: FN773539]	*Dodartia orientalis* L.; N. Hölzl M34434; (M); [EMBL: FN794057]	*Dodartia orientalis* L.; N. Hölzl M34434; (M); [EMBL: FN794091]
Elytraria	Acanthaceae	*Elytraria imbricata* (Vahl) Persoon; J. Calónico S.&A. Domínguez M. 4883; (M); [EMBL:FN773540]	*Elytraria imbricata* (Vahl) Persoon; [GenBank: AF061819.1]	*Elytraria imbricata* (Vahl) Persoon; P. Döbbeler 4189; (M); [EMBL:FN794092]
Euphrasia	Orobanchaceae	*Euphrasia stricta* D. Wolff ex J.F. Lehmann; Borsch 3785; (BONN); [EMBL:FN773541]	*Euphrasia stricta* D. Wolff ex J.F. Lehmann; Borsch 3785; (BONN); [EMBL:FN794058]	*Euphrasia stricta* D. Wolff ex J.F. Lehmann; Borsch 3785; (BONN); [EMBL:FN794093]
Forsythia	Oleaceae	*Forsythia suspensa* Vahl; [GenBank:EU281175.1]	*Forsythia suspensa* Vahl; [GenBank:EU281157.1]	*Forsythia suspensa* Vahl; [GenBank:AF225231.1]
Genlisea	Lentibulariaceae	*Genlisea aurea* A.St.-Hil.; [GenBank:AF531814.1]	*Genlisea aurea* A.St.-Hil.; [GenBank:AF482614]	*Genlisea aurea* A.St.-Hil.; [GenBank:AF482540]
Genlisea	Lentibulariaceae	*Genlisea hispidula* Stapf; [GenBank:AF531815]	*Genlisea hispidula* Stapf; [GenBank:AF488528.1]	*Genlisea hispidula* Stapf; [GenBank:AF488523.1]
Globularia	Plantaginaceae	*Globularia nudicaulis* L.; [GenBank:AY667473]	*Globularia trichosantha* Fisch. & C.A.Mey.; [GenBank: AY591321]	*Globularia repens* Lam.; [GenBank:AY492206]
Gratiola	Plantaginaceae	*Gratiola officinalis* L.; [GenBank:AF531777]	*Gratiola brevifolia* Raf.; [GenBank:AY727201 and AY727237]	*Gratiola pilosa* Michx.; [GenBank:AJ609182]
Halleria	Stilbaceae	*Halleria tetragona* Baker; [GenBank:AY667476.1]	*Halleria elliptica* L.; [GenBank:AJ621108]	*Halleria lucida* L.; [GenBank:AJ609181]
Harpagophytum	Pedaliaceae	*Harpagophytum grandidieri* Baill.; [GenBank: AF531813]	*Harpagophytum grandidieri* Baill.; [GenBank: AF482610]	*Harpagophytum grandidieri* Baill.; Kai Müller KM707; (BONN); [EMBL:FN794094]
Harveya	Orobanchaceae	*Harveya alba* Hepper; E. Fischer 11547; (BONN); [EMBL:FN773564]	*Harveya alba* Hepper; E. Fischer 11547; (BONN); [EMBL:FN794078]	*Harveya alba* Hepper; E. Fischer 11547; (BONN); [EMBL:FN794095]
Hydrotriche	Plantaginaceae	*Hydrotriche hottoniaeflora* Zucc.; E. Fischer 10264; (BONN); [EMBL:FN773542]	*Hydrotriche hottoniaeflora* Zucc.; E. Fischer 10264; (BONN); [EMBL:FN794059]	*Hydrotriche hottoniaeflora* Zucc.; E. Fischer 10264; (BONN); [EMBL:FN794096]
Ibicella	Martyniaceae	*Ibicella lutea* v.Eselt; [GenBank:AF531778]	*Ibicella lutea* v.Eselt; Kai Müller KM735; (BONN); [EMBL:FN794060]	*Ibicella lutea* v.Eselt; Kai Müller KM735; (BONN); [EMBL:FN794097]
Jacaranda	Bignoniaceae	*Jacaranda mimosifolia* D.Don; [GenBank:AJ429328.1]	*Jacaranda mimosifolia* D.Don; [GenBank:EF105070.1]	*Jacaranda mimosifolia* D.Don; [GenBank:AJ431039.1]
Jasminum	Oleaceae	*Jasminum nudiflorum* Lindl.; [GenBank:AF531779.1]	*Jasminum nudiflorum* Lindl.; [GenBank:AF531779.1]	*Jasminum nudiflorum* Lindl.; [GenBank:AF531779.1]
Jovellana	Calceolariaceae	*Jovellana violacea* G.Don; [GenBank:AJ580487.1]	*Jovellana violacea* G.Don; K.H. & W. Rechinger 63014; (M); [EMBL:FN794061]	*Jovellana violacea* G.Don; K.H. & W. Rechinger 63014; (M); [EMBL:FN794098]
Kigelia	Bignoniaceae	*Kigelia africana* Benth.; [GenBank:AF051988]	*Kigelia africana* Benth.; [GenBank:EF105072]	-
Kohleria	Gesneriaceae	*Kohleria spicata* Oerst.; [GenBank:AJ580486.1]	*Kohleria spicata* Oerst.; [GenBank:AJ439820.1]	*Kohleria ocellata* Fritsch in Engl. & Prantl; B. Schäferhoff 70; (BONN); [EMBL:FN794099]
Lamium	Lamiaceae	*Lamium maculatum* L.; [GenBank:AF531780]	*Lamium amplexicaule* L.; [GenBank:AB266235]	*Lamium album* L.; [GenBank:AJ431044]
Lantana	Verbenaceae	*Lantana camara* L.; [GenBank:AF315303.1]	*Lantana camara* L.; [GenBank:AF231884.1]	*Lantana camara* L.; [GenBank:EU348856.1]
Limnophila	Plantaginaceae	*Limnophila aromatica* (Lam.) Merr.; Schäferhoff 52; (BONN); [EMBL:FN773543]	*Limnophila aromatica* (Lam.) Merr.; Schäferhoff 52; (BONN); [EMBL:FN794062]	*Limnophila aromatica* (Lam.) Merr.; Schäferhoff 52; (BONN); [EMBL:FN794100]
Limosella	Scrophulariaceae	*Limosella aquatica* L.; Kai Müller & Andreas Worberg 258; (BONN); [EMBL:FN773544]	*Limosella aquatica* L.; Kai Müller & Andreas Worberg258; (BONN); [EMBL:FN794063]	*Limosella grandiflora* Benth.; [GenBank:AJ609170]
Lindenbergia	Orobanchaceae	*Lindenbergia philippinensis* Benth.; [GenBank: AF051990]	*Lindenbergia philippinensis* Benth.; [GenBank: AJ608586.1]	*Lindenbergia sp.*; [GenBank:AJ431049]
Lindernia	Linderniaceae	*Lindernia brevidens* Skan; E. Fischer 8022; (BONN); [EMBL:FN773545]	*Lindernia brevidens* Skan; [GenBank:AY492182]	*Lindernia brevidens* Skan; [GenBank:AY492213]
Littorella	Plantaginaceae	*Littorella uniflora* (L.) Asch.; N. Korotkova, K. Lewejohann & W. Lobin 2; (BONN); [EMBL: FN773546]	*Littorella uniflora* (L.) Asch.; N. Korotkova, K. Lewejohann & W. Lobin 2; (BONN); [EMBL: FN794064]	*Littorella uniflora* (L.) Asch.; N. Korotkova, K. Lewejohann & W. Lobin 2; (BONN); [EMBL: FN794101]

Table 1 Taxa, specimens and GenBank acession numbers for sequences used in the present study *(Continued)*

Mazus	Phrymaceae	*Mazus rugosus* Lour.; E. Fischer 10250; (BONN); [EMBL:FN773547]	*Mazus rugosus* Lour.; E. Fischer 10250; (BONN); [EMBL:FN794065]	*Mazus stachydifolius* Maxim.; AJ609167
Mecardonia	Plantaginaceae	*Mecardonia procumbens* Small; [GenBank: AY492152.1]	*Mecardonia procumbens* Small; [GenBank:AY492184]	*Mecardonia procumbens* Small; [GenBank:AY492215]
Micranthemum	Linderniaceae	*Micranthemum umbrosum* (J.F.Gmel.) Blake; Schäferhoff 43; (BONN); [EMBL:FN773548]	*Micranthemum umbrosum* (J.F.Gmel.) Blake; [GenBank:AY492186]	*Micranthemum umbrosum* (J.F.Gmel.) Blake; [GenBank:AY492217]
Micrargeria	Orobanchaceae	*Micrargeria filiformis* (Schum. Thonn.) Hutch. Dalziel; E. Fischer 10299; (BONN); [EMBL:FN773549]	*Micrargeria filiformis* (Schum. Thonn.) Hutch. Dalziel; E. Fischer 10299; (BONN); [EMBL:FN794066]	*Micrargeria filiformis* (Schum. Thonn.) Hutch. Dalziel; E. Fischer 10299; (BONN); [EMBL:FN794102]
Mimulus	Phrymaceae	*Mimulus guttatus* D.C.; [GenBank:AY667471]	*Mimulus micranthus* A. Heller; [GenBank:AY575534]	*Mimulus aurantiacus* Curtis; [GenBank:AJ609163]
Mitraria	Gesneriaceae	*Mitraria coccinea* Cav.; B. Schäferhoff 65; (BONN); [EMBL:FN773550]	*Mitraria coccinea* Cav.; B. Schäferhoff 65; (BONN); [EMBL:FN794067]	*Mitraria coccinea* Cav.; B. Schäferhoff 65; (BONN); [EMBL:FN794103]
Myoporum	Scrophulariaceae	*Myoporum montanum* R.Br.; [GenBank:AF531808]	*Myoporum montanum* R.Br.; [GenBank:AJ296513]	*Myoporum mauritianum* A.DC.; [GenBank:AJ609161]
Ocimum	Lamiaceae	*Ocimum basilicum* L.; [GenBank:AY177670.1]	*Ocimum basilicum* L.; [GenBank:AY570462.1]	*Ocimum basilicum* L.; [GenBank:AJ505351.1]
Oftia	Scrophulariaceae	*Oftia africana* Bocq. Ex Baill.; Schäferhoff 66.; (BONN); [EMBL:FN773551]	*Oftia africana* Bocq. Ex Baill.; Schäferhoff 66.; (BONN); [EMBL:FN794068]	*Oftia africana* Bocq. Ex Baill.; [GenBank:AJ609156.1]
Olea	Oleaceae	*Olea europaea* L.; [GenBank:AM229542.1]	*Olea europaea* L.; [GenBank:AM229542.1]	*Olea europaea* L.; [GenBank:AM229542.1]
Orobanche	Orobanchaceae	*Orobanche caryophyllacea* Sm.; [GenBank:AF051992]	*Orobanche coerulescens* Stephan; [GenBank: AY881137]	*Orobanche hederae* Duby; [GenBank:AJ431050]
Otacanthus	Plantaginaceae	*Otacanthus coeruleus* Lindl.; [GenBank:AY667459]	*Otacanthus sp.*; [GenBank:AY492188]	*Otacanthus sp.*; [GenBank:AY492219]
Paulownia	Paulowniaceae	*Paulownia tomentosa* (Thunb.) Steud.; [GenBank: AF051997]	*Paulownia tomentosa* (Thunb.) Steud.; [GenBank: AY423122]	*Paulownia tomentosa* (Thunb.) Steud.; [GenBank: AJ431051]
Pedicularis	Orobanchaceae	*Pedicularis sylvatica* L.; [GenBank:AF531781]	*Pedicularis cheilanthifolia* Schrenk; [GenBank: AY881133]	*Pedicularis attollens* A. Gray; [GenBank:EF103821]
Petrea	Verbenaceae	*Petrea racemosa* Nees; Schäferhoff 55; BG Bonn 11113; (BONN); [EMBL:FN773552]	*Petrea racemosa* Nees; Schäferhoff 55; BG Bonn 11113; (BONN); [EMBL:FN794069]	*Petrea racemosa* Nees; Schäferhoff 55; BG Bonn 11113; (BONN); [EMBL:FN794104]
Philcoxia	Plantaginaceae	*Philcoxia minensis* V.C.Souza & Giul.; [GenBank: EF467901]	*Philcoxia minensis* V.C.Souza & Giul.; [GenBank: EF467889.1]	-
Phryma	Phrymaceae	*Phryma leptostachya* L.; [GenBank:AJ429341.1]	*Phryma leptostachya* L.; [GenBank:DQ532471.1]	*Phryma leptostachya* L.; [GenBank:AJ431053.1]
Phyla	Verbenaceae	*Phyla nodiflora* (L.) Greene; Schäferhoff 56; BG Bonn 4146; (BONN); [EMBL:FN773553]	*Phyla nodiflora* (L.) Greene; Schäferhoff 56; BG Bonn 4146; (BONN); [EMBL:794070]	*Phyla nodiflora* (L.) Greene; Schäferhoff 56; BG Bonn 4146; (BONN); [EMBL:FN794105]
Pinguicula	Lentibulariaceae	*Pinguicula agnata* Casper; [GenBank:AF531782]	*Pinguicula agnata* Casper; [GenBank:AF482617]	*Pinguicula agnata* Casper; [GenBank:AF482543.1]
Pinguicula	Lentibulariaceae	*Pinguicula alpina* L.; [GenBank:AF531783]	*Pinguicula alpina* L.; [GenBank:AF482618]	*Pinguicula alpina* L.; [GenBank:AF482544.1]
Pinguicula	Lentibulariaceae	*Pinguicula lusitanica* L.; [GenBank:DQ010661]	*Pinguicula lusitanica* L.; [GenBank:AF482625.1]	*Pinguicula lusitanica* L.; [GenBank:AF482551.1]
Plantago	Plantaginaceae	*Plantago media* L.; [GenBank:AY667474.1]	*Plantago media* L.; [GenBank:AY101920]	*Plantago argentea* Chaix; [GenBank:AJ431056.1]
Plocosperma	Plocospermataceae	*Plocosperma buxifolium* Benth.; [GenBank:AJ429315]	*Plocosperma buxifolium* Benth.; T.Borsch, H.Flores, S. Zumaya 377; (BONN); [EMBL:FN794071]	*Plocosperma buxifolium* Benth.; T.Borsch, H.Flores, S. Zumaya 377; (BONN); [EMBL:FN794106]
Polypremum	Tetrachondraceae	*Polypremum procumbens* L.; [GenBank:AJ429351.1]	*Polypremum procumbens* L.; [GenBank:AJ430938.1]	*Polypremum procumbens* L.; [GenBank:AJ431063.1]
Proboscidea	Martyniaceae	*Proboscidea louisiana* (Mill.) Thell.; [GenBank: AF531809]	*Proboscidea louisiana* (Mill.) Thell.; [GenBank: AJ608573]	*Proboscidea louisiana* (Mill.) Thell.; Kai Müller KM706; BG Bonn 17132; (BONN); [EMBL:FN794107]
Rehmannia		*Rehmannia elata* N.E.Br.; Hong-Qing Li 2004-0607; (HSNU); [EMBL:FN773554]	*Rehmannia glutinosa* Steud.; [GenBank:AY423124]	*Rehmannia angulata* (Oliv.) Hemsl.; [GenBank: AJ609145]
Rhynchoglossum	Gesneriaceae	*Rhynchoglossum gardneri* Theobald & Grupe; B. Schäferhoff 67; (BONN); [EMBL:FN773555]	*Rhynchoglossum obliquum* Blume; [GenBank: AY423133.1]	*Rhynchoglossum gardneri* Theobald & Grupe; B. Schäferhoff 67; (BONN); [EMBL:FN794108]

Table 1 Taxa, specimens and GenBank acession numbers for sequences used in the present study *(Continued)*

Salvia	Lamiaceae	*Salvia coccinea* Juss. ex Murr.; [GenBank:AY840147.1]	*Salvia coccinea* Juss. ex Murr.; [GenBank:AY506617.1]	*Salvia guaranitica* A.St.-Hil. ex Benth.; [GenBank: AJ505421.1]
Schlegelia	Schlegeliaceae	*Schlegelia parviflora* (Oerst.) Monach.; [GenBank: AJ429345.1]	*Schlegelia parviflora* (Oerst.) Monach.; [GenBank: AJ608570.1]	*Schlegelia parviflora* (Oerst.) Monach.; [GenBank: AJ431057.1]
Scoparia	Plantaginaceae	*Scoparia dulcis* L.; E. Fischer 10254; (BONN); [EMBL: FN773556]	*Scoparia dulcis* L.; E. Fischer 10254; (BONN); [EMBL: FN794072]	*Scoparia dulcis* L.; E. Fischer 10254; (BONN); [EMBL: FN794109]
Scrophularia	Scrophulariaceae	*Scrophularia chrysantha* Jaub. & Spach; B. Schäferhoff 68; (BONN); [EMBL:FN773557]	*Scrophularia canina* L.; [GenBank:AY423123]	*Scrophularia arguta* [Soland.]; [GenBank:AJ431061]
Sesamum	Pedaliaceae	*Sesamum indicum* L.; [GenBank:AJ429340.1]	*Sesamum indicum* L.; [GenBank:AF479010.1]	*Sesamum indicum* L.; [GenBank:AJ609226.1]
Seymeria	Orobanchaceae	*Seymeria pectinata* Pursch; [GenBank:AF051999.1]	*Seymeria laciniata* Standl.; [GenBank:EF103898.1]	*Seymeria laciniata* Standl.; [GenBank:EF103820.1]
Stachytarpheta	Verbenaceae	*Stachytarpheta cayennensis* (L.C. Rich.) Vahl; E. Martínez S. 37128; (M); [EMBL:FN773558]	*Stachytarpheta cayennensis* (L.C. Rich.) Vahl; [GenBank:AJ608567.1; (M)	*Stachytarpheta cayennensis* (L.C. Rich.) Vahl; [GenBank:AJ299259.1; (M)
Stemodia	Plantaginaceae	*Stemodia durantifolia* Sw.; [GenBank:AY492164.1]	*Stemodia glabra* Spreng.; [GenBank:AJ608566.1]	*Stemodia durantifolia* Sw.; [GenBank:AY492225]
Stemodiopsis	Linderniaceae	*Stemodiopsis ruandensis* Eb.Fisch.; E. Fischer 10352; (BONN); [EMBL:FN773559]	*Stemodiopsis ruandensis* Eb.Fisch.; E. Fischer 10352; (BONN); [EMBL:794073]	*Stemodiopsis ruandensis* Eb.Fisch.; E. Fischer 10352; (BONN); [EMBL:FN794110]
Stilbe	Stilbaceae	*Stilbe ericoides* L.; [GenBank:AJ429350.1]	*Stilbe ericoides* L.; [GenBank:AJ430937.1]	*Stilbe ericoides* L.; [GenBank:AJ431062.1]
Streptocarpus	Gesneriaceae	*Streptocarpus bindseili* Eb.Fisch.; [GenBank:AF531810]	*Streptocarpus bindseili* Eb.Fisch,; E. Fischer 1006; Ruanda; (KOBL, BR); [EMBL:794074]	*Streptocarpus bindseili* Eb.Fisch,; E. Fischer 1006; Ruanda; (KOBL, BR); [EMBL:FN794111]
Tetrachondra	Tetrachondraceae	*Tetrachondra patagonica* Skotsb.; [GenBank: AJ429352.1]	*Tetrachondra patagonica* Skotsb.; [GenBank: AJ430939.1]	*Tetrachondra patagonica* Skotsb.; [GenBank: AJ431064.1]
Tetranema	Plantaginaceae	*Tetranema roseum* (M.Martens & Galeotti) Standl. & Steyerm.; [GenBank:AY667475]	*Tetranema roseum* (M.Martens & Galeotti) Standl. & Steyerm.; [GenBank:AY492192]	*Tetranema roseum* (M.Martens & Galeotti) Standl. & Steyerm.; [GenBank:AY492226.1]
Thomandersia	Thomandersiaceae	*Thomandersia hensii* De Wild. Et T. Durand; D. Champluvier 5351; (M); [EMBL:FN773560]	*Thomandersia hensii* De Wild. Et T. Durand; D. Champluvier 5351; (M); [EMBL:794075]	*Thomandersia hensii* De Wild. Et T. Durand; D. Champluvier 5351; (M); [EMBL:FN794112]
Thunbergia	Acanthaceae	*Thunbergia alata* Sims; [GenBank:AF531811]	*Thunbergia alata* Sims; [GenBank:AF061820]	*Thunbergia alata* Sims; [GenBank:AJ609131]
Torenia	Linderniaceae	*Torenia stolonifera* Boj. Ex Benth.; E. Fischer 10257; (BONN); [EMBL:FN773561]	*Torenia stolonifera* Boj. Ex Benth.; E. Fischer 10257; (BONN); [EMBL:794076]	*Torenia stolonifera* Boj. Ex Benth.; E. Fischer 10257; (BONN); [EMBL:FN794113]
Utricularia	Lentibulariaceae	*Utricularia subulata* L.; [GenBank:AF531821]	*Utricularia subulata* L.; [GenBank:AF482676]	*Utricularia subulata* L.; [GenBank:AF482599.1]
Utricularia	Lentibulariaceae	*Utricularia multifida* R.Br.; [GenBank:AF531848]	*Utricularia multifida* R.Br.; [GenBank:AF482659]	*Utricularia multifida* R.Br.; [GenBank:AF482583]
Utricularia	Lentibulariaceae	*Utricularia biloba* R. Br.; B. Schäferhoff 69; cult. BG Bonn 19853; (BONN); [EMBL:FN773561]	*Utricularia biloba* R. Br.; [GenBank:AF482634]	*Utricularia biloba* R. Br.; [GenBank:AF482561.1]
Verbena	Verbenaceae	*Verbena rigida* Spreng.; [GenBank:AF531820]	*Verbena rigida* Spreng.; Kai Müller KM742; BG Bonn 4147; (BONN); [EMBL:794077]	*Verbena rigida* Spreng.; [GenBank:AJ431065]
Vitex	Lamiaceae	*Vitex trifolia* L.; [GenBank:AB284175.1]	*Vitex trifolia* L.; [GenBank:AJ505539.1]	*Vitex trifolia* L.; [GenBank:AJ505416.1]
outgroup				
Coffea	Rubiaceae	*Coffea arabica*; [GenBank:EF044213]	[GenBank:EF044213]	[GenBank:EF044213]
Nicotiana	Solanaceae	*Nicotiana tabacum*; [GenBank:NC001879.2]	[GenBank:NC001879.2]	[GenBank:NC001879.2]
Solanum	Solanaceae	*Solanum tuberosum*; [GenBank:DQ231562]	[GenBank:DQ231562]	[GenBank:DQ231562]

Key: Voucher information (collector and number, garden accession number if from living collection, herbarium acronym in braces) is provided for sequences newly generated in this study.

Table 2 Primers used in the present study

Name	Sequence 5'-3'	Design
trnK3914Fdi	GGGGTTGCTAACTCAACGG	Johnson and Soltis [120]
LE1R	ATAGAAATAGATTCGTTC	Müller et al. [13]
LE4R	TTCGCCTGAAAATCCGTAACC	Müller et al. [13]
LE5R	CAAGGTTCCTTGCRCCAACC	this study
ACmatK500F	TTCTTCTTTGCATTTATTACG	Müller and Borsch [121]
LindmatK1714R	CTCCAAAGAAAGYCAGTTCCTCTT	this study
LindmatK1580F	TCAATTCATTCAACWTTTCCC	this study
LE2F	TGGTACGGAGTCAAAKTC	Müller et al. [13]
trnK2R	AACTAGTCGGATGGAGTAG	Johnson and Soltis [120]
trntC2	TATGGCGAAATTGGTAGACGC	this study
trntF	ATTTGAACTGGTGACACGAG	Taberlet et al. [122]
rpsF	GTGTGTAGAAAGCAACGTGCGACTT	Oxelman et al. [123]
rpsR2	TCGGGATCGAACATCAATTGCAAC	Oxelman et al. [123]

performed with 10,000 replicates, each using TBR branch swapping and holding only one tree [70]. We measured the additional information provided by SIC-coded indels by the difference in decay indices (computed with PRAP2) for each node, comparing analyses with and without indels.

Bayesian Inference of Phylogeny

Bayesian inference (BI) of phylogeny was done with help of MrBayes v3.1.2 [71]. The model of best fit for the combined dataset as well as for each of the three partitions (*trnK/matK*, *rps16* and *trnL-F)* was found to be GTR+G+I model was found as the optimal one using jModelTest v.0.1.1 [72]. The indel partition was co-analyzed together with the DNA partition, with the restriction site (binary) model applied to the gap characters and the ascertainment (coding) bias set to "variable". Default priors were used, i.e. flat dirichlets (1.0, 1.0) for state frequencies and instantaneous substitution rates, a uniform prior (0.0, 50.0) for the shape parameter of the gamma distribution, a uniform prior (0.0, 1.0) for the proportion of invariable sites, a uniform topological prior, an exponential prior Exp (10.0) for branch lengths. Four categories were used to approximate the gamma distribution. Two runs with 5 million generations each were run, and four chains were run in parallel for each run, with the temperature set to 0.2. The chains were sampled every 100th generation, and the burnin was set to 5000. To check for convergence of the independent runs under a given model, it was ensured that the plots of both runs indicated that the stationary phase was reached, that the potential scale reduction factor approached 1 for all parameters, and that no supported conflicting nodes were found among the consensus trees generated from each run. Convergence and effective sampling sizes (ESS) of all parameters were assessed with halp of Tracer v1.5 [73].

Maximum likelihood analyses

For maximum likelihood (ML) analyses RAxML v7.0.0 [74] was used. During the search for the best tree, the GTRGAMMA model was used, while the slightly simpler GTRCAT model was employed by RAxML during the 500 bootstrap replicates. Support values from all types of analysis were mapped on the tree topology from the ML analysis and conflicting nodes were identified with help of TreeGraph2 [75].

Topological tests

Topological tests were used to see whether alternative topologies could be rejected with confidence. Specifically it was tested whether evidence against Byblidaceae being sister to Lentibulariaceae was strong. Under parsimony, the Templeton and Winning-sites (sign) tests were used ("NonparamTest" option in Paup*), while under the likelihood criterion, the Approximately Unbiased test (AU-Test) [76] along with the more classical Shimodaira-Hasegawa test (SH-test [77]), as implemented in consel 0.1j [78], were employed.

Ancestral state reconstruction

We inferred ancestral states for ten selected morphological characters. Information on character states was compiled from different sources [79,1,27,80] and is given in Table 3. We took the fully resolved best tree from the RAxML search, and traced the evolution of these characters on that topology via maximum likelihood, using the "multistate" command in BayesTraits [81].

Results

Sequence statistics and results from tree searches

Sequences of *trnK/matK*, *trnL-F* and *rps16* yielded an alignment of 7809 characters, of which 1739 were excluded from subsequent analysis because of uncertain homology. The alignment is available from TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S10963); detailed sequence statistics are given in Table 4. Consensus trees from parsimony analyses were well resolved and supported. The MP trees from substitutions only were 13118 steps long (CI 0.419, RI 0.504,), those based on substitution and indel characters had a length of 14719 steps (CI 0.453, RI 0.507,). Comparison of decay values of substitution data versus substitutions plus SIC-coded indels showed higher decay values for most nodes when indel information was included (see

Table 3 Morphological characters traced in the present study

Taxon/character	1	2	3	4	5	6	7	8	9	10
Outgroup	0	0	0	?	0	0	?	?	0	0
Plocospermataceae	0/1	0	0	0	0	0	0	0	0	0
Carlemanniaceae	1	0	2	0	0	0	0	0	0	0
Oleaceae	1	0	2	0	0	0	0	0	0	0
Tetrachondraceae	1	0	1	0	0	?	0	?	0	0
Calceolariaceae	1	1	2	0	1	1	0	1	0	0
Gesneriaceae	0	1	1	0	1	1	0	1	0	0
Plantaginaceae	0	1	0/1/2	0	0	1	1	?	0	0
Gratiolaceae	0	1	1	0	0	1	1	0	0	0
Scrophulariaceae	0	1	1	0	0	1	1	0/1	0	0
Byblidaceae	0	0	0	0	0	1	1	0	1	0
Linderniaceae	0	1	1	1	0	1	1	0/1	0	0
Stilbaceae	0	1	1	0	0	1	1	0	0	0
Lamiaceae	0	1	1	0	0	1	1	0	0	0
Mazoideae	0	1	1	0	0	1	1	0	0	0
Phrymoideae	0	1	1	0	0	1	1	0	0	0
Paulowniaceae	0	1	1	0	0	1	1	0	0	0
Rehmannia	0	1	1	0	0	?	1	1	0	0
Orobanchaceae	0	1	1	0	0	1	1	1	0	1
Thomandersiaceae	0	1	1	0	0	1	1	0	0	0
Pedaliaceae	0	1	1	0	0	1	1	0	0	0
Bignoniaceae	0	1	1	0	0	1	1	0	0	0
Verbenaceae	0	1	1	0	0	1	1	0	0	0
Schlegeliaceae	0	1	1	0	0	1	1	0	0	0
Martyniaceae	0	1	1	0	0	1	1	0	0	0
Acanthaceae	0	1	1	0	0	1	1	0	0	0
Lentibulariaceae	0	1	2	0	0	1	1	0	1	0

Key: 1: merosity 0 = pentamerous 1 = tetramerous; 2: symmetry 0 = polysymmetric 1 = monosymmetric; 3: number of stamens 0 = 5 1 = 4 2 = 2; 4: geniculate stamens 0 = absent 1 = present; 5: pair flowered cymes 0 = absent 1 = present; 6: Anthraquinones from shicimic acid metabolism 0 = absent 1 = present; 7: biosynthetic route II decarboxylated iridoids 0 = absent 1 = present; 8: alveolated seeds 0 = absent 1 = present; 9: Carnivory 0 = absent 1 = present; 10: Parasitism 0 = absent 1 = present.

Additional file 2, Figure S1). Trees from coding *rbcL* and *ndhF* seqences were far less resolved than those from our three marker combined analysis (Additional file 3 Figure S2 and Additional file 4, Figure S3). The tree topology from the ML analysis is shown in Figure 2, collapsing nodes support by less than 50% in at least one of the tree methodological approaches. BI and ML trees generally showed slightly higher resolution and statistical support than trees from MP searches. Effective sampling sizes (ESS) of all parameters from the Bayesian analysis were > 150. A phylogram from BI with branch lengths indicating relative substitution rates is given in Figure 3.

Resolution of the backbone of the Lamiales phylogeny

The precise branching pattern of the nine first-branching families in the Lamiales tree (Plocospermataceae, Carlemanniaceae, Oleaceae, Tetrachondraceae, Calceolariaceae, Gesneriaceae, Plantaginaceae (incl. Gratioleae), Scrophulariaceae) is inferred with very high or maximum (most cases) support (Figure 2). A total of 16 nodes determining this branching pattern among families along the spine of the basal Lamiales grade receive very high or maximum support by all (most cases) or at least two out of three inference methods. An additional 19 of the nodes indicating delimitation and relative position of the remaining 15 more derived families receive very high or maximum support by at least one out of three analytic approaches.

Phylogenetic position of *Hydrostachys*

In our blastn searches, all sequences (*rbcL*, *atpB*, 18s rDNA, 26s rDNA, *ndhF*, *matK*) reached highest similarity scores to other *Hydrostachys* sequences, followed by sequences from Cornales taxa (Hydrangeaceae, Cornaceae, Loasaceae), with the exception of the *matK* sequence of *Hydrostachys multifida* (AY254547) of Hufford et al. [82] used in the study of Burleigh et al. [31]. This sequence showed highest similarity with *Hydrangea hirta* and a number of sequences from *Avicennia*. When included in the present *trnK/matK* alignment, the high similarity of sequence AY254547 to *Avicennia* is obvious. A blast search of the newly generated *matK* sequence of *Hydrostachys* [EMBL: FN8112689] resulted in best matches with taxa from Cornales. Aligning and analyzing the newly generated *trnK*/*matK*, *rps16* and *trnL-F* sequences, *Hydrostachys* is resolved outside

Table 4 Sequence statistics for the rapidly evolving chloroplast markers used

charset	#chars	#chars*	length range	mean	S.D.	%divergence*	S.E.*	%variable*	%informative*	%GC
dataset	7809	6070	2211-4503	3.926.44	482.561	10.15	0.187	51.417	36.063	34.212
trnK/matK	3699	3035	454-2645	2.228.78	446.491	10.367	0.264	60.362	43.229	43.229
trnLF	1997	1577	489-1104	882.881	72.353	9.086	0.402	40.076	28.155	28.155
rps16	2113	1458	0-929	814.772	122.607	10.792	0.464	45.062	29.698	29.698

* calculated based on the alignment with hotspots excluded.

Standard errors calculated based on 100 bootstrap replicates.

Key: Characters = number of characters in the alignment matrix; Length range = actual sequence length in nucleotides (including hotspots; minimal and maximal value observed); SD = standard deviation of mean length; S.E. = Standard error; % divergence (range) = pairwise sequence distance in percent (uncorrected p distance, overall mean); % variable = percentage of variable positions; % informative = percentage of parsimony informative positions; % GC = GC content.

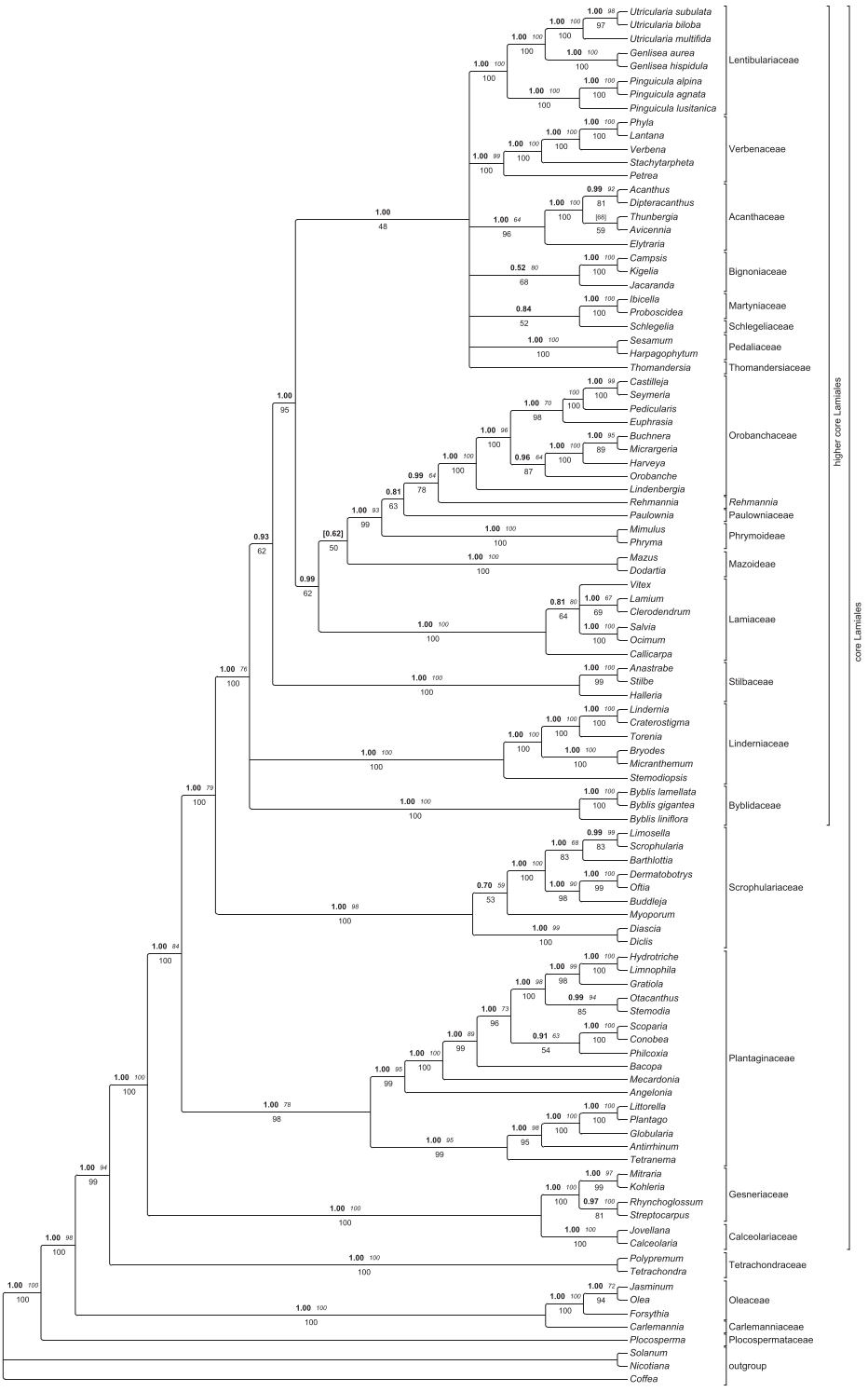


Figure 2 Phylogeny of Lamiales inferred from parsimony, likelihood and Bayesian analysis of sequences from plastid *trnK/matK, trnL-F* and *rps16*. Topology from the maximum likelihood tree depicted, collapsing nodes not supported by > = 50% in at least one of the three analyses. Bold numbers above branches are posterior probabilities from Bayesian inferences, italic numbers above branches are MP bootstrap values, number below branches indicate ML bootstrap proportions. Numbers in brackets indicate that the respective node was not supported by all three methodological approaches. The bracketed number then indicates the strongest support found for any node that contradicts the shown node [69]. Familial annotation according to APG III [28]. For Phrymaceae monophyly is not confirmed, so subfamilies are annotated; *Rehmannia* is currently not assigned to a family.

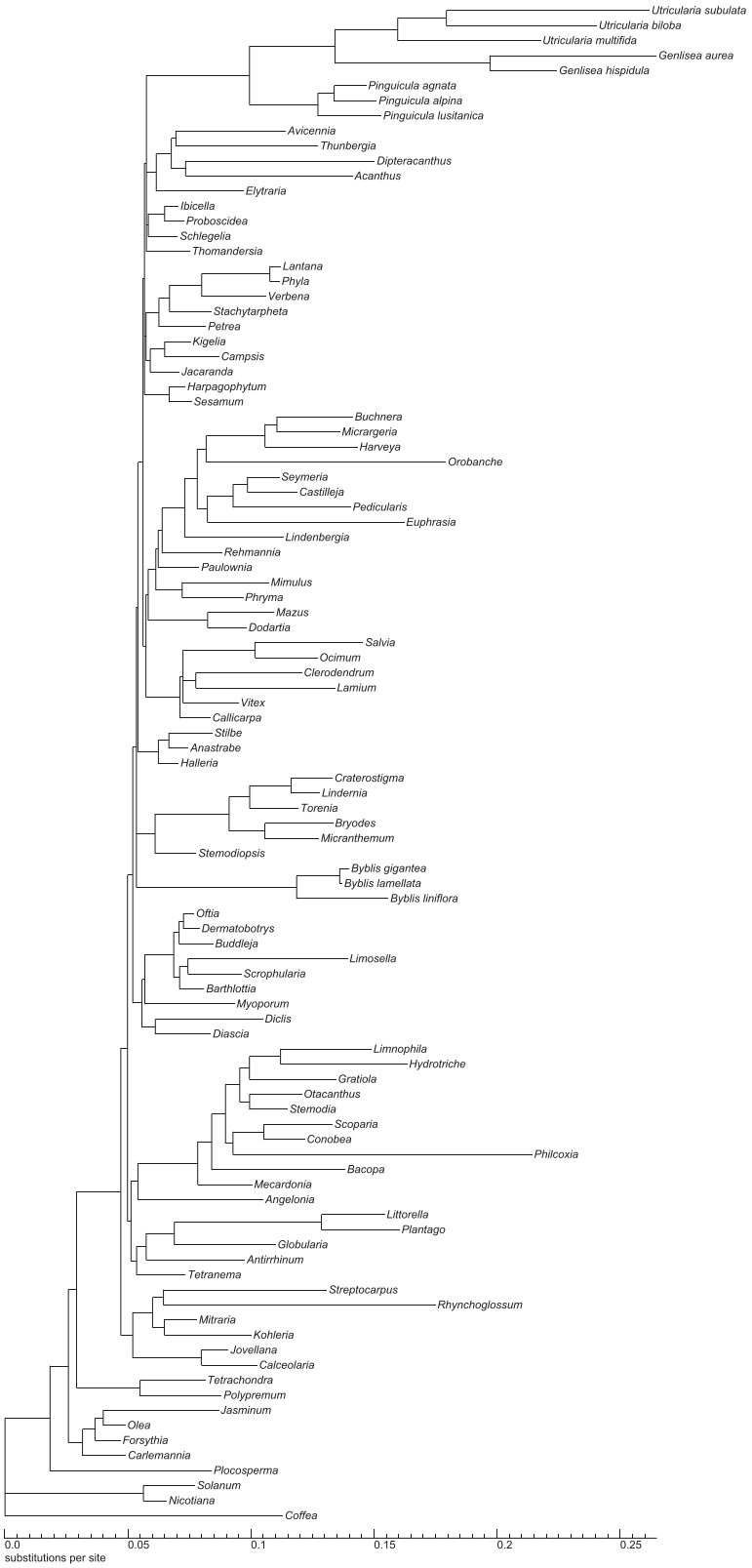


Figure 3 Phylogram from Bayesian Inference of phylogeny with branch lengths giving the relative substitution rates using the GTR+G+I model.

Lamiales. Parsimony analysis of the newly generated *matK* sequence in the context of the angiosperm *matK* data set [35] evidently places the newly generated *matK* sequence of *Hydrostachys* outside Lamiales, although its precise position within asterids remains unresolved in the 50%-majority-rule-bootstrap tree (Additional file 5, Figure S4).

Position of carnivorous lineages

In neither the Bayesian nor the maximum likelihood analysis Byblidaceae were found closely related to Lentibulariaceae. In MP analyses, the position of Byblidaceae receives no bootstrap support; interestingly, however, the strict consensus from all shortest trees depicts Byblidaceae as sister to Lentibulariaceae, regardless of the inclusion of indels. Because of this incongruence, albeit unsupported, topological tests were employed to further investigate the position of Byblidaceae. Under a parsimony framework, the Templeton and sign tests find the ML topology (Byblidaceae not closely related to Lentibulariaceae) not to be significantly less parsimonious than the shortest tree (Table 5), indicating that even under parsimony there is no significant evidence against the ML position of Byblidaceae or for its sister-group relationship to Lentibulariaceae. The AU-Test and SH-Test indicate that a sister-group relationship of Byblidaceae and Lentibulariaceae is significantly less likely than the maximum likelihood and Bayesian consensus topology.

Results from ancestral state reconstruction

Ancestral state reconstruction indicated the probabilities of the individual character states to be expected along branches as shown in Figure 4.

Discussion

Lamiales sensu APGIII [28] (including Carlemanniaceae and Plocospermataceae) receive maximal support in the present study which is the first to sample taxa from these two families in a multigene study; a single gene study [36] did not provide support for the branching order of the early branching lamialean families.

The phylogenetic position of *Hydrostachys*

Hydrostachys as a rheophyte with tuber-like rhizomes, fibrous roots, and no stomata is a morphologically highly aberrant genus [32], which has always hampered inference of its phylogenetic affinities based on morphology. Embryological characters such as endosperm development and the apical septum in the ovary [83] might be interpreted as supporting a placement of *Hydrostachys* in Lamiales [31]. The first molecular study, however, placed it within Cornales [34]. In all previous phylogenetic studies, the genus was found on a long branch, indicating strongly elevated substitutional rates - a fact that could have misled previous phylogenetic inferences [33]. Burleigh et al. [31] recently used a 5-gene data matrix to infer an angiosperm phylogeny, and resolved *Hydrostachys* as nested in Lamiales, branching right after Oleaceae. Results from our re-sequencing and re-analysis, along with a blast screening of existing GenBank sequences, strongly suggest that this placement most likely was due to an erroneous *matK* sequence used in their study. That sequence was first published by Hufford et al. [82] but is identical to one published earlier by Hufford et al. [62], although citing a different voucher. Interestingly, Burleigh et al. [31] report that the 3-gene matrix (*rbcL*, *atpB*, 18S) places *Hydrostachys* in Cornales, while in the 5-gene matrix (additional *matK* and 26S data), *Hydrostachys* is found in Lamiales. The authors suggest the *matK* sequence to be the driving force for this result. Indeed, the most likely incorrect *matK* sequence misinforms phylogenetic inference, even though only one out of five genes provides the erroneous signal. If nothing else, this demonstrates the strong phylogenetic signal and potential of *matK* for phylogenetic analyses at the given phylogenetic depth. Phylogenetic reconstruction using our newly generated sequences in the context of the three-marker matrix compiled here and in the context of the angiosperm *matK* alignment clearly places *Hydrostachys* outside Lamiales, which is consistent with earlier findings [36,84,85] and with the analysis of two unpublished *matK* sequences by Kita and Kato (AB038179, AB038180).

Table 5 Results from topology tests

		Templeton	Winning-sites	Approxiomately Unbiased	Shimodaira-Hasegawa
topology	Length	P	P	P	P
tree 1	13123	0.2971	0.4049	1.000	0.994
tree 2	13118			5e-004	0.006

Key: Maximum Parsimony: Templeton- and Winning-sites tests. Tree 1: optimal tree from RAxML search (Figure 2), tree 2: optimal tree from MP ratchet search, where Byblidaceae appear as sister to Lentibulariaceae. P = Approximate probability of getting a more extreme test statistic under the null hypothesis of no difference between the two trees (two-tailed test). The shortest tree (tree 2) is not significantly different from the ML topology (tree 1, Figure 2). Maximum Likelihood: Approximatly Unbiased- and Shimodaira-Hasegawa tests. The ML topology (tree 1, Figure 2) is significantly different from and more likely than the MP alternative (tree 2).

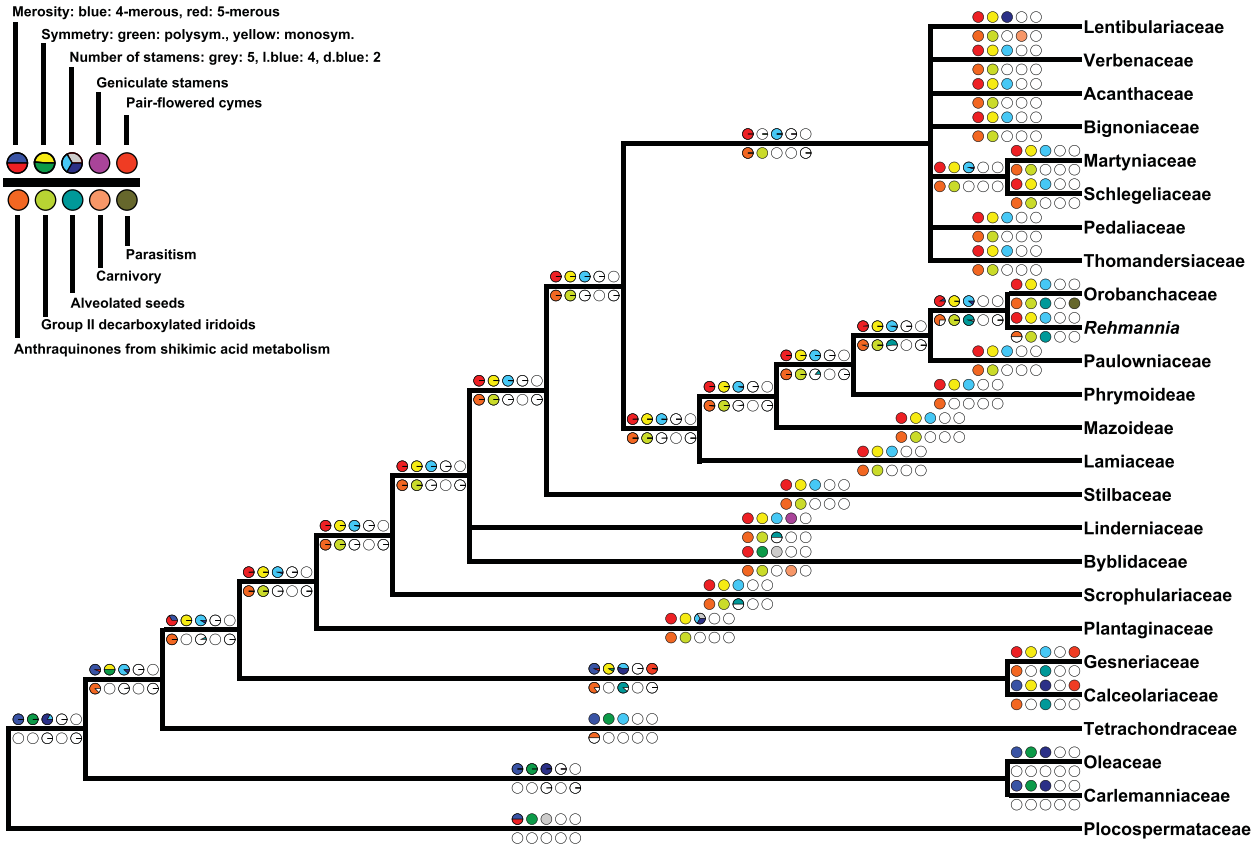


Figure 4 Evolution of selected morphological characters in Lamiales. ML ancestral state reconstruction on the ML topology (Figure 2) simplified to represent families by only one OTU and collapsing nodes not supported by > = 50% in at least one of the analyses. Pie charts give probabilities of character states; white indicates absence in case of binary (presence-absence) characters, while color indicates presence. Otherwise, colors indicate states as shown in legend.

A robust hypothesis on the basal grade in Lamiales

The Central American Plocospermataceae branch first in Lamiales (Figure 2), a scenario also found earlier in all studies that sampled this monotypic family [26,35,36]. A clade consisting of Carlemanniaceae plus Oleaceae branches second. A close relationship between these two families was found weakly supported (64% BS) previously [36] based on *rbcL* sequences, and was also observed in a study dealing with plastome rearrangements in Oleaceae [35], when Carlemanniaceae appeared sister to Oleaceae despite being set to as outgroup. We find the sister group relationship between Carlemanniaceae and Oleaceae with maximum support.

Tetrachondraceae are recovered with maximum support in all three analyses as third branch in Lamiales. While this relationship has been observed previously [36,26], statistical support for it has increased significantly in our study (59% MP BS support in Savolainen et al. [36] versus PP 1.00, 100% ML BS, 94% MP BS, support in our tree). The family comprises two genera, *Tetrachondra* and *Polypremum*, both of which were sampled here. The genus *Tetrachondra* has a disjunct distribution (New Zealand/South America) and comprises the two aquatic or semi-aquatic species, while the monotypic *Polypremum* is found from southern U.S. to the northern part of South America.

Relationships within core Lamiales

The core Lamiales (sensu [35], all Lamiales excluding Carlemanniaceae, Oleaceae, Plocospermataceae, and Tetrachondraceae; Figure 2) are unambiguously recovered by our analysis. As first branch within this core group a maximally supported clade composed of Calceolariaceae and Gesneriaceae (Figure 1f, g) is found. The phylogenetic affinities of both families had remained unclear so far [45,38,2] but both share the presence of cornoside and absence of iridoids [86]. Gesneriaceae are a large (ca. 3200 species), predominantly pantropical family of herbaceous perennials (rarely woody shrubs and small trees), about one fifth of them growing as epiphytes [87]. In contrast to many other lamialean families, molecular phylogenetics confirmed

their traditional circumscription, as proposed by Bentham in 1876 [88].

Plantaginaceae

Next in the basal grade of core Lamiales is a clade comprising Plantaginaceae as currently defined [28] (PP 1.00, 100% ML BS, 84% MP BS), in which a major split separates two groups from each other. All former studies focusing on Plantaginaceae relationships found a major dichotomy within this family [38,22,39,89]. Rahmanzadeh et al. [2] argued that the finding of a well supported clade including genera from Gratioleae together with unclear relationships of this group to other families is handled best with the recognition of a separate family. Thus, Gratiolaceae were resurrected [2]. Current phylogenies allow both the recognition of two families, as well as the treatment of Plantaginaceae with two major subfamilies. Since the taxon sampling is still far from being complete, and clear morphological characters for either of the groups are lacking, we solely accept Plantaginaceae throughout this manuscript. Rahmanzadeh et al. [2] tentatively assigned 36 genera to their Gratiolaceae, 13 of which were included in our phylogenetic study. Among the genera proposed to be part of Gratiolaceae, the widespread genus *Limosella* was found in Scrophulariaceae [22,39], and the present analysis confirms placement of *Limosella* in Scrophulariaceae. *Stemodiopsis* is found in Linderniaceae, while *Lindenbergia* is sister to the remaining Orobanchaceae. According to Olmstead et al. [38] and Rahmanzadeh et al. [2], Angelonieae (two genera: *Angelonia* and *Monopera*) appears closely related to Gratioleae. Gratioleae have an integument 3-6 cells across, with large, transversely elongated endothelial cells in vertical rows; this causes its seeds to have longitudinal ridges. The exotestal cells have hook-like thickenings [1]. Stevens et al. [1] suggest Angelonieae (integument 5-12 cells across) should also be included in Gratioleae. However, a denser taxon sampling will be needed to further test what belongs in this clade-regardless of the taxonomic level on which it might be recognized.

Scrophulariaceae

Scrophulariaceae in their new circumscription, including former Buddlejaceae and Myoporaceae, are the sister to all other higher core Lamiales (PP 1.00, 100% ML BS, 79% MP BS). This was already indicated by previous studies [2,39] and is confirmed here with high confidence. A vastly expanded circumscription of Scrophulariaceae that was presented as a possibility in APGIII [28] would thus mean that all higher core Lamiales would have to be included in order to respect the principle of monophyletic families. Such a classification would have to include a morphologically very heterogeneous assemblage of lineages with more than 17.000 species and does therefore not appear as very helpful.

Higher core Lamiales (HCL) and the evolution of carnivory

The remaining families Acanthaceae, Bignoniaceae, Byblidaceae, Lamiaceae, Lentibulariaceae, Linderniaceae, Orobanchaceae, Paulowniaceae, Pedaliaceae, Phrymaceae, Schlegeliaceae, Stilbaceae, Thomandersiaceae, and Verbenaceae form a clade strongly supported by BI (PP 1.00) and ML (100% ML BS) analysis, but only moderately supported (76% MP BS) in MP trees (referred to as "higher core Lamiales", or HCL clade, in the following). There is no morphological synapomorphy known for this clade.

A monophyletic origin of carnivory in Lamiales has been discussed since the introduction of molecular phylogenetics to the field of angiosperm systematics (see chapter on Lamiales in [90]). In the earliest analyses of *rbcL* sequences, the genus *Byblis* was found sister to Lentibulariaceae, but this placement gained only weak statistical support [19]. Later, an analysis of three coding plus three non-coding chloroplast markers [26] found Byblidaceae as sister to Lentibulariaceae with 65% jackknife support. This is the highest statistical support ever reported for this relationship, but only one *Byblis* species and one *Pinguicula* species were sampled in that study.

Based on our data, a close relationship of carnivorous Byblidaceae and Lentibulariaceae is extremely unlikely. The placement of Byblidaceae next to Lentibulariaceae, as found in previous studies and even in single MP tree topologies of the current study, has been rejected at highest significance levels by our topological tests and is contradicted with substantial statistical support by our ML and BI trees. It might be due to long branch attraction, to which MP is much more susceptible than the other two approaches [91].

Accordingly, carnivory evolved at least twice within Lamiales, in congruence with Müller et al. [13]. Our data still do not provide enough resolution to identify the immediate sister group of Lentibulariaceae. The family appears in a weakly supported group together with Acanthaceae, Thomandersiaceae and Martyniaceae/Schlegeliaceae and Bignoniaceae, Pedaliaceae and Verbenaceae. An earlier study, sampling only one species from Lentibulariaceae (*Pinguicula*), found *Elytraria* (Acanthaceae) as sister to Lentibulariaceae [39] with 52% parsimony BS. In contrast, the monophyly of Acanthaceae, including *Elytraria*, was strongly supported in a more recent study sampling 85 taxa from Acanthaceae [92]. In congruence with that, we find *Elytraria* sister to remaining Acanthaceae.

The lack of resolution in higher core Lamiales still hampers a clear identification of the precise degree of relatedness to Martyniaceae, two strongly glandular members of which (*Ibicella* and *Proboscidea*) have been reported to attract and catch numerous arthropods, and

thus have been classified as "protocarnivorous". Recent tests for protease activity of glands of the two respective genera were negative [93]; however, putatively mutualistic arthropods have been reported to be associated with each genus [94], from which the plant might benefit in a manner similar to the symbiosis observed in the African *Roridula* (Roridulaceae, Ericales) [93].

Next relatives to the supposedly carnivorous or "protocarnivorous" genus *Philcoxia* are found in Gratioleae, as previously suggested [21]. Without any doubt, Gratioleae have no close connection to Lentibulariaceae, despite some morphological similarity. Should further tests identify *Philcoxia* as a truly carnivorous plant, this would be the third independent origin of the syndrome within the order.

Further insights into the family circumscriptions in higher core Lamiales

Linderniaceae

The exact position of Linderniaceae within higher core Lamiales remains unclear. It is found unresolved in tritomy together with Byblidaceae and a clade including Acanthaceae, Bignoniaceae, Lamiaceae, Lentibulariaceae, Martyniaceae, Orobanchaceae, Paulowniaceae, Pedaliaceae, Phrymaceae, Schlegeliaceae, Stilbaceae, Thomandersiaceae, and Verbenaceae. Only the maximum likelihood tree depicts Linderniaceae and Byblidaceae forming a poorly supported clade. The centers of diversity of this family are in Southeast Asia and tropical Africa. Among them, desiccation tolerant plants like *Craterostigma* are found.

Stilbaceae and remaining families

Within the remaining families, the African Stilbaceae branch first; this scenario gains convincing support from Bayesian Inference (PP 0.93), weak support from ML bootstrapping (62% ML BS), and lacks parsimony bootstrap support. Molecular phylogenetic studies had expanded the traditional circumscription of Stilbaceae [38,39,95,96] to 11 genera (3 of which we sampled here) with a predominantly South African distribution. Only *Nuxia* extends to tropical Africa and the Arabian Peninsula.

One of two major clades in this assembly comprises Lamiaceae, Phrymaceae, Paulowniaceae, *Rehmannia*, and Orobanchaceae. Although this clade also was recovered previously [39], this is the first time it receives support from BI and ML. Within that group, Lamiaceae are sister to the remaining taxa, supported by 50% ML BS (our study), and PP 0.92 and 58% MP BS value [39]. We find subfamily Mazoideae of Phrymaceae sister to a clade including *Paulownia*, Phrymaceae subfamily Phrymoideae, *Rehmannia* and Orobanchaceae. Herein, *Rehmannia* is weakly linked to Orobanchaceae, while the relationship between *Paulownia* and Phrymoideae remains unresolved. Previous studies dealing with the next relatives of Orobanchaceae found either *Paulownia* [38], or *Phryma* and *Paulownia* together, but as unresolved tritomy [26], or *Mimulus* and *Paulownia* as successive sisters to Orobanchaceae [2] but did not include *Rehmannia* and/or *Triaenophora*.

With regard to Orobanchaceae relationships, the most extensive sampling in terms of both taxa and character number are that of Xia et al. [43] and Albach et al. [50]. The authors found *Rehmannia* and *Triaenophora* together as sister clade to Orobanchaceae, which should either be included in Orobanchaceae, as suggested by Albach et al. [50], or be recognized as a new family. As a morphological synapomorphy, Orobanchaceae, *Rehmannia* and *Triaenophora* share alveolated seeds [43]. Although a well resolved phylogeny of Orobanchaceae exists, it still remains to be tested using plastid sequence data whether the non-parasitic *Lindenbergia* alone is sister to the remaining Orobanchaceae, or if *Lindenbergia* plus the hemiparasitic genera *Siphonostegia*, *Schwalbea*, *Monochasma*, *Cymbaria* and *Bungea* are in the respective position [49].

Including taxa from both subfamilies of Phrymaceae in a context of putative relatives, no evidence for the monophyly of Phrymaceae was found [37,39]. Only Beardsley and Olmstead [51] found weak support for a monophyletic Phrymaceae, but this result is probably due to the specific sampling used. In that study [51], chloroplast data alone did not support this clade, while nuclear data and the combined analysis did so. The incongruence might be caused by a plastid-nuclear genome incongruity, which must be confirmed by additional data.

The two subfamilies of Phrymaceae, Phrymoideae and Mazoideae, do not form a clade in any of the trees in Xia et al. [43] or Albach et al. [50], and the branching order of Mazoideae, Phrymoideae and *Paulownia* is inconsistent in different analyses of these studies. Hence, the authors abstain from assigning these groups to families. In the light of our data we suggest to segregate Mazoideae from Phrymaceae and elevate it to family rank.

The position of Lamiaceae distinct from Verbenaceae (Figure 2) is an important and noteworthy finding. It ends a century-old discussion on close relationships of a Lamiaceae-Verbenaceae complex [88,97,98]. Molecular phylogenetic analysis rather concluded that Lamiaceae may not be monophyletic with respect to Verbenaceae [99]. However, analyses of *rbcL* [100,99] were not conclusive about their relationships and even a combined *matK/trnK* analysis [2] did not provide sufficient support for Lamiaceae and Verbenaceae.

The families Acanthaceae, Bignoniaceae, Lentibulariaceae, Martyniaceae, Pedaliaceae, Schlegeliaceae,

Thomandersiaceae, and Verbenaceae form a clade in our Bayesian and ML analyses (PP 1.00, ML BS 48%). For all families for which more than one taxon was sampled, monophyly is confirmed, but there is only little resolution of intra-familial relationships in that clade, especially in MP trees. In the work of Oxelman et al. [39], a corresponding clade was found, including the families mentioned above, except Pedaliaceae. We find weak support for Schlegeliaceae to be sister to Martyniaceae, while Oxelman et al. [39] found Martyniaceae, Verbenaceae and Schlegeliaceae in a clade (PP 0.82). Wortley et al. [42] found *Thomandersia* weakly linked to Schlegeliaceae, however, our data do not exhibit evidence for support such a relationship. A close examination of the floral anatomy of *Thomandersia* [101] could not improve the knowledge on its relationships.

Implications for the evolution of floral symmetry and other characters

Within Lamiales, both polysymmetric and monosymmetric (zygomorphic) flowers occur. Next to the typical pentamerous flowers, some groups exhibit tetramerous morphology. With the most highly resolved phylogeny of Lamiales to date, the evolution of floral symmetry and flower merosity within the order can be studied in more detail than previously possible. Assuming the ancestral asterid flower to be pentamerous and polysymmetric, Plocospermataceae as the most basal family of Lamiales, share this plesiomorphic character state (Figure 4). Regarding the evolution of tetramery, there are two possible scenarios. In the first, tetramery evolved once after the branching of Plocospermataceae in Lamiales, with two reversals to pentamery in both Gesneriaceae and then independently in all Lamiales branching after the Calceolariaceae/Gesneriaceae clade, this possibility is the one which is favoured by our ML ancestral state reconstruction. In the second scenario, tetramery evolved three times independently in (i) Oleaceae/Carlemanniaceae clade, (ii) Tetrachondraceae, and (iii) Calceolariaceae. Both options require three changes in flower merosity, and thus are equally parsimonious. However, there are details in floral development that differ among the tetramerous families. In Oleaceae, sepals are initiated in orthogonal positions, and petals are in diagonal position, whereas in Tetrachondraceae, sepals are initiated in diagonal, and petals in orthogonal position [102]. Initiation in Calceolariaceae follows that in Oleaceae; data for Carlemanniaceae are missing. Because tetramery in the early branching lineages of Lamiales is different for each group on more detailed level, independent gains seem more likely than a general shift towards tetramery and two independent reversals to pentamery. Tetramerous flowers are also found in the more derived Gratioleae, Veroniceae and Plantagineae (Plantaginaceae). Based on mixed evidence for fusion and loss of flower parts in these groups, multiple origins of tetramery within Plantaginaceae have been assumed. For the Plantaginaceae, Bello et al. [103] hypothesize two shifts from pentamery to tetramery: (i) in *Amphianthus*, which has recently been shown to be nested in *Gratiola* [89], and (ii) in a clade consisting of *Aragoa*, *Plantago* and *Veronica*. An independent shift to tetramery has been suggested by Albach et al. [104] based on loss of a sepal in Veroniceae and fusion in *Plantago* and *Aragoa*. But in these taxa the upper lip is composed out of two petals. Evidence for this is vascularization with two midribs, teratologic, pentamerous flowers, and an evolutionary row from pentamerous to tetramerous flowers within this tribe [98,82]. The evolution of flower symmetry can be easily reconstructed. Lamiales descended from a polysymmetric ancestor, and early branching lineages in Lamiales share this character state. After branching of Tetrachondraceae, the ancestor of the following taxa once acquired monosymmetric flowers, accompanied by a reduction from five stamens to four stamens plus one staminode. There are multiple transitions back to actinomorphic flowers in Lamiales, e.g. in the case of *Plantago* (Plantaginaceae) [103,105], in some taxa in Lamiaceae, Scrophulariaceae, Gesneriaceae, and in all Byblidaceae. The corolla of Byblidaceae is treated here as actinomorphic, although the curved stamens introduce a slight element of zygomorphy.

Further morphological characters

Several morphological or biochemical characters lend further support to some of our hypothesized phylogenetic relationships in Lamiales. Carlemanniaceae and Oleaceae share the characteristic of having only two stamens, while the first-branching Plocospermataceae have five stamens, and the lineages branching later in the evolution of Lamiales generally have four stamens. The sister-group relationship between Calceolariaceae and Gesneriaceae is further confirmed by two morphological characters shared by these families (see Figure 4): (i) the thyrsic inflorescence with pair flowered cymes, and (ii) aulacospermous alveolated seeds [102]. Aulacospermous seeds are otherwise only found in Linderniaceae (*Crepidorhopalon, Hartliella*). However, an aberrant type of aulacospermous seeds is found in some genera of Scrophulariaceae s.str.. Here not all cells of the endothelium protrude into the endosperm and the ontogeny is different from Calceolariaceae, Gesneriaceae and Linderniaceae [44,106]. With regard to chemical compounds, Plocospermataceae, Oleaceae and Carlemanniaceae have no anthraquinones from the shikimic acid metabolism, Tetrachondraceae have not been examined for the occurrence of these compounds, and all other lineages in Lamiales possess them. Consequently, these anthraquinones have evolved immediately before or

immediately after branching of Tetrachondraceae. Group II decarboxylated iridoids most likely evolved once after the branching of Calceolariaceae + Gesneriaceae, since they are shared by all taxa branching after this clade [1]. The close relationship between *Rehmannia* and Orobanchaceae is supported by the shared occurrence of alveolated seeds.

Divergence ages in Lamiales

There have been several attempts to estimate Asterid divergence ages, using fossil calibration points outside Lamiales. By means of the earliest relaxed clock dating method NPRS [107], Wikström et al. [108] provided estimates for Lamiales stem group (sga) and crown group ages (cga) of 74 mya and 64 mya, respectively. Using a more sophisticated approach (PL, [107]), the later results of Bremer at al. [109] and Janssens et al. [110] were quite congruent, estimating the stem group age at 106 and 104 mya, and the crown group age at 97 and 95 mya, respectively. The recent study of Magallon and Castillo [111] presents a diversification hypothesis for all angiosperms derived from constraining minimal ages of 49 nodes with fossil data. This setup resulted in a sga of 80 mya and a cga of 63 mya for Lamiales, maybe because of the strongly reduced taxon sampling among Lamiales compared to Bremer et al. [109]. Furthermore, the highest diversification rates among angiosperms were found in Lamiales [112]. This rapid radiation could be a reason for the difficulty in untangling the relationships in Lamiales, as previously supposed [2]. The very short branches among the representatives of Higher Core Lamiales (see Figure 3) are putatively indicative of a rapid radiation. So far, reliable relaxed-clock estimates for the age of major Lamiales lineages have been lacking for two reasons, one of which is the scantiness of useful fossil calibration points. Only few fossils, sometimes with questionable assignment [113], are known from Lamiales. They include a mummified *Byblis* seed (middle Eocene[114]), a fruit from Bignoniaceae (middle Eocene, [115]), *Justicia*-like pollen (Neogene, [116]), and vegetative parts from *Hippuris* (Hippuridaceae), *Fraxinus* (Oleaceae), and *Chilopsis* (Bignoniaceae) from Oligocene [117]. The second reason for the absence of dating attempts in Lamiales has been the uncertainty with respect to the phylogenetic position of the families within Lamiales. We believe that our study represents good progress with regard to this second problem. Nevertheless, we refrain from trying to obtain divergence age estimated based on our data at this point, because (i) the sparseness of reliable and useful fossil calibration points would force us to either use an insufficient number of calibration points or use calibration points that themselves are molecular-clock based estimates with a substantial error margin, and (ii) because the remaining uncertainties in the branching order within Lamiales would translate into inferring clade ages with unsatisfyingly wide confidence intervals.

Conclusions

Utility of chloroplast markers for Lamiales phylogenetics

Phylogenetic analysis of combined *trnK*/*matK*, *trn*L-F and *rps*16 intron sequences enhanced both resolution and statistical support compared to previous studies. Addition of the more slowly evolving protein coding *rbcL* and *ndhF* genes to our three-marker dataset did not increase resolution and support values of trees to the slightest degree (Additional file 6, Figure S5), and analyses of each of the coding markers alone yield highly unresolved topologies.

Despite the step forward reported here, more data need to be compiled to clarify the affinities within the derived Lamiales, especially for finding the next relatives of carnivorous lineages and a better understanding of the path to carnivory in the order. A recent simulation study argued for accumulating many more characters from slow evolving markers, and recommends 10,000-20,000 characters for Lamiales [40]. Apart from the much greater effort required by this strategy, the simulation approach taken by the authors does not allow a rejection of the utility of non coding markers. This is because the distribution of rates and homoplasy at individual sites, which seems to be a very important factor determining phylogenetic utility [57], was not taken into account by the authors. Moreover, simulations were exclusively based on substitutional patterns derived from functionally highly constrained *ndhF* and *rbcL* data sets with a scarce taxon sampling and a very rough estimation of phylogeny by neighbor-joining. A currently popular approach in large scale angiosperm phylogenetics takes this idea one step further and uses concatenated coding sequences extracted from complete cp genome sequences (e.g. [118]).

However, regardless of the markers and number of characters used, it has emerged as highly crucial to maintain a high taxon sampling density while accumulating more characters [40,112,119]. Although the cost for complete cp genome sequences have dropped dramatically in the past years, in particular when only protein coding regions are targeted and no assembly is aimed at, the cost/benefit ratio so far has prevented researchers from taking this avenue for resolving the Lamiales phylogeny. For such an approach, it is currently unclear whether an appropriate number of taxa could be upheld while keeping costs at a reasonable level, and whether the information content in even a large number of slowly evolving protein coding genes would significantly exceed that in just a few more

quickly evolving cp genome regions. In view of the substantial progress made here with this kind of marker, adding further data from non-protein coding chloroplast regions seems a promising strategy that, alone or in combination with phylogenomic approaches, might finally provide us with a clear picture of Lamiales evolution.

Additional material

Additional file 1: Table S1: Taxa, specimens and GenBank acession numbers for sequences used in the 5 gene analysis. Voucher information.

Additional file 2: Figure S1: A comparison of decay values. Numbers above branches give decay values from nucleotide data matrix; numbers below branches that from nucleotides plus coded indels.

Additional file 3: Figure S2: Tree from *rbcL* analysis. Strict consensus of 100 MP bootstrap replicates performed.

Additional file 4: Figure S3: Tree from *ndhF* analysis. Strict consensus of 100 MP bootstrap replicates performed.

Additional file 5: Figure S4: Tree from angiosperm-wide *matK* analysis of the Hilu et al. 2003 dataset plus our newly generated *Hydrostachys* sequence. Strict consensus of 100 MP bootstrap replicates performed.

Additional file 6: Figure S5: Tree from combined *trnK/matK, trnL-F, rps16, rbcL, ndhF* analysis. 100 bootstrap replicates performed.

Acknowledgements
This study was funded by DFG grant "Carnivory in Lamiales: understanding character evolution, substitution rate plasticity, and genome miniaturization", MU2875/2, to K.F.M. Additional funding was obtained from the DFG project "Mutational dynamics of non-coding genomic regions and their potential for reconstructing evolutionary relationships in eudicots" (BO1815/2-1 and/-2; QU153/2-1 and/2-2) to T.B. and Dietmar Quandt. Thanks to Nadja Korotkova for a photograph used in Figure 1. The authors would like to thank the staff of the Bonn Botanical Gardens for cultivating plants analyzed in the present study, and the curators of the respective herbaria (BONN, M) for providing material for DNA extraction. We also want to thank Richard Olmstead and two anonymous reviewers for very helpful comments that helped to improve the manuscript.

Author details
[1]Institute for Evolution and Biodiversity, University of Muenster, Hüfferstraße 1, 48149 Münster, Germany. [2]Department Biology, Systematic Botany and Mycology, Ludwig-Maximilians-Universität München, Menzinger Straße 67, D-80638 Munich, Germany. [3]Institut für Integrierte Naturwissenschaften - Biologie, Universität Koblenz-Landau, Universitätsstraße 1, 56070 Koblenz, Germany. [4]Institut für Biologie und Umweltwissenschaften (IBU), Carl von Ossietzky Universität Oldenburg, Carl von Ossietzky-Str. 9-11, 26111 Oldenburg, Germany. [5]Botanischer Garten und Botanisches Museum Berlin-Dahlem and Institute for Biology, Dahlem Center of Plant Sciences (DCPS), Freie Universität Berlin, Königin Luise-Straße 6-8, 14195 Berlin, Germany.

Authors' contributions
B.S. generated data and drafted the manuscript. K.F.M. was responsible for the conception of the study and helped writing the manuscript. D.C.A. provided data and improved the manuscript. A.F. and T.B. provided plant material. T.B. contributed during manuscript preparation. A.F., E.F. and G.H. improved the manuscript. T.B., E.F., and D.C.A. contributed to the conception of the study during its initial phase, G.H. in its final phase. A.F. contributed during manuscript preparation. All authors have given final approval of the version to be published.

Received: 25 May 2010 Accepted: 12 November 2010
Published: 12 November 2010

References
1. Stevens PF: **Angiosperm Phylogeny Website.** 2001 [http://www.mobot.org/MOBOT/research/APweb/], Version 7, May 2006.
2. Rahmanzadeh R, Müller KF, Fischer E, Bartels D, Borsch T: **Linderniaceae and Gratiolaceae (Lamiales) are further lineages distinct from Scrophulariaceae.** *Plant Biology* 2005, **7**:67-78.
3. Bartels D: **Desiccation tolerance studied in the resurrection plant *Craterostigma plantagineum*.** *Integr Comp Biol* 2005, **45**:696-701.
4. Young ND, Steiner KE, dePamphilis CW: **The evolution of prasitism in Scrophulariaceae/Orobanchaceae: plastid gene sequences refute an evolutionary transition series.** *Ann MO Bot Gard* 1999, **86**:876-893.
5. Richies CR, Parker C: **Parasitic plants as weeds.** *Parasitic plants* London, UK: Chapman & Hall; 1995, 226-255.
6. Lloyd FE: **Carnivorous plants.** Massachusetts: Waltham; 1942.
7. Juniper BE, Robins RJ, Joel DM: **The carnivorous plants.** London: Academic press; 1989.
8. Brugger J, Rutishauser R: **Bau und Entwicklung landbewohnender *Utricularia*-Arten.** *Bot Helv* 1989, **99**:91-146.
9. Rutishauser R, Sattler R: **Complementarity and heuristic value of contrasting models in structural botany: 3. Case study on shoot-like "leaves" and leaf-like "shoots" in *Utricularia macrorhiza* and *U. purpurea* (Lentibulariaceae).** *Bot Jahrb* 1989, **111**:121-137.
10. Rutishauser R, Isler B: **Developmental genetics and morphological evolution of flowering plants, especially Bladderworts (*Utricularia*): Fuzzy arberian morphology complements classical morphology.** *Ann Bot* 2001, **88**:1173-1202.
11. Barthlott W, Porembski S, Fischer E, Gemmel B: **First protozoa-trapping plant found.** *Nature* 1998, **392**:447.
12. Greilhuber J, Borsch T, Müller KF, Worberg A, Porembski S, Barthlott W: **Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size.** *Plant Biology* 2006, **8**:770-777.
13. Müller KF, Borsch T, Legendre L, Porembski S, Theisen I, Barthlott W: **Evolution of carnivory in Lentibulariaceae and the Lamiales.** *Plant Biology* 2004, **6**:477-490.
14. Müller KF, Borsch T, Legendre L, Porembski S, Barthlott W: **Recent progress in understanding the evolution of Lentibulariaceae.** *Plant Biology* 2006, **8**:748-757.
15. Hartmeyer S: **Carnivory of *Byblis* revisited-A simple method for enzyme testing on carnivorous plants.** *Carniv Pl Newslett* 1997, **26**:34-38.
16. Plachno BJ, Adamec L, Lichtscheidl IK, Peroutka M, Adlassnig W, Vrba J: **Fluorescence labelling of phosphatase activity in digestive glands of carnivorous plants.** *Plant Biology* 2006, **8**:813-820.
17. Lang F: **Untersuchungen über Morphologie, Anatomie und Samenentwicklung von *Polypompholyx* und *Byblis gigantea*.** *Flora* 1901, 149-206.
18. Conran JG: **The embryology and relationships of the Byblidaceae.** *Aust Sys Bot* 1996, **9**:243-254.
19. Albert VA, Williams SE, Chase MW: **Carnivorous plants: Phylogeny and structural evolution.** *Science* 1992, **257**:1491-1495.
20. Taylor P, Souza VC, Giulietti AM, Harley RM: ***Philcoxia*: A new genus of Scrophulariaceae with three new species from Eastern Brazil.** *Kew Bulletin* 2000, **55**:155-163.
21. Fritsch P, Almeda F, Martins AB, Cruz BC, Estes D: **Rediscovery and phylogenetic placement of *Philcoxia minensis* (Plantaginaceae), with a test of carnivory.** *Proc Calif Acad Sci* 2007, **58**:447-467.
22. Albach DC, Meudt HM, Oxelman B: **Piecing together the "new" Plantaginaceae.** *Am J Bot* 2005, **92**:297-315.
23. McDade LA, Moody ML: **Phylogenetic relationships among Acanthaceae: evidence from noncoding trnL-trnF chloroplast DNA sequences.** *Am J Bot* 1999, **86**:70-80.
24. Olmstead RG, Bremer B, Scott KM, Palmer JD: **A parsimony analysis of the Asteridae s.l. based on rbcL sequences.** *Ann MO Bot Gard* 1993, **80**:700-722.
25. Olmstead RG, Kim KJ, Jansen RK, Wagstaff SJ: **The phylogeny of the Asteridae sensu lato based on chloroplast ndhF gene sequences.** *Mol Phylogenet Evol* 2000, **16**:96-112.

26. Bremer B, Bremer K, Heidari N, Erixon P, Olmstead RG, Anderberg AA, Källersjö M, Barkhordarian E: **Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels.** *Mol Phylogenet Evol* 2002, **24**:274-301.
27. Soltis DE, Soltis PS, Endress PK, Chase MW: **Phylogeny and evolution of angiosperms.** Sunderland, Massachusetts, USA: Sinauer Associates; 2005.
28. APG III: **An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III.** *Bot J Linn Soc* 2009, **161**:105-121.
29. Dahlgren G, (ed): **Systematische Botanik.** Berlin Heidelberg New York: Springer; 1987.
30. Takhtajan A: **Diversity and Classification of Flowering Plants.** New York: Columbia University Press; 1997.
31. Burleigh JG, Hilu K, Soltis D: **Inferring phylogenies with incomplete data sets: a 5-gene, 567-taxon analysis of angiosperms.** *BMC Evol Biol* 2009, **9**:61.
32. Xiang Q, Moody ML, Soltis DE, Fan CZ, Soltis PS: **Relationships within Cornales and circumscription of Cornaceae–matK and rbcL sequence data and effects of outgroups and long branches.** *Mol Phylogenet Evol* 2002, **24**:35-57.
33. Fan C, Xiang Q: **Phylogenetic analyses of Cornales based on 26S rRNA and combined 26S rDNA-matK-rbcL sequence data.** *Am J Bot* 2003, **90**:1357-1372.
34. Hempel A, Reeves PA, Olmstead R, Jansen RK: **Implications of rbcL sequence data for higher order relationships of the Loasaceae and the anomalous aquatic plant *Hydrostachys* (Hydrostachyaceae).** *Plant Syst Evol* 1994, **194**:25-37.
35. Hilu KW, Borsch T, Müller KF, Soltis DE, Soltis PS, Savolainen V, Chase M, Powell M, Alice LA, Evans R, Sauquet H, Neinhuis C, Slotta TA, Rohwer JG, Campbell CS, Chatrou L: **Angiosperm phylogeny based on matK sequence information.** *Am J Bot* 2003, **90**:1758-1776.
36. Savolainen V, Fay MF, Albach DC, Backlund A, Van der Bank M, Cameron KM, Johnson LA, Lledó MD, Pintaud J-, Powell M, Sheaham MC, Soltis DE, Soltis PS, Weston P, Whitten WM, Wurdack KJ, Chase MW: **Phylogeny of the eudicots: a nearly complete familial analysis based on rbcL gene sequences.** *Kew Bulletin* 2000, **55**:257-309.
37. Tank DC, Beardsley PM, Kelchner SA, Olmstead RG: **L. A. S. JOHNSON REVIEW No. 7. Review of the systematics of Scrophulariaceae s.l. and their current disposition.** *Aust J Bot* 2006, **19**:289-307.
38. Olmstead RG, DePamphilis CW, Wolfe AD, Young ND, Elisons WJ, Reeves PA: **Disintegration of the Scrophulariaceae.** *Am J Bot* 2001, **88**:348-361.
39. Oxelman B, Kornhall P, Olmstead RG, Bremer B: **Further disintegration of Scrophulariaceae.** *Taxon* 2005, **54**:411-425.
40. Wortley AH, Rudall PJ, Harris DJ, Scotland RW: **How much data are needed to resolve a difficult phylogeny? Case study in Lamiales.** *Syst Biol* 2005, **54**:697-709.
41. Andersson S: **On the phylogeny of the genus *Calceolaria* (Calceolariaceae) as inferred from ITS and plastid matK sequences.** *Taxon* 2006, **55**:125-137.
42. Wortley AH, Harris DJ, Scotland RW: **On the Taxonomy and Phylogenetic Position of *Thomandersia*.** *Syst Botany* 2007, **32**:415-444.
43. Xia Z, Wang Y, Smith JF: **Familial placement and relations of *Rehmannia* and *Triaenophora* (Scrophulariaceae s.l.) inferred from five gene regions.** *Am J Bot* 2009, **96**:519-530.
44. Fischer E: **Scrophulariaceae.** In *The Families and Genera of Vascular Plants.* Edited by: Kubitzki K. Berlin: Springer; 2004:333-432.
45. Olmstead RG, Reeves PA: **Evidence for the polyphyly of the Scrophulariaceae based on chloroplast rbcL and ndhF sequences.** *Ann MO Bot Gard* 1995, **82**:176-193.
46. APG2: **An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II.** *Bot J Linn Soc* 2003, **141**:399-436.
47. dePamphilis CW, Young ND, Wolfe AD: **Evolution of plastid gene rps2 in a lineage of hemiparasitic and holoparasitic plants: many losses of photosynthesis and complex patterns of rate variation.** *Proc Natl Acad Sci USA* 1997, **94**:7367-7372.
48. Wolfe A, dePamphilis C: **The effect of relaxed functional constraints on the photosynthetic gene rbcL in photosynthetic and nonphotosynthetic parasitic plants.** *Mol Biol Evol* 1998, **15**:1243-1258.
49. Bennett JR, Mathews S: **Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A.** *Am J Bot* 2006, **93**:1039-1051.
50. Albach DC, Yan K, Jensen SR, Li H: **Phylogenetic placement of *Triaenophora* (formerly Scrophulariaceae) with some implications for the phylogeny of Lamiales.** *Taxon* 2009, **58**:749-756.
51. Beardsley PM, Olmstead RG: **Redefining Phrymaceae: The placement of *Mimulus*, tribe Mimuleae, and *Phryma*.** *Am J Bot* 2002, **89**:1093-1102.
52. Magin N, Classen R, Gack C: **The morphology of false anthers in *Craterostigma plantagineum* and *Torenia polygonioides* (Scrophulariaceae).** *Can J Bot* 1989, **67**:1931-1937.
53. Fischer E: **Systematik der afrikanischen Lindernieae (Scrophulariaceae).** *Trop Subtrop Pflanzenwelt* 1992, **82**:1-365.
54. Spangler RE, Olmstead RG: **Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences rbcL and ndhF.** *Ann MO Bot Gard* 1999, **86**:33-46.
55. Nakai T: **Classes, Ordines, Familiae, Subfamiliae, Tribus, Genera nov quae attinet ad plantas Koreanas.** *J Jap Bot* 1949, **24**:8-14.
56. Reveal J: **Newly required suprageneric names in vascular plants.** *Phytologia* 1995, **79**:68-76.
57. Müller KF, Borsch T, Hilu KW: **Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: Contrasting matK, trnT-F, and rbcL in basal angiosperms.** *Mol Phylogenet Evol* 2006, **41**:99-117.
58. Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W: **Non-coding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms.** *J Evol Biol* 2003, **16**:558-576.
59. Worberg A, Quandt D, Barniske A-, Löhne C, Hilu KW, Borsch T: **Phylogeny of basal eudicots: Insights from non-coding and rapidly evolving DNA.** *Org Divers Evol* 2007, **7**:55-77.
60. Borsch T, Quandt D: **Mutational dynamics and phylogenetic utility of noncoding chloroplast DNA.** *Plant Syst Evol* 2009, **282**:169-199.
61. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: **Basic local alignment search tool.** *J Mol Biol* 1990, **215**:403-410.
62. Hufford L, Moody ML, Soltis DE: **A phylogenetic analysis of Hydrangeaceae based on sequences of the plastid gene matk and their combination with rbcl and morphological data.** *Int J Plant Sci* 2001, **162**:835-846.
63. Albach DC, Soltis PS, Soltis DE, Olmstead RG: **Phylogenetic analysis of asterids based on sequences of four genes.** *Ann MO Bot Gard* 2001, **88**:163-212.
64. Müller J, Müller KF, Neinhuis C, Quandt D: **PhyDE - Phylogenetic Data Editor.** 2006 [http://www.phyde.de].
65. Kelchner SA: **The evolution of non-coding chloroplast DNA and its application in plant systematics.** *Ann MO Bot Gard* 2000, **87**:482-498.
66. Simmons MP, Ochoterena H: **Gaps as characters in sequence-based phylogenetic analyses.** *Syst Biol* 2000, **49**:369-381.
67. Müller KF: **SeqState: Primer design and sequence statistics for phylogenetic DNA datasets.** *Appl Bioinformatics* 2005, **4**:65-69.
68. Müller KF: **PRAP - computation of Bremer support for large data sets.** *Mol Phylogenet Evol* 2004, **31**:780-782.
69. Swofford DL: **PAUP*. Phylogenetic Analysis Using Parsimony (* and other Methods).** Sinauer Associates, Sunderland, Massachussets; 1998.
70. Müller KF: **The efficiency of different search strategies in estimating parsimony jackknife, bootstrap, and Bremer support.** *BMC Evol Biol* 2005, **5**:58.
71. Ronquist F, Huelsenbeck JP: **MrBayes 3: Bayesian phylogenetic inference under mixed models.** *Bioinformatics* 2003, **19**:1572-1574.
72. Posada D: **jModelTest: phylogenetic model averaging.** *Mol Biol Evol* 2008, **25**:1253-1256.
73. Andrew Rambaut, Alexei JDrummond: **Tracer.** Edinburgh: Institute for Evolutionary Biology; 2009.
74. Stamatakis A: **RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models.** *Bioinformatics* 2006, **22**:2688-2690.
75. Stöver B, Müller KF: **TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses.** *BMC Bioinformatics* 2010, **11**:7.
76. Shimodaira H: **An approximately unbiased test of phylogenetic tree selection.** *Syst Biol* 2002, **51**:492-508.
77. Shimodaira H, Hasegawa M: **Multiple comparisons of log-likelihoods with applications to phylogenetic inference.** *Mol Biol Evol* 1999, **16**:1114-1116.

78. Shimodaira H, Hasegawa M: **CONSEL: for assessing the confidence of phylogenetic tree selection.** *Bioinformatics* 2001, **17**:1246-1247.
79. Watson L, Dallwitz M: **The families of flowering plants: descriptions, illustrations, identification, and information retrieval.** 1992.
80. Mabberley D: **The plant-book: a portable dictionary of the vascular plants.** Cambridge: Cambridge University Press; 2008.
81. Pagel M, Meade A, Barker D: **Bayesian Estimation of Ancestral Character States on Phylogenies.** *Systematic Biology* 2004, **53**:673-684.
82. Hufford L, McMahon MM, Sherwood AM, Reeves G, Chase MW: **The major clades of Loasaceae: Phylogenetic analysis using the plastid matK and trnL-trnF regions.** *Am J Bot* 2003, **90**:1215-1228.
83. Jäger-Zürn I: **Zur Frage der systematischen Stellung der Hydrostachyaceae auf Grund ihrer Embryologie, Blüten-und Infloreszenzmorphologie.** *Plant Syst Evol* 1965, **112**:621-639.
84. Soltis DE, Soltis PS, Chase MW, Mort ME, Albsch DC, Zanis M, Savolainen V, Hahn WH, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS: **Angiosperm phylogeny inferred from 18S rDNA, rbcL, and atpB sequences.** *Bot J Linn Soc* 2000, **133(81)**:381--461.
85. Albach D: **Phylogenetic placement of the enigmatic angiosperm *Hydrostachys*.** *Taxon* 2001, **50**:781-805.
86. Jensen SR, Albach DC, Ohno T, Grayer RJ: ***Veronica*: Iridoids and cornoside as chemosystematic markers.** *Biochem Syst Ecol* 2005, **33**:1031-1047.
87. Smith JF, Wolfram JC, Brown KD, Carroll CL, Denton DS: **Tribal Relationships in the Gesneriaceae: evidence from DNA sequences of the chloroplast gene ndhF.** *Ann MO Bot Gard* 1997, **84**:50-66.
88. Bentham G: **Gesneriaceae.** In *Genera Plantarum 2* Edited by: Bentham G, Hooker JD 1876, 990-1025, Reeve.
89. Estes D, Small RL: **Phylogenetic relationships of the monotypic genus *Amphianthus* (Plantaginaceae tribe Gratioleae) inferred from chloroplast DNA sequences.** *Syst Botany* 2008, **33**:176-182.
90. Soltis DE, Soltis PS, Endress PK, Chase MW: **Phylogeny and Evolution of Angiosperms.** 2005.
91. Felsenstein J: **Cases in which parsimony or compatibility methods will be positively misleading.** *Syst Biol* 1978, **27**:401-410.
92. McDade LA, Daniel TF, Kiel CA: **Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales).** *Am J Bot* 2008, **95**:1136-1152.
93. Plachno BJ, Adamec L, Huet H: **Mineral nutrient uptake from prey and glandular phosphatase activity as a dual test of carnivory in semi-desert plants with glandular leaves suspected of carnivory.** *Ann Bot-London* 2009, **104**:649-654.
94. Rice B: **Reassessing commensal-enabled carnivory in *Proboscidea* and *Ibicella*?** *Carniv Pl Newslett* 2008, 15-19.
95. Bremer B, Olmstead RG, Struwe L, Sweere JA: **rbcL sequences support exclusion of *Retzia*, *Desfontainia*, and *Nicodemia* from the Gentianales.** *Plant Syst Evol* 1994, **190**:213-230.
96. Oxelman B, Backlund M, Bremer B: **Relationships of the Buddlejaceae s.l. investigated using parsimony jackknife and branch support analysis of chloroplast ndhF and rbcL sequence data.** *Syst Botany* 1999, **24**:164-182.
97. Cronquist A: **The Evolution and Classification of Flowering Plants.** New York: The New York Botanical Garden;, 2 1988.
98. Briquet J: **Labiatae.** In *Die natürlichen Pflanzenfamilien. Volume 4/3a.* Edited by: Engler A, Prantl K. Leipzig: Engelmann; 1895:132-182.
99. Cantino PD: **Evidence for a polyphyletic origin of the Labiatae.** *Ann MO Bot Gard* 1992, 361-379.
100. Wagstaff SJ, Hickerson L, Spangler R, Reeves PA, Olmstead RG: **Phylogeny in Labiatae s.l., inferred from cpDNA sequences.** *Plant Syst Evol* 1998, **209**:265-274.
101. Wortley AH, Scotland RW, Rudall PJ: **Floral anatomy of *Thomandersia* (Lamiales), with particular reference to the nature of the retinaculum and extranuptial nectaries.** *Bot J Linn Soc* 2005, **149**:469.
102. Mayr EM, Weber A: **Calceolariaceae: floral development and systematic implications.** *Am J Bot* 2006, **93**:327-343.
103. Bello MA, Rudall PJ, González F, Fernández-Alonso JL: **Floral morphology and development in *Aragoa* (Plantaginaceae) and related members of the order Lamiales.** *Int J Plant Sci* 2004, **165**:723-738.
104. Albach DC, Martinez-Ortega MM, Fischer MA, Chase MW: **Evolution of Veroniceae: A phylogenetic perspective.** *Ann MO Bot Gard* 2004, **91**:275-302.
105. Endress P: **Symmetry in flowers: diversity and evolution.** *Int J Plant Sci* 1999, **160**:S3-S23.
106. Hartl D: **Das alveolierte Endosperm bei Scrophulariaceen, seine Entstehung, Anatomie und taxonomische Bedeutung.** *Beiträge zur Biologie der Pflanzen* 1959, **35**:95-110.
107. Sanderson MJ: **Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach.** *Mol Biol Evol* 2002, **19**:101-109.
108. Wikström N, Savolainen V, Chase MW: **Evolution of the angiosperms: calibrating the family tree.** *Proc R Soc Lond [Biol]* 2001, **268**:2211-2220.
109. Bremer K, Friis EM, Bremer B: **Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification.** *Syst Biol* 2004, **53**:496-505.
110. Janssens SB, Knox EB, Huysmans S, Smets EF, Merckx VS: **Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change.** *Mol Phylogenet Evol* 2009, **52**:806-824.
111. Magallon S, Castillo A: **Angiosperm diversification through time.** *Am J Bot* 2009, **96**:349-365.
112. Zwickl DJ, Hillis DM: **Increased taxon sampling greatly reduces phylogenetic error.** *Syst Biol* 2002, **51**:588-598.
113. Nie Z, Sun H, Beardsley PM, Olmstead RG, Wen J: **Evolution of biogeographic disjunction between eastern Asia and eastern North America in *Phryma* (Phrymaceae).** *American Journal of Botany* 2006, **93**:1343-1356.
114. Conran JG, Christophel DC: **A fossil Byblidaceae seed from Eocene South Australia.** *Int J Plant Sci* 2004, **165**:691-694.
115. Pigg KB, Wehr WC: **Tertiary Flowers, Fruits, and Seeds of Washington State and Adjacent Areas-Part III.** *Wash Geol* 2002, **30**:3-16.
116. Germeraad J, Hopping C, Muller J: **Palynology of tertiary sediments from tropical areas.** *Review of Palaeobotany and Palynology* 1968, **6**:189-198, 200-210, 212-228, 230-259, 261, 263-348.
117. Axelrod DI: **The Late Oligocene Creede Flora, Colorado.** Berkeley, Los Angeles, London: University of California Press; 1987**130.**
118. Jansen RK, Cai Z, Raubeson LA, Daniell H, dePamphilis CW, Leebens-Mack J, Müller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee S, Peery R, McNeal JR, Kuehl JV, Boore JL: **Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns.** *Proc Natl Acad Sci USA* 2007, **104**:19369-19374.
119. Qiu Y, Li L, Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC: **The deepest divergences in land plants inferred from phylogenomic evidence.** *Proc Natl Acad Sci USA* 2006, **103**:15511-15516.
120. Johnson LA, Soltis DE: **Phylogenetic inference in Saxifragaceae s.str. and *Gilia* (Polemoniaceae) using matK sequences.** *Ann MO Bot Gard* 1995, **82**:149-175.
121. Müller KF, Borsch T: **Phylogenetics of Amaranthaceae based on matK/trnK sequence data evidence from parsimony, likelihood, and Bayesian analyses.** *Ann MO Bot Gard* 2005, **92**:66-102.
122. Taberlet P, Gielly L, Pautou G, Bouvet J: **Universal primers for amplification of 3 noncoding regions of chloroplast DNA.** *Plant Mol Biol* 1991, **17**:1105-1109.
123. Oxelman B, Lidén M, Berglund D: **Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae).** *Plant Syst Evol* 1997, **206**:393-410.

doi:10.1186/1471-2148-10-352
Cite this article as: Schäferhoff *et al.*: Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. *BMC Evolutionary Biology* 2010 **10**:352.

Article II

Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae)

Fleischmann, A., Schäferhoff, B., Heubl, G., Rivadavia, F., Barthlott, W., Müller, K.F., 2010. *Molecular Phylogenetics and Evolution* 56: 768-783.

Molecular Phylogenetics and Evolution 56 (2010) 768–783

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae)

Andreas Fleischmann [a,*], Bastian Schäferhoff [b,d], Günther Heubl [a], Fernando Rivadavia [c], Wilhelm Barthlott [d], Kai F. Müller [b,d]

[a] Department of Biology, Systematic Botany and Mycology, Ludwig-Maximilians-Universität München, Menzinger Straße 67, D-80638 Munich, Germany
[b] Institute for Evolution and Biodiversity, Westfälische Wilhelms-Universität, Hüfferstraße 1, D-48149 Münster, Germany
[c] Daniel Burnham Ct., San Francisco, CA 94109, USA
[d] Nees-Institut für Biodiversität der Pflanzen, Friedrich-Wilhelms Universität Bonn, Meckenheimer Allee 170, D-53115 Bonn, Germany

ARTICLE INFO

Article history:
Received 19 December 2009
Revised 1 March 2010
Accepted 7 March 2010
Available online 11 March 2010

Keywords:
Carnivorous plants
Genlisea
Lentibulariaceae
trnK/matK
rps16
trnQ–rps16
Phylogeny

ABSTRACT

The carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae) comprises at least 22 species distributed in South and Central America as well as in Africa (including Madagascar). It has only recently been shown to be a true carnivore, specialized in protozoa and other small soil organisms. Here we present a statistically highly supported phylogeny of *Genlisea* based on three chloroplast loci. The most recent common ancestor of *Genlisea* most likely was of Neotropical origin and characterized by pedicels that are recurved in fruit, a strongly glandular inflorescence, and bivalvate capsule dehiscence. The further evolution of various morphological characters during the diversification of the genus is discussed. The two previously suggested subgenera *Tayloria* and *Genlisea* correspond to the two major clades found in our analyses. In subgenus *Genlisea*, three clades can be clearly distinguished based on molecular and morphological characters and on biogeographic patterns, which led us to propose a new sectional classification.

1. Introduction

The genus *Genlisea* A. St.-Hil. belongs to the carnivorous plant family Lentibulariaceae (Lamiales, asterids I, APG III, 2009) and comprises at least 22 species (a few more undiagnosed taxa await their formal description (Fleischmann et al., in preparation), and unlike preceding treatments recognizing 21 species (Fischer et al., 2000; Taylor, 1991b), the authors do not consider *G. subviridis* Hutch. conspecific with *G. africana* Oliv.). *Genlisea* is distributed in South and Central America and in Africa (including Madagascar), with centers of highest species diversity in south-eastern Brazil, the Guiana Highlands and the Flora Zambesia area (Fig. 2).

Recently, the genus *Genlisea* has attended increased scientific interest, because some of its members posses the smallest nuclear genomes recorded among angiosperms, with ultrasmall holoploid genome sizes of 63 and 64 Mbp in *G. margaretae* and *G. aurea*, respectively (Greilhuber et al., 2006). Surprisingly, both the smallest and the largest nuclear genomes in Lentibulariaceae are found in *Genlisea*, resulting in about 24-fold variation of genome size in the genus (Greilhuber et al., 2006). Moreover, some chloroplast regions exhibit what is among the highest DNA substitutional rates in angiosperm chloroplasts (Müller et al., 2004). These attributes make *Genlisea* a perfect model system for studying factors governing substitutional rate and genome size shifts. The evolution of these ultrasmall genomes is focus of ongoing research (Greilhuber et al., in preparation).

1.1. Carnivory in Genlisea

All species of *Genlisea* lack roots which have been replaced by rhizophylls (Fig. 1A–C) that are derived of leaves and epiascidiate in ontogeny (Reut, 1993; Juniper, 1986; Juniper et al., 1989). The achlorophyllous subterraneous rhizophylls are Y-shaped, consisting of a vesicular bulb-like basal part and a tubular neck ending in two helically twisted arms (Fig. 1B). These trap leaves attract and capture soil protozoa (Barthlott et al., 1998; Płachno et al., 2008) and also – presumably unselectively – trap small invertebrates and algae (e.g. Darwin, 1875; Goebel, 1891; Juniper et al., 1989; Lloyd, 1942; Heslop-Harrison, 1975; Studničkа, 2003c; Płachno et al., 2005b; Plachno and Wolowski, 2008). The prey enters the rhizophylls through small openings along the apical part of the two helical arms, or through the slightly larger opening ("trap mouth"; Lloyd, 1942) at the branching point of these arms. From there it is further directed

* Corresponding author. Fax: +49 89 172 638.
E-mail address: fleischmann@lrz.uni-muenchen.de (A. Fleischmann).

1055-7903/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2010.03.009

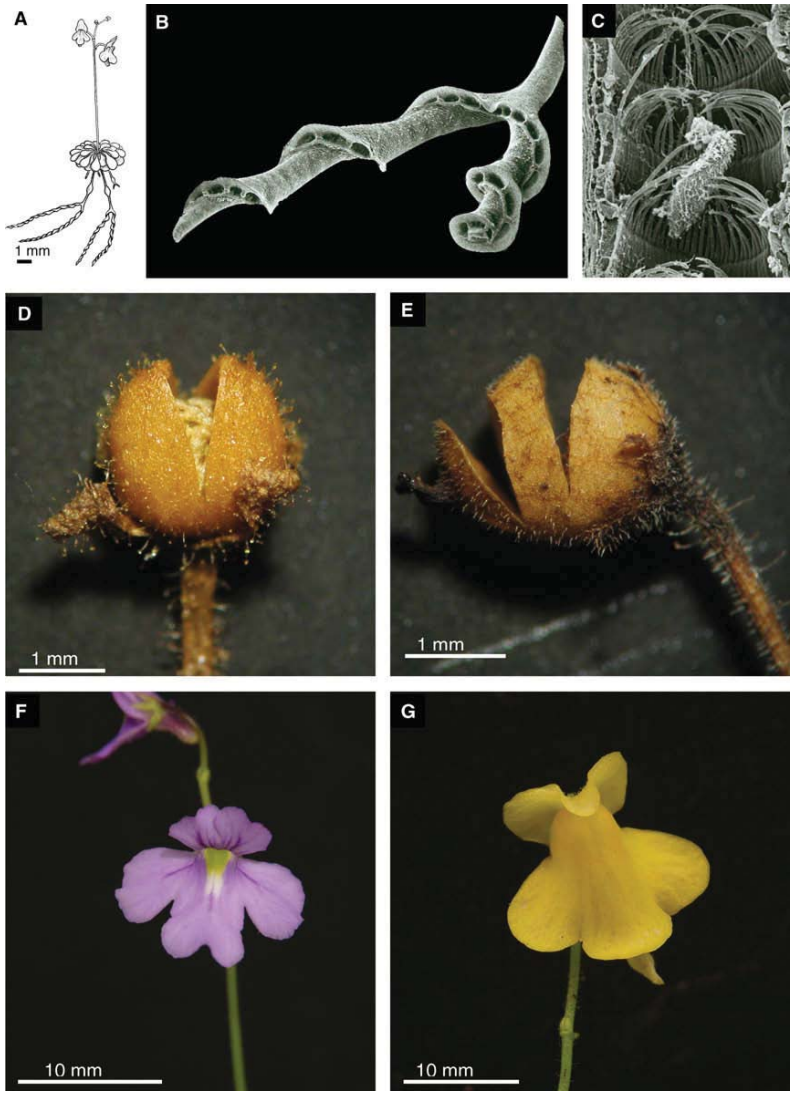


Fig. 1. (A) General habit of *Genlisea*. (B) Scanning electron micrograph of a *Genlisea* trap (*Genlisea margaretae*), showing the achlorophyllous, forked, and helically twisted rhizophyll. (C) Interior of the same trap, showing inward-pointing bristles and a trapped ciliate. (D and E) Capsule dehiscence. (D) Longitudinally bivalvate opening of subgenus *Tayloria* (shown: *Genlisea violacea*). (E) Multiple circumscissile opening of subgenus *Genlisea* (shown: *Genlisea pygmaea*). (F and G) Flowers of cultivated specimens. (F) Salveriform flower of subgenus *Tayloria* (shown: *G. violacea*). (G) Snap-dragon flower of subgenus *Genlisea* (shown: *G. aurea*).

into the tubular interior of the vesicular trap leaf by rows of retrorse 2–3 celled "detentive hairs" (Lloyd, 1942; Reut, 1993; Fig. 1C), that allow only unidirectional movement towards the distal part of the trap (comparable to an "eel trap" (Darwin, 1875) or a "lobster pot" (Heslop-Harrison, 1975)). The prey accumulates in a hollow chamber at the distal part of the trap leaf (e.g. Płachno et al., 2005b; Lloyd, 1942; Reut, 1993), where it is probably killed by anoxia inside the traps (Adamec, 2007) and digested by proteolytic enzymes secreted from internal gland hairs (Płachno et al., 2005a; Barthlott et al., 1998; Heslop-Harrison, 1975). However, the function of the trapping mechanism is still not understood in detail, and there is at least some contradictory evidence whether *Genlisea* rhizophylls may act as active (Juniper et al., 1989; Studnička, 1996, 2003a,b) or – more likely – passive traps (Płachno et al., 2008, 2005b; Adamec, 2003). Minor interspecific differences in trap morphology, especially in the shape of the detentive hairs, have been taken into consideration for species distinction by some authors (Fromm-Trinta, 1979; Reut, 1993), but were considered to be of low systematic value by others (Taylor, 1991a; Fischer et al., 2000).

1.2. General morphology and habitats

Genlisea plants are small annual or (more frequently) perennial herbs. In addition to the carnivorous leaves, the plants bear a second, non-modified leaf type that is chlorophyllous, epiterrestric, usually spathulate to lanceolate, and forms a more or less dense rosette (Fig. 1A), usually on a short rhizome in perennial species. Only *G. repens* forms a creeping elongated rhizome. The inflorescence is basically a raceme (Fig. 1A), quite often one-sided, simple or branched, and the scape can either be glabrous or hairy, with glandular or setaceous hairs. Bracts and bracteoles are lanceolate in general, and the pedicels are either glabrous, shortly hairy, or possess long- or short-stalked glandular hairs. The same types of indumentum can be found on the calyx and ovary. The zygomorphic flowers of *Genlisea* (Fig. 1A, F and G) follow the typical "snap-dragon" pattern (bearing a spur and gibbous palate) also observed in related members of the order Lamiales. The corolla is mostly yellow or violet, rarely whitish or cream, and interestingly yellow colored flowers are restricted to the South American

species. The ovary is either glabrous or covered with short eglandular or glandular hairs, and the indumentum of generative organs is used as an essential character for species classification (Taylor, 1991a; Fromm-Trinta, 1984; Fischer et al., 2000).

The carnivorous nature of *Genlisea* represents an adaptation to nutrient poor habitats, and the plants are confined to perennial or seasonally wet seepage areas and shallow soils of dripping walls over rock, marshy grasslands and swamps, quartzitic sand plains or flushes on the outcrops of inselbergs and ferricretes (Fischer et al., 2000; Fromm-Trinta, 1979; Rivadavia, 2007).

1.3. Taxonomic history and previous phylogenetic work

Two subgenera have been proposed for *Genlisea* (Fischer et al., 2000, based on the taxonomic section concept of Fromm-Trinta, 1977), which can be distinguished by the different capsule dehiscence (Fig. 1D and E). While in subgenus *Tayloria* capsules open septicidally by two (rarely four, cf. Taylor, 1989) longitudinal valves (Fig. 1D), subgenus *Genlisea* has poricidous capsules opening by an apical lid and often along additional ring-like slits below the lid, resulting in a unique multiple-circumscissile (Taylor, 1989) or sometimes even spiral dehiscence (Stopp, 1958; Fig. 1E). Further differences between both subgenera can be observed in pollen morphology (Fromm-Trinta, 1981; Taylor, 1989) and in the ultrastructure of digestive glands in the trap interior as well as in the distribution pattern of the quadrifid glands along the hollow distal part of the trap leaves (Płachno et al., 2007).

Subgenus *Tayloria* comprises three species (as well as a few undescribed or neglected taxa; Rivadavia, 2002) endemic to the highlands of south-eastern Brazil, whereas members of subgenus *Genlisea* can be found in both Africa and the Neotropics (however, not a single species occurs on both continents; Fig. 2).

Fischer et al. (2000) suggest three distinct morphological groups for the African species. These groups are distinguished based on fruiting characteristics, life history (annuality vs. perenniality) and ovary indumentum and were not assigned names or taxonomic rank.

While this classification already reflects an underlying concept of the evolution of *Genlisea*, no detailed study on phylogenetic relationships of the whole genus have been undertaken hitherto, except some preliminary phylogenetic reconstructions including few species. Both Jobson et al. (2003) and Müller et al. (2004, 2006) gave support for the monophyly of the two subgenera *Genlisea* and *Tayloria*. In addition, Müller et al. (2004) revealed that the African species of subgenus *Genlisea* are not a monophyletic group, but gave rise to the Neotropical species. Based both on morphological and molecular data, the closest allies of *Genlisea* have always been accepted to be *Pinguicula* and *Utricularia*. Molecular studies further revealed *Pinguicula* to be sister of the other two genera (Müller et al., 2004, 2006; see also Barthlott et al., 2007 for a general overview). As to the systematic position of the family, recent molecular systematic approaches stopped a long debate caused by equivocal morphological evidence (revised by Casper, 1962) and clearly placed them in the core-group of the order Lamiales in the asterid I clade of core eudicots (e.g. Müller et al., 2001, 2004, 2006; APG III, 2009). However, the sister group of Lentibulariaceae still is not known for sure and is subject of ongoing research (Schäferhoff et al., in preparation).

1.4. Aims of this study

A prerequisite for the above-mentioned studies on genome size plasticity and DNA substitution rates in *Genlisea* is a well-supported hypothesis on its organismal evolution, which is simultaneously the starting point for exemplarily analyzing morphological variation and ecological differentiation in the context of carnivory.

The availability of probably the largest living plant collection of *Genlisea* at the Bonn Botanical Gardens and the private plant collection of A.F., as well as the well preserved herbarium material collected by F.R. (SPF), enabled us to extract DNA from fresh tissue for a high percentage of the recognized species.

In this study, the chloroplast gene *matK* was sequenced (along with the adjacent non-coding regions of the *trnK* intron), as well as the intron of the ribosomal protein S16 gene *rps16* (Oxelman

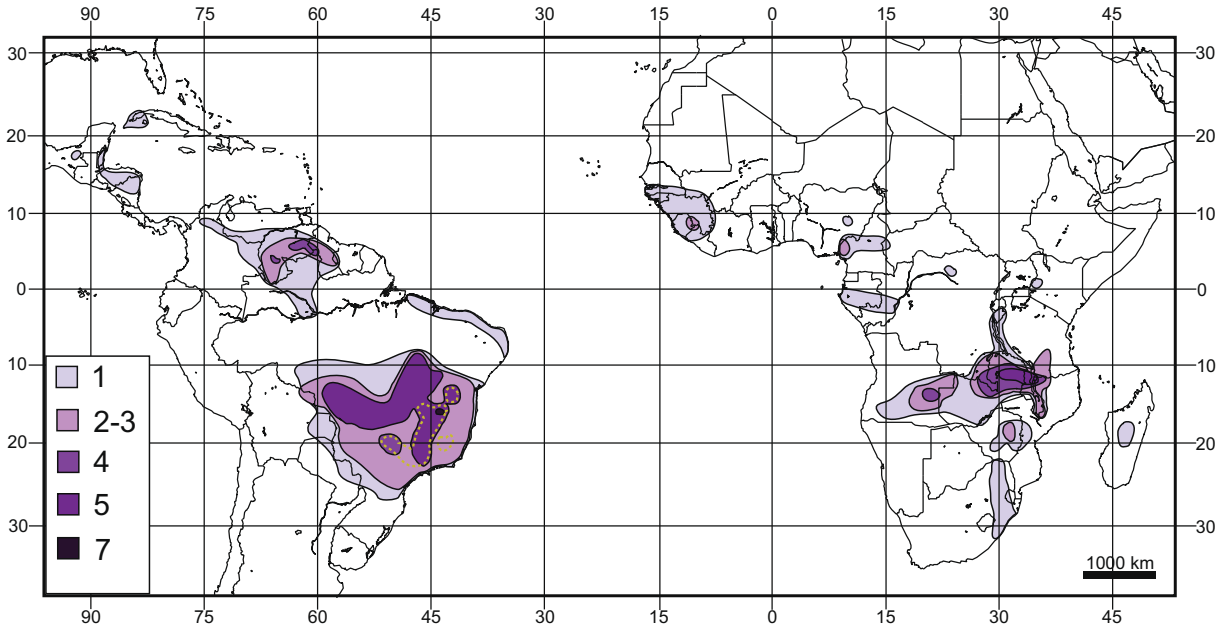


Fig. 2. Distribution of *Genlisea*, with species numbers indicated. Dashed yellow line marks the range of subgenus *Tayloria*. Data from Fischer et al. (2000), Fromm-Trinta (1978), Taylor (1967, 1991), Olvera and Martínez (2002), Rivadavia and Fleischmann (pers. obs.).

et al., 1997) and the flanking *trnQ–rps16* intergenic spacer region (Calviño and Downie, 2007), all of which had previously been demonstrated to be useful markers for phylogenetic reconstruction in crown Lamiales *viz.* Lentibulariaceae (Cieslack et al., 2005; Jobson et al., 2003; Müller and Borsch, 2005; Müller et al., 2001, 2004, unpublished data).

Based on these data, we aim to reconstruct the evolutionary history of *Genlisea* using three complementary analytic approaches and evaluate the phylogenetic significance and ancestral state shifts of selected morphological traits, life history and of biogeographic distribution patterns. Finally, we evaluate previously suggested infrageneric taxonomic groups (formal and informal ones) in the light of the phylogenetic results and suggest a new sectional classification.

2. Materials and methods

2.1. Plant samples

The data set comprises 39 accessions representing 19 out of the currently accepted 22 *Genlisea* species, plus three so far undescribed taxa, as well as three outgroup taxa (one representative of the other two genera in Lentibulariaceae, *Utricularia* L. and *Pinguicula* L., respectively, plus *Verbena* (Verbenaceae) as representative of a closely related family in Lamiales. All infrageneric taxa and distinguished groups (Fischer et al., 2000; Fromm-Trinta, 1977, 1981; Taylor 1991b) are represented. The four undiagnosed taxa (Fleischmann et al., in preparation) were collected by F.R. in Brazil and are of close affinity to *G. violacea* (*G.* aff. *violacea* 'giant'; *G.* sp. 'Itacambira'), G. repens (G.sp. 'Gran Sabana') and *G. filiformis* (*G.* aff. *filiformis*), respectively. Voucher specimens are deposited at Munich Herbarium (M), Bonn Herbarium (BONN), or the São Paulo Herbarium (SPF). Table 1 lists species names, current taxonomy, vouchers and GenBank accession numbers for the included taxa.

2.2. Amplification and sequencing

DNA was isolated from herbarium specimens and from fresh or silica-gel dried tissues from cultivated plants (preferably corollas, due to the lower risk of soil-borne contaminants (see Müller et al., 2004), occasionally green leaves), using a NucleoSpin® Plant Kit (Macherey–Nagel, Düren, Germany) following the manufacturer's protocol (Macherey-Nagel, 2007).

Purification of genomic DNA was achieved using QiaQick columns (Qiagen Inc., Valencia, California).

For PCR amplification of the *trnK* intron, 50 µl volumes were used, containing 2 µl DNA template, 10 µl dNTP mix (1.25 mM each), 2 µl of each primer (100 pmol/µl), and 0,25 µl Taq Polymerase (5 U/µl; Promega). The PCR profile was 1:30 min at 96 °C, 1 min at 50 °C, 1:30 min at 72 °C, 35 cycles of 0:30 min at 96 °C, 1 min at 50 °C, 1:30 min at 72 °C, and a final extension of 10 min at 72 °C. In most taxa, the region was amplified in two overlapping halves. Primers (MWG Biotech., Germany) used for amplification and sequencing of the *trnK* intron were trnK3914Fdi and trnK2R (Johnson and Soltis, 1995), LE4R (Müller et al., 2004), ACmatK500F (Hilu et al., 2003), as well as the specifically designed primers LE5R (5′-CAA GGT TCC TTG RCC AAC C-3′), GenliseaR (5′-TTC SCC TGA AAA TCM GTA ACC-3′), Utrnk1387F (5′-GAA ATT CCW TTT TAT CTW CGA G-3′). Primer design was semi-automated with help of SeqState (Müller, 2005a). Due to the location of the universal amplification primers, both forward and reverse primers started sequencing approx. 30 bp within the intron (relative to the sequence of *Nicotiana tabacum* L., GenBank Accession No. NC001879).

Primers and PCR protocols for amplification and sequencing of the *rps16* intron followed Oxelman et al. (1997). In addition the two internal sequencing primers rps16-234F (5′-ACC AAC TTC GTA AAT GTA TCT TAC-3′) and rps16-706R (5′-TTC ATT TCT TGA GTG GTC TT-3′) were designed to fill sequence gaps in certain accessions of *Genlisea*.

Out of the 19 primers specifically designed by Calviño and Downie (2007) for the entire *trnQ–trnk* region of certain Apiaceae taxa, the primer pair trnQ and rps161R proved to work well for PCR amplification and DNA sequencing of the *trnQ–rps16* spacer in Lentibulariaceae, as well as for several other taxa of Lamiales (Schäferhoff, in preparation; Scheunert, in preparation). The PCR profile for amplifications of the *trnQ–rps16* intergenic spacer did not follow that published in Calviño and Downie (2007), but was simplified as follows: 5 min at 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 54 °C, 2 min at 72 °C, final extension of 10 min at 72 °C.

PCR products were electrophoresed in a 1.2% agarose gel (Neeo-agarose, Roth) and bands were excised and subsequently purified using the QiaQuick gel extraction kit (Qiagen Inc., Valencia, California or the AVE-Gene gel extraction kit (AVE-Gene, Korea). For cycle sequencing, either the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq DNA polymerase FS (Applied Biosystems) was used, or the cycle sequencing reaction, cleanup and electrophoresis were performed at Macrogen Inc., Korea. GenBank references for all sequences used in this study are presented in Table 1.

Sequence pherograms and basecalls were checked using PhyDE v0.995 (Müller et al., 2005; www.phyde.de).

2.3. Sequence alignment and phylogenetic analysis

An initial multiple sequence alignment (MSA) was obtained with MAFFT (Katoh and Toh, 2008), which apparently incorporates the best-performing MSA algorithms to date (e.g. Wang et al., in press; Simmons et al., submitted for publication). Based on this, sequence alignments were improved manually using PhyDE in order to accommodate direct and inverted repeats that are ignored by currently available automated alignment tools. Sequence statistics (Table 3) were obtained via SeqState, which was also used to code indels via simple indel coding (SIC, Simmons and Ochoterena, 2000). Maximum likelihood (ML) and Maximum parsimony (MP) analyses were conducted using PAUP* v4.0b10 (Swofford, 2002).

MP analyses used the parsimony ratchet (Nixon, 1999). PRAP (Müller, 2004) was used to output command files to be executed in PAUP (Swofford, 2002), using 10 random addition cycles with 200 ratchet iterations each, in which 25% of characters receiving weight 2. For tree evaluation, 10,000 bootstrap replicates were run to minimize standard errors of bootstrap proportions. Searches for the optimal trees were kept less intense, using only one tree to swapped upon (TBR) in each replicate (Müller, 2005b). Identical settings were used for a second set of MP analyses that additionally employed information from SIC-coded indels.

For ML analyses, a fast likelihood ratchet approach (Morrison, 2007) as newly implemented in PRAP (Müller, 2004) v.2.0 was used (http://bioinfweb.info/Software/PRAP2). PRAP-generated command files that were handed over to PAUP (Swofford, 2002). Finding the ML tree ideally includes (i) rapidly getting a starting tree not too far from the optimal score; (ii) move rapidly to a (near-) optimal tree island, (iii) getting the best tree within the island (Morrison, 2007). Step (i) was achieved by calculating a BioNJ tree using LogDet distances, followed by one round of NNI and then one round of SPR branch swapping, optimizing the substitution model parameters between these steps. Similar to the ratchet originally described for parsimony, step (ii) included alternating between branch swapping on the original matrix and branch swapping on a matrix with 25% of characters upweighted. Differences to Nixon's strategy for parsimony include that SPR branch swapping was used, only ten iterations were performed, and during the weighted analyses, only one tree was saved. ApproxLim

Table 1
Taxa used in the present study. Sequences marked with * published in Müller et al. (2004), or *(1) Jobson and Albert (2002), *(2) Bremer et al. (2002), all other sequence data newly generated.

Species	voucher (country, collector, number & herbarium)	Lab code LE...	*Genlisea* subgenus	geographic distribution	*trnK*	*rps16*	*trnQ-rps16*
Genlisea africana Oliv.	Zambia, Central, Kapiri Mposhi, *F. Rivadavia & A. Fleischmann 73* (M; SPF)	268	*Genlisea*	Africa (Central)	FN641702	FN641735	FN641781
Genlisea aurea A.St.-Hil.	Brazil, Mato Grosso, Chapada dos Guimarães, *M.R.F. Cardoso 121* (SPF)	360	*Genlisea*	South America (Brazil)	FN641695	FN641743	FN641773
Genlisea aurea A.St.-Hil.	Brazil, Goiás, Chapada dos Veadeiros, *F. Rivadavia & V. Batista 2609* (SPF)	368	*Genlisea*	South America (Brazil)	FN641694	FN641744	FN641772
Genlisea aurea A.St.-Hil.	Brazil, Goiás, Cristalina, *F. Rivadavia & V. Batista 2645* (SPF)	288	*Genlisea*	South America (Brazil)	FN641714	FN641745	FN641771
Genlisea aurea A.St.-Hil.	Brazil, São Paulo, Campos do Jordão, *F. Rivadavia, M.A.K. Fontana, M. Peixoto & L. Wix 1182* (SPF)	366	*Genlisea*	South America (Brazil)	FN641693	FN641746	FN641770
Genlisea barthlottii S.Porembski, Eb.Fisch. & B.Gemmel	Sierra Leone, Northern, Makene, *A. Fleischmann s. n.* (M)	308	*Genlisea*	Africa (tropical West)	FN641704	FN641732	FN641784
Genlisea filiformis A.St.-Hil.	Brazil, Tocantins, Jalapão, *F. Rivadavia 2190* (SPF)	291	*Genlisea*	South America to Mexico	FN641691	FN641748	FN641768
Genlisea filiformis A.St.-Hil.	Brazil, Bahia, Guaibim, *F. Rivadavia 2106* (SPF)	320	*Genlisea*	South America to Mexico	FN641690	FN641749	FN641767
Genlisea aff. filiformis	Brazil, Pará, Vigia, *F. Rivadavia 2101* (SPF)	321	*Genlisea*	South America (Brazil)	FN641685	FN641755	FN641761
Genlisea aff. filiformis	Brazil, Maranhão, Barreirinhas, *F. Rivadavia 2495* (SPF)	298	*Genlisea*	South America (Brazil)	FN641684	FN641756	FM641760
Genlisea glabra P.Taylor	Venezuela, Bolívar, Churí-tepui, *s.n.* (M)	476	*Genlisea*	South America (Guianas)	FN641692	FN641747	FN641769
Genlisea glandulosissima R.E.Fr.	Zambia, Northern, Kasama, *F. Rivadavia & A. Fleischmann 30* (M; SPF)	263	*Genlisea*	Africa (Central)	FN641700	FN641738	FN641778
Genlisea glandulosissima R.E.Fr.	Zambia, Northern, Lake Chila, *F. Rivadavia & A. Fleischmann 43* (M; SPF)	265	*Genlisea*	Africa (Central)	FN641699	FN641739	FM641777
Genlisea guianensis N.E.Br.	Venezuela, Bolívar, Ucaima, *J. Bogner 1067* (M)	293	*Genlisea*	South America (Brazil + Guianas)	FN641697	FN641741	FN641775
Genlisea guianensis N.E.Br.	Brazil, Goiás, Cristalina, *F. Rivadavia 2655* (SPF)	292	*Genlisea*	South America (Brazil + Guianas)	FN641696	FN641742	FN641774
Genlisea hispidula Stapf	South Africa, cultivated BGM (M)	294	*Genlisea*	Africa (South and Central)	*AF531815	FN641731	FN641785
Genlisea hispidula Stapf	South Africa, Gauteng, Witbank, *F. Rivadavia & A. Fleischmann N4* (M; SPF)	305	*Genlisea*	Africa (South and Central)	FN641705	FN641730	FN641786
Genlisea lobata E.Fromm-Trinta	Brazil, Minas Gerais, Serra da Araponga, *F. Rivadavia, F. Pinheiro, L.S. Leoni & J. Mullins 517* (SPF)	296	*Tayloria*	South America (Brazil)	FN641711	FN641723	FN641793
Genlisea margaretae Hutch.	Zambia, Luapula, Mansa, *F. Rivadavia & A. Fleischmann 17* (M; SPF)	260	*Genlisea*	Africa (Central) + Madagascar	FN641701	FN641737	FN641779
Genlisea margaretae Hutch.	Madagascar, BG Bonn 11400 (BONN)	309	*Genlisea*	Africa (Central) + Madagascar	*AF531816	FN641736	FN641780
Genlisea pygmaea A.St.-Hil.	Brazil, Tocantins, Jalapão, *F. Rivadavia 2264* (SPF)	276	*Genlisea*	South America	FN641686	FN641754	FN641762
Genlisea repens Benj.	Venezuela, Amazonas, Cerro Neblina, *F. Rivadavia, GertJH & S. McPherson 1876* (SPF)	322	*Genlisea*	South America	FN641689	FN641751	FN641765
Genlisea repens Benj.	Brazil, Minas Gerais, Uberaba, *P.M. Gonella, F. Rivadavia, N. Silva & R. Palis 10* (SPF)	371	*Genlisea*	South America (Brazil)	FN641687	FN641753	FN641763
Genlisea roraimensis N.E.Br.	Venezuela, Bolívar, Kukenán-tepui, *s.n.* (M)	314	*Genlisea*	South America (Venezuela)	*AF531817	FN641750	FN641766
Genlisea sanariapoana Steyerm.	Venezuela, Amazonas, Pto. Ayacucho, *A. Gröger & S. Llamozas 1118* (M)	301	*Genlisea*	South America (Guianas)	FN641698	FN641740	FN641776
Genlisea sp. 'Gran Sabana'	Venezuela, Bolívar, Gran Sabana, *F. Rivadavia 2567* (SPF)	283	*Genlisea*	South America (Venzuela)	FN641688	FN641752	FN641764
Genlisea stapfii A.Chev.	Sierra Leone, Northern, Kabala, *H. Schäfer s.n.* (M)	306	*Genlisea*	Africa (tropical West)	*AF531818	FN641733	FN641783
Genlisea subglabra Stapf	Zambia, Northen, Kasama, *F. Rivadavia & A. Fleischmann 53* (M; SPF)	266	*Genlisea*	Africa (Central)	FN641706	FN641729	FN641787
Genlisea subviridis Hutch.	Zambia, Central, Kapiri Mposhi, *F. Rivadavia & A. Fleischmann 80* (M; SPF)	269	*Genlisea*	Africa (Central)	FN641703	FN641734	FN641782
Genlisea uncinata P.Taylor & E.Fromm-Trinta	Brazil, Bahia, Mucugê, *F. Rivadavia 471* (SPF)	367	*Tayloria*	South America (Brazil)	*AF531819	FN641718	FN641798
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Milho Verde, *F. Rivadavia 2560* (SPF)	304	*Tayloria*	South America (Brazil)	FN641716	FN641728	FN641788
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Diamantina, *F. Rivadavia 2551* (SPF)	282	*Tayloria*	South America (Brazil)	FN641707	FN641726	FN641790
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Serra do Caraça, *F. Rivadavia 110* (SPF)	302	*Tayloria*	South America (Brazil)	FN641709	FN641724	FN641792
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Couto de Magalhães de Minas, *F. Rivadavia 2532* (SPF)	303	*Tayloria*	South America (Brazil)	FN641715	FN641727	FN641789
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Serra do Cipó, *F. Rivadavia 1947* (SPF)	312	*Tayloria*	South America (Brazil)	FN641708	FN641725	FN641791
Genlisea violacea A.St.-Hil.	Brazil, Minas Gerais, Serra de Ibitipoca, *F. Rivadavia 1952* (SPF)	376	*Tayloria*	South America (Brazil)	FN641710	FN641722	FN641794
Genlisea aff. violacea 'giant'	Brazil, Minas Gerais, Itacambira, *F. Rivadavia & R. Gibson 1365* (SPF)	295	*Tayloria*	South America (Brazil)	FN641717	FN641720	FN641796
Genlisea aff. violacea 'giant'	Brazil, Minas Gerais, Grão Mogol, *F. Rivadavia 277* (SPF)	364	*Tayloria*	South America (Brazil)	FN641713	FN641719	FN641797
Genlisea sp. 'Itacambira'	Brazil, Minas Gerais, Itacambira, *F. Rivadavia & F. Pinheiro 1139* (SPF)	365	*Tayloria*	South America (Brazil)	FN641712	FN641721	FN641795
Pinguicula alpina L.	*L. Legendre s.n.* (BONN)	023	-	Europe, Asia	*AF531783	*(1)AF482544	FN641759
Utricularia multifida R.Br.	Australia, Western Australia, near Walepole, cult. *A. Fleischmann s.n.*	256	-	Western Australia	*AF531848	*(1)AF482583	FN641758
Verbena rigida Spreng.	cult. BG Bonn 4147, *K. Müller 742* (BONN)	073	-	Europe	*AF531820	*(2)AJ431065	FN641760

Table 2
Morphological character matrix. Characters and their states as follows (referred to in Fig. 4): 1 capsule dehiscence: 0 bivalvate, 1 circumscissile. 2 capsule position in fruit: 0 reflexed or recurved, 1 upright. 3 life history: 0 perennial, 1 annual. 4 indumentum of the scape: 0 glandular (in combination with eglandular hairs in *G. pygmaea*, *G.* aff. *filiformis* and *G. filiformis*), 1 glabrous. 5 indumentum of the capsule: 0 glandular, 1 glabrous (few eglandular hairs in *G. hispidula*). 6 corolla color: 0 violet (rarely white), 1 yellow. 7 corolla upper lip: 0 entire, 1 bilobate. 8 rosette: 0 lax, few leaves, 1 dense, many leaves. 9 leave shape: 0 spathulate, 1 linear to lanceolate. 10 biogeography: 0 America, 1 Africa. If both states occur in one taxon, bracts unite them.

Species	Character number									
	1	2	3	4	5	6	7	8	9	10
G. subviridis	1	1	1	0	0	0	0	0	0	1
G. africana	1	1	[01]	0	0	0	0	0	0	1
G. barthlottii	1	1	1	0	0	0	0	0	0	1
G. stapfii	1	1	1	0	0	0	0	0	0	1
G. margareatae	1	0	0	0	0	0	0	1	0	1
G. glandulossisima	1	0	0	0	0	0	0	1	0	1
G. hispidula	1	1	0	1	1	0	0	0	0	1
G. subglabra	1	1	0	1	1	0	0	0	0	1
G. aurea	1	1	0	0	0	1	0	1	0	0
G. pygmaea	1	1	[01]	0	0	1	0	1	0	0
G. aff. filiformis	1	1	1	0	0	1	0	1	0	0
G. filiformis	1	1	1	0	0	1	0	1	0	0
G. repens	1	1	0	1	1	1	0	1	0	0
G. sp. 'Gran Sabana'	1	1	0	1	1	1	0	1	0	0
G. roraimensis	1	1	0	0	1	1	0	1	0	0
G. glabra	1	1	0	1	1	0	0	1	0	0
G. guianensis	1	1	0	1	0	0	0	1	1	0
G. sanariapoana	1	1	0	0	0	0	0	1	0	0
G. uncinata	0	0	0	0	0	0	0	0	0	0
G. sp. 'Itacambira'	0	0	1	0	0	0	1	0	0	0
G. violacea, type'	0	0	1	0	0	0	1	0	0	0
G. aff. *violacea* 'giant'	0	0	0	0	0	0	1	0	0	0
G. violacea 'short spur'	0	0	1	0	0	0	1	0	0	0
G. lobata	0	0	1	0	0	0	1	0	0	0
Utricularia multifida	0	n.a.	1	1	1	0	1	1	0	n.a.
Pinguicula alpina	0	n.a.	0	0	0	0	1	0	0	n.a.

was set to 2 and not adjusted. This strategy was found to be more successful in converging to the ML tree than the strategies implemented in other ML inference packages (Morrison, 2007). Using jModelTest (Posada, 2008) and the AIC, the best model was found to be GTR+G. To assess confidence in clades, bootstrapping was performed by executing PRAP-generated command files in PAUP. Using optimized parameter values and the maximum likelihood topology from the likelihood ratchet search, SPR branch swapping was performed for each bootstrapped data matrix, and the proportion of iterations in which a given clade was recovered was mapped onto the maximum likelihood tree with TreeGraph v.2.0 (see below).

Bayesian analyses were conducted in MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003), again using the GTR+Γ model. Default priors were used, i.e. flat dirichlets (1.0, . . ., 1.0) for state frequencies and instantaneous substitution rates, a uniform prior (0.0, 50.0) for the shape parameter of the gamma distribution, a uniform prior (0.0, 1.0) for the proportion of invariable sites, a uniform topological prior, an exponential prior Exp(10.0) for branch lengths. Four categories were used to approximate the gamma distribution. Two runs with 2 million generations each were run, and four chains were run in parallel for each run, with the temperature set to 0.2. The chains were sampled every 100th generation. To check for convergence of the independent runs under a given model, it was made sure that (i) plots of both runs indicate that stationary phase was reached in both runs, (ii) potential scale reduction factor approached 1 for all parameters; (iii) no supported conflicting nodes were found among the consensus trees generated from each run.

Statistical support from congruent or conflicting nodes found with the different analytic approaches was merged onto one tree and visualized with help of TreeGraph v.2.0 (Stöver and Müller, 2010; http://treegraph.bioinfweb.info).

2.4. Non-sequence data and reconstruction of ancestral states

Morphological characteristics and dimensions, as well as information on geographical distribution and ecological habit were either based on personal observation of plants in natural habitats in Brazil, Venezuela, tropical West and Central Africa and from greenhouse-grown plants (A.F. and F.R.), or taken from herbarium specimens or taxonomic literature (Fischer et al., 2000; Fromm-Trinta, 1977, 1981, 1984; Rivadavia, 2000, 2002, 2007; Taylor, 1967; Taylor and Fromm-Trinta, 1983). The morphological and biogeographical data were coded as binary characters (Table 2).

Anatomical and morphological characters of the rhizophylls of most species represented in the taxon sampling were obtained from literature (Fromm-Trinta, 1979; Lloyd, 1942; Płachno et al., 2008; Reut, 1993) and SEM photographs (Płachno, unpublished data) and mapped on the tree topology *a posteriori* to infer the phylogenetic significance of this character, as discussed by various authors (Fischer et al., 2000; Fromm-Trinta, 1979; Płachno et al., 2007; Reut, 1993; Taylor, 1991a). Information on pollen morphology was inferred from literature (Fromm-Trinta, 1979, 1981; Taylor, 1989) and a palynological survey on all *Genlisea* species sampled for this study (Fleischmann, unpublished data).

The reconstruction of ancestral states was done with Mesquite v2.71 (Maddison and Maddison, 2009), using the trace all characters option. Parsimony ancestral state optimization was chosen throughout all analyses.

3. Results

Basic sequence and alignment statistics are provided in Table 3. Phylogenetic signal in the three sequenced plastid regions is highly congruent, as statistically well-supported contradictions among the individual tree topologies could not be observed.

Table 3
Sequence statistics for the markers used. Characters = number of characters in the alignment matrix); Length range = actual sequence length in nucleotides (including hotspots; minimal and maximal value observed); SD = standard deviation of mean length; % divergence (range) = pairwise sequence distance in percent (uncorrected *p* distance, overall mean, lowest and highest scores in brackets); ti/tv ratio (transition/transversion ratio based on *p* distances; range in brackets); % variable = percentage of variable positions; % informative = percentage of parsimony informative positions; % GC = GC content.

Marker	Characters	Length range[a]	Mean[a]	SD[a]	% Divergence	SE	(Range)	ti/tv	SE	(Range)
Combined dataset	4184	2628–4196	3719.36	323.818	9.161	0.289	(0.282–22.096)	0.658	0.038	(0.053–1.429)
trnQ	1062	279–857	576.692	91.941	11.600	0.554	(0.286–27.434)	0.646	0.078	(0.000–2.000)
rps16	511	789–851	814.282	14.523	7.663	0.730	(0.000–15.927)	0.690	0.121	(0.000–3.000)
trnK/matK	2611	1261–2503	2336.72	306.887	8.655	0.298	(0.147–27.778)	0.703	0.053	(0.000–3.143)

Marker	% Variable	% Informative	% GC[a]	% A[a]	% C[a]	% G[a]	% T[a]
Combined dataset	37.643	21.917	32.829	32.724	15.518	17.311	34.447
trnQ	36.768	22.444	27.166	35.917	14.259	12.907	36.917
rps16	32.485	18.200	34.602	33.310	16.107	18.494	32.088
trnK/matK	42.279	22.411	33.650	31.742	15.688	17.962	34.609

[a] Calculated with hotspots included (statistics for which correct alignment not needed).

The tree based on a dataset that combines all three markers is shown in Fig. 3, along with bootstrap support values from ML and MP analyses (LB and PB, respectively) and posterior probabilities from Bayesian analyses (PP). When information from SIC-coded indels was used in the MP analyses, an identical topology was found and bootstrap values were virtually identical. The monophyly of *Genlisea* is supported by all analyses (100 LB, 100 PB, 1.00 PP; Fig. 3), with *Utricularia* as sister genus (100 LB, 100 PB, 1.00 PP), confirming the results of previous phylogenetic studies (Jobson et al., 2003; Müller et al., 2004, 2006).

Within *Genlisea*, two well-supported (100 LB, 100 PB, 1.00 PP) sister clades are revealed, which agree with the two subgenera proposed for the genus based on morphological characters (Fischer et al., 2000; Fromm-Trinta, 1977). First-branching in the Brazilian-endemic *Tayloria*-clade (100 LB, 100 PB, 1.00 PP) is the large perennial *Genlisea uncinata* Taylor and Fromm-Trinta from the highlands of Bahia state in north-eastern Brazil, followed by the so far undescribed perennial taxon *G.* aff. *violacea* 'giant' from central Minas Gerais state (100 LB, 100 PB, 1.00 PP). Sister to this species is an unresolved polytomy (92 LB, 93 PB, 1.00 PP) consisting of the annual *G. violacea* A. St.-Hil. from the type collection area in the coastal mountains of southern Minas Gerais, the undescribed annual *G.* sp. 'Itacambira' from central Minas Gerais, and a clade (100 LB, 99 PB, 1.00 PP) that consists of the sister pair (93 LB, 95 PB, 1.00 PP) of the annual *G. lobata* E. Fromm-Trinta from eastern Minas Gerais bordering Espírito Santo state, and a clade (87 LB, 82 PB, 1.00 PP) made up of

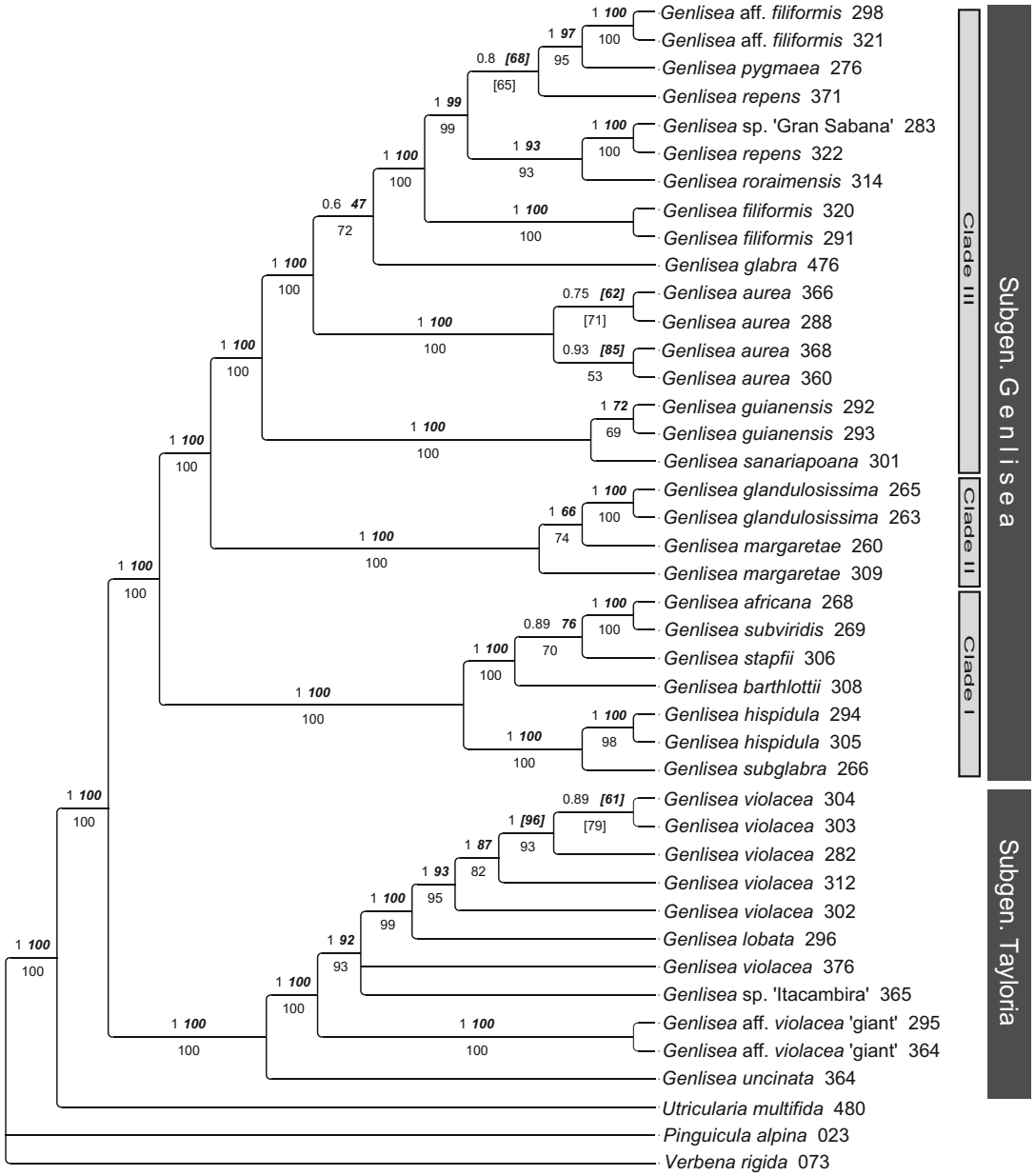


Fig. 3. Majority-rule-consensus tree from the Bayesian MCMC-analysis of combined *trnK*, *rps16* and *trnQ–rps16* datasets. Numbers above branches show Bayesian posterior probabilities (left), maximum likelihood bootstrap proportions (right); numbers below branches are maximum parsimony bootstrap proportions values. Numbers in brackets indicate that the respective node was not supported by all three methodological approaches. The bracketed number then indicates the strongest support found for any node that contradicts the shown node (cf. Stöver and Müller, 2010).

collections of smaller forms of *G. violacea* with narrow cylindrical spur from northern Minas Gerais. Thus *G. violacea* is revealed as paraphyletic assemblage here.

Subgenus *Genlisea* consists of three well-supported (100 LB, 100 PB, 1.00 PP) subclades (Fig. 3), named "clade I–III" here to facilitate the following discussion. Clade I exclusively comprises African species: the perennial sister pair (100 LB, 100 PB, 1.00 PP) *G. hispidula* Stapf and *G. subglabra* Stapf is sister (100 LB, 100 PB, 1.00 PP) to the annual species *G. africana* Oliv., *G. barthlottii* S. Porembski, Eb. Fisch. and B. Gemmel, *G. stapfii* A. Chev. and *G. subviridis* Hutch. The African clade I is sister to a clade (100 LB, 100 PB, 1.00 PP) containing African and South American species arranged in two statistically highly supported monophyletic groups, here referred to as clades II and III, respectively (both 100 LB, 100 PB, 1.00 PP). First-branching is clade II, comprising the two African species *G. margaretae* Hutch. and *G. glandulosissima* R.E. Fr.; however, the sampled individuals from *G. margaretae* are reconstructed as paraphyletic, with the accession from Madagascar being sister to a pair of central tropical African *G. margaretae* and *G. glandulosissima* (66 LB, 74 PB, 1.00 PP). The perennial violet-flowered African species *G. margaretae* and *G. glandulosissima* are sister to a monophyletic group (clade III) of exclusively South American species.

The first-branching sister pair (100 LB, 100 PB, 1.00 PP) of the two large perennial violet-flowered species *G. guianensis* N.E. Br. and *G. sanariapoana* Steyerm. is revealed as sister to all yellow-flowered species of *Genlisea* (100 LB, 100 PB, 1.00 PP). Only the Venezuelan *G. glabra* P. Taylor that has a pale lilac corolla falls within the yellow-flowered species, however with weak support (47 LB, 72 PB, 0.6 PP). It is sister to a clade (100 LB, 100 PB, 1.00 PP) comprising species that occur both north and south of the Amazon (*G. filiformis* A. St.-Hil., *G. pygmaea* A. St.-Hil., *G. repens* Benj., and the undiagnosed *G.* aff. *filiformis*) as well as the Venezuelan endemic *G. roraimensis* N.E. Br. and the so far undiagnosed taxon *G.* sp. 'Gran Sabana'. "*Genlisea repens*" turned out to be a polyphyletic assemblage, with the "typical" rhizome forming plants from Brazil branching basal (weak-supported; 0.8 PP) from a clade containing the sister pair (97 LB, 95 PB, 1.00 PP) *G.* aff. *filiformis* and *G. pygmaea*. In contrast, the rosette-forming Venezuelan collection of *G. repens* fell within a clade of the Venezuelan *G. roraimensis* and *G.* sp. 'Gran Sabana' (93 LB, 93 PB, 1.00 PP). Basally branching from the "G. glabra-yellow species" sister clade is the Brazilian-endemic *G. aurea* A. St.-Hil. (100 LB, 100 PB, 1.00 PP), which is quite variable morphologically regarding general habit and corolla size. Indeed the accessions sampled are retrieved as two distinct clades, although weakly supported by Bayesian analyses only (0.75 PP and 0.93 PP, respectively).

Phylogenetic reconstructions revealed no notable differences in branch lengths between species of *Genlisea* (Fig. 5), indicating a more or less constant evolution rate in the genus and the absence of lineage effects or rate differences correlated with life history. Preliminary data also suggest that species with a comparatively large nuclear genome (e.g. *G. hispidula*; Greilhuber et al., 2006) do not seem to have measurably different substitution rates (as approximated via branch lengths) compared to species with ultrasmall genomes (e.g. *G. aurea*; Greilhuber et al., 2006). The evolution of genome size in Lentibulariaceae and potential correlates is the subject of parallel research to be published elsewhere (Greilhuber et al., in preparation).

4. Discussion

4.1. Subgenus Tayloria

Members of subgenus *Tayloria* constitute the smaller out of the two major clades reconstructed in *Genlisea* (Fig. 3). All its members are characterized by pedicels that are recurved or reflexed in fruit (Fig. 4), and by strongly glandular inflorescence parts. The bivalvate capsule dehiscence (Fig. 1D) is similar to that of species in the first-branching lineage of Lentibulariaceae, *Pinguicula* (Casper, 1966). Therefore, *Tayloria* can be assumed to represent the "basal lineage" within *Genlisea*, in the sense of exhibiting plesiomorphic states for at least some characters and therefore more or less representing the assumed suite of character states found in the immediate common ancestor of contemporary *Genlisea*.

The first-branching *G. uncinata* is a distinct and comparatively large perennial species inhabiting a small remote highland area in Bahia state of north-eastern Brazil (Rivadavia, 2000; Taylor 1991a; Taylor and Fromm-Trinta, 1983). It differs from all other *Genlisea* species in its unique papillose seed morphology (Taylor, 1991a), and from the other members of subgenus *Tayloria* in the large and robust habit, in a distinctive flower morphology (the upper lip of *G. uncinata* is not bilobate as observed in all other species of *Tayloria* (Figs. 1F and 4), the spur is distinctively hooked; Fig. 3), and a thick and very glandular inflorescence with strongly recurved pedicels in fruit (i.e. the pedicel supporting the capsule is curved inwards; Fig. 4). The entire upper lip is a character shared with members of subgenus *Genlisea* (Figs. 1G and 4), and lends morphological support to the basal position of *G. uncinata* found with molecular data. The morphological distinctness of *G. uncinata* may be explained by its status as a relict lineage that survived in a remote area. The sister group of *G. uncinata* is characterized by a bilobate upper corolla lip (Fig. 4) and pedicels that are just slightly recurved or reflexed in fruit. Except for the first-branching *G.* aff. *violacea* 'giant', which represents an as yet undescribed but distinctive, large perennial taxon (Fleischmann et al., in preparation), all remaining species of subgenus *Tayloria* are annuals (Figs. 4 and 5). However, annuality in *Tayloria* seems to be induced by environmental conditions of their natural habitats (Rivadavia, 2002, 2007), as all of them can grow as polycarpic facultative perennials in cultivation (Rivadavia and Fleischmann, pers. obs.). *G. violacea* is retrieved as paraphyletic with *G. lobata* nested within. However, morphological diversity observed between different populations of *G. violacea* indicates that this aggregate obviously represents more than one species, resulting in several species that could be separated based on flower characteristics (Benjamin, 1847; Sylvén, 1908). The type population of *G. violacea* from Ibitipoca, Minas Gerais state (Saint-Hilaire, 1833), as represented by sample 376 in the present study, is characterized by a rather large spur that is widened towards the tip. In contrast, all other accessions of *G. violacea* sampled in this study display a short cylindrical spur. They perfectly match *G. cylindrica* Sylvén, a species previously separated from *G. violacea* based on floral characters (Sylvén, 1908), but later regarded as conspecific with the latter (Fromm-Trinta, 1979; Taylor 1991b). Interestingly, both *G. lobata* and *G.* sp. 'Itacambira' exhibit a narrow tubular spur, separating them from the earlier branching *G.* aff. *violacea* 'giant'. Variation in spur morphology within subgenus *Tayloria* could be explained by adaptation to different pollinators, as quite often several taxa of the subgenus are found growing sympatrically (Rivadavia, pers. obs.), and frequently associated with species of subgenus *Genlisea*.

4.2. African species of subgenus Genlisea

The distinction of three groups among the African species (group I–III), as already suggested by Fischer et al. (2000) based on inflorescence indumentum and the arrangement of pedicel and capsule in fruit, is supported here. However, the authors' division of the African species is not reflected by three subclades of a large "African clade". Instead, the African species of *Genlisea* are paraphyletic, consisting of an exclusively African clade (group II + III, our "clade I") that is sister to a clade of African species

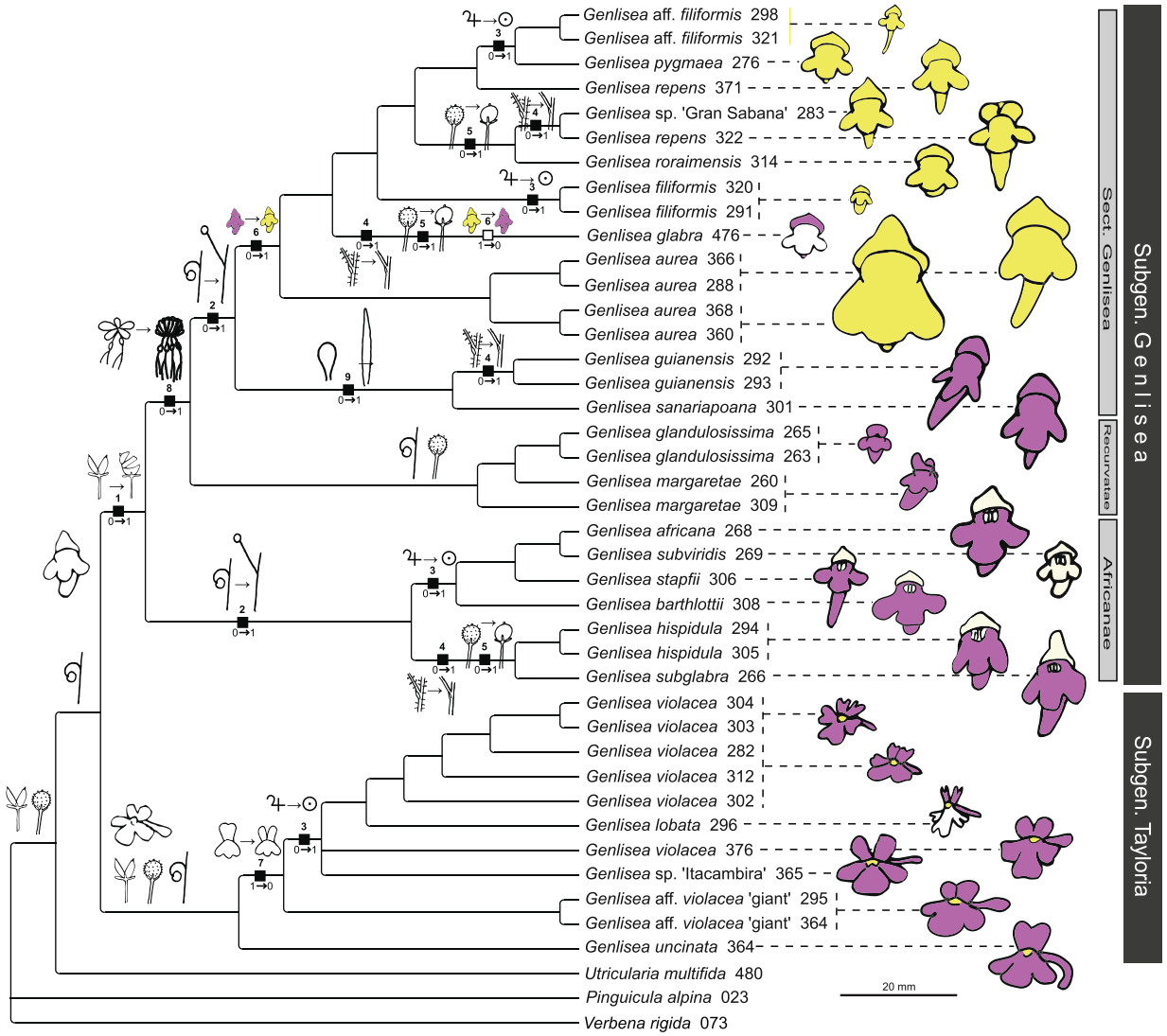


Fig. 4. Evolution of selected characters in *Genlisea*. Transitions appear as filled boxes on the branches, white boxes indicate reversals. Characters and character states follow Table 2, character numbers shown above boxes, state transitions below. Implied plesiomorphic states coded as 0, implied apomorphic states as 1. Small diagrammatic sketches illustrate character states above the respective boxes.

(group I), which in turn is sister to a large group of South American species.

Clade I exclusively comprises species with seed capsules held upright in fruit (as the pedicels do not reflex after anthesis, Fig. 4). It contains two highly supported subclades that differ in life history and inflorescence indumentum (Figs. 4 and 5). The two perennial species *G. hispidula* and *G. subglabra*, both without glandular hairs on inflorescence or ovary ("group III" sensu Fischer et al., 2000), are sister to a group of annual species with at least partially glandular scapes and ovaries (*G. barthlottii*, *G. stapfii*, *G. subviridis* and *G. africana*; the recently described *G. taylorii* from tropical eastern Angola (Fischer et al., 2000), which could not be included for this study, is expected to belong to this clade, too). Interestingly, *G. barthlottii*, which shows a tendency to perennial growth in suitable habitats and in cultivation (Fleischmann, pers. obs.), is indicated in a basal position sister to the obligate annual (monocarpic) African species.

Fischer et al. (2000) assigned *G. angolensis* (material could not be obtained for the present study) to this group of annual glandular species, due to the glandular ovaries and pedicels that are not reflexed in fruit. However, the life form of this semi-aquatic species is not known with certainty, and at least vegetative parts are reminiscent of large perennial species like the aquatic Neotropical *G. guianensis*. Based on observations made on the few available herbarium specimens of this species, *G. angolensis* could represent a link between the glabrous perennial African species and the annual glandular species. Obtaining fresh material of *G. angolensis* for successful sequencing is therefore a priority for our ongoing work in the genus.

Genlisea subviridis, which was put into synonymy with *G. africana* (Fischer et al., 2000; Taylor, 1991b), is considered as a distinct species by the authors of the present study, since it differs from *G. africana* in flower and capsule morphology (for a taxonomical revision of the genus: Fleischmann et al., in preparation). Moreover, both species seem to be separated well ecologically, too (Fleischmann and Rivadavia, pers. obs., 2006). All members of clade I share large to medium sized flowers (8–10–18 mm long), with a violet, mauve or bluish, rarely whitish corolla lower lip that bears a dis-

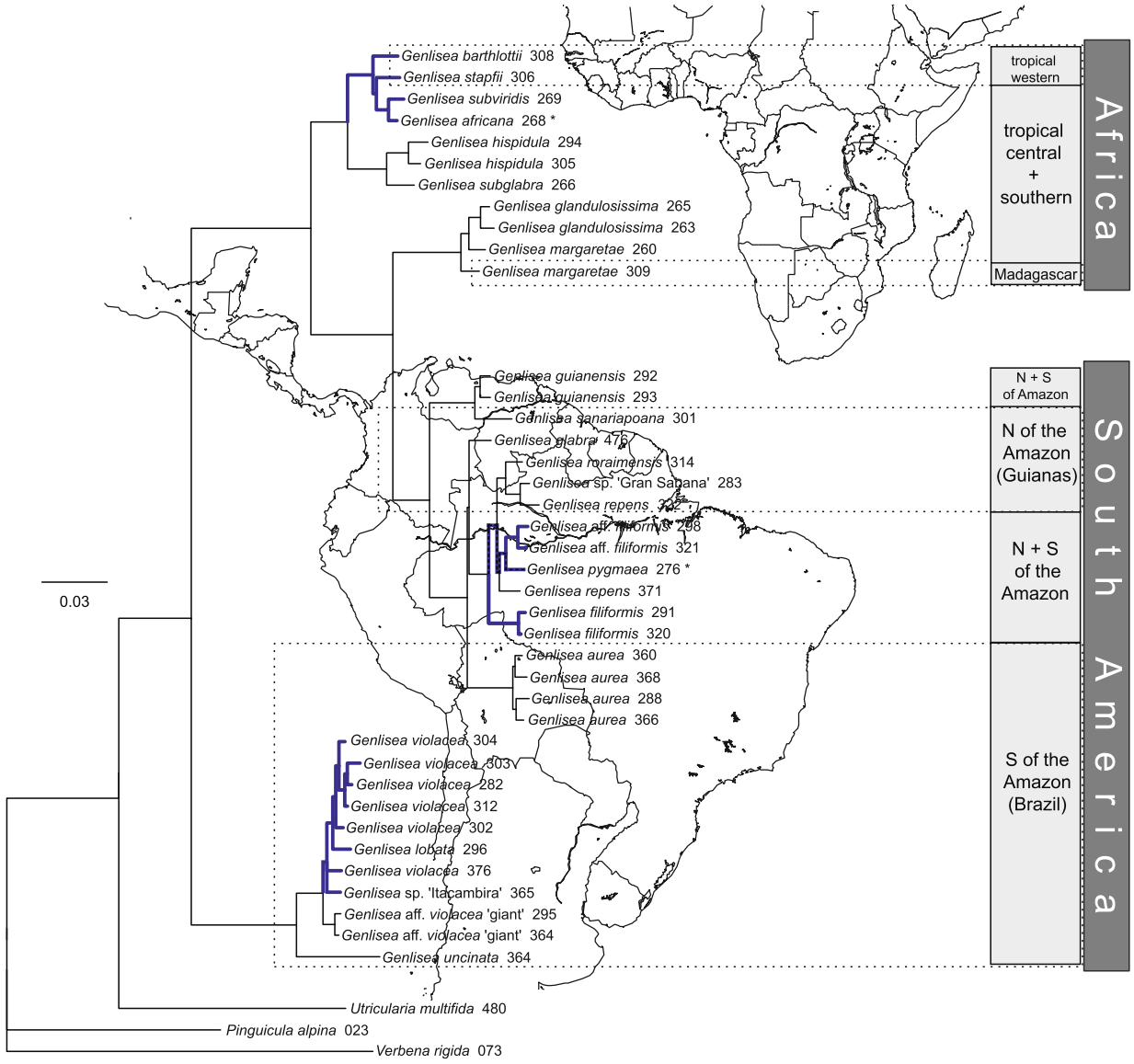


Fig. 5. Bayesian phylogram of *Genlisea* based on the combined *trnK*, *rps16* and *trnQ–rps16* datasets, with *Utricularia* and *Pinguicula* (Lentibulariaceae) and *Verbena* (Verbenaceae) as outgroup, showing biogeographical distribution and life history of *Genlisea*. Highlighted branches: annual life history; species that occur both as annuals or perennials naturally by ∗. Branch lengths follow scale given.

tinct pale white mark on the palate with conspicuous dark purple reticulate veins (Fig. 4). In contrast, the lower lip usually is paler or white. All other members of subgenus *Genlisea* have a uniformly colored corolla.

The African species in clade II ("group I" *sensu* Fischer et al., 2000; *G. margaretae* and *G. glandulosissima*) are perennials characterized by glandular ovaries and capsules, and by pedicels that are strongly reflexed in fruit (Figs. 4 and 5). *Genlisea pallida*, which could not be included in the present study due to lack of suitable material, is likely to group with this clade as well, as already proposed by Fischer et al. (2000). Furthermore, all members of this group can be distinguished from African members of clade I by their overall smaller flowers (6–10 mm long), and by a corolla uniform in color (except for some darker veins on the exterior of the tube in some specimens), but always without a distinct pale marking on the palate with dark reticulate venation.

The paraphyly of *G. margaretae* in the present study might be explained by the fact that the basal branching accession originates from a disjunct population of *G. margaretae* in central Madagascar. This Madagascan plant had been treated as a distinct species, *G. recurva*, by Bosser (1958). It is, however, identical to *G. margaretae* in overall morphology and is therefore regarded as synonym of the latter (Taylor, 1991b; Fischer et al., 2000). The Madagascan *G. margaretae* is sister to *G. margaretae* from Central Africa plus the Zambian endemic *G. glandulosissima*, two species which frequently can be found growing sympatrically, and natural hybrids between those two species occur occasionally (Hutchinson, 1946; Fischer et al., 2000; and Fleischmann and Rivadavia, pers. obs.). The hybrid of *G. margaretae* and *G. glandulosissima* was already mentioned by Hutchinson (1946) from the common *locus classicus* of both parent species in northern Zambia, and this is also is the locality where the respective material used for the present study was collected. Therefore it is possible that *G. margaretae* from some Central African populations (including the type location) displays a certain degree of introgression with *G. glandulosissima* (and therefore clusters as

sister to that species), whereas the plastid genome of the isolated populations of *G. margaretae* on the island of Madagascar had not been influenced by past hybridization events, and thus still posses a more ancestral plastome. Further phylogenetic reconstruction using nuclear markers as well as haplotype networks may help to reveal the role of reticulate evolution in *Genlisea*.

4.3. South American species of subgenus Genlisea

Within the South American clade of subgenus *Genlisea*, a purple flower color represents the plesiomorphic character state (found in the large perennial species *G. guianensis*, *G. sanariapoana*, which are sister to all other South American species having yellow flowers, as well as in the African ancestors; Fig. 3). Thus a yellow corolla color evolved only once within the genus; they are synapomorphic for the clade comprising *G. aurea*, *G. pygmaea*, *G. repens*, *G. filiformis*, *G.* aff. *filiformis*, *G. roraimensis* and *G.* sp. 'Gran Sabana' (Figs. 3 and 4). However, a violet flower color has evolved a second time independently in *G. glabra* of isolated high tepui summits of central Venezuela (Figs. 3 and 4).

G. aurea is split into two clades (Fig. 3), the members of both show minor morphological differences. Typical *G. aurea* as circumscribed by Saint-Hilaire (1833) is represented by the accessions from Chapada dos Veadeiros, Goiás state (Accession 368). These are robust plants with thick scapes and the largest flowers in the genus, up to 3 cm in length and over 2 cm in width (Rivadavia, 2007). Interestingly, these are the samples of *G. aurea* for which ultrasmall genomes have been reported (Greilhuber et al., 2006), whereas other accessions of *G. aurea* exhibit larger *C*-values, at least twice the size of the ones published (Greilhuber, unpublished data). The latter clade belong to plants that are more slender in general appearance, have thinner but taller scapes and smaller flowers with a spur that is distinctly longer than the corolla lower lip. These have been described as *G. minor* A. St.-Hil. by the generic author (Saint-Hilaire, 1833), or assigned to the name *G. ornata* var. *gracilis* by Merl (Luetzelburg, 1923), however, have been considered conspecific with *G. aurea* in subsequent treatments (Fromm-Trinta, 1979; Taylor, 1991b). Indeed, both taxa are separated by few characters only, and the more slender *G. minor* of lower altitudes had been discussed as mere ecotype of the more robust appearance of montane *G. aurea* that is induced by altitudinal conditions (Saint-Hilaire, 1833). However, the morphological differences between both taxa seem to remain stable both in wild populations (Rivadavia, pers. obs.) and in cultivated specimens (Fleischmann, pers. obs.), but both lineages need further study before a separation on a taxonomic rank can be considered.

4.4. Phylogenetic significance of the rhizophyll anatomy

Without living material for all sampled species and intense microscopic studies, it has not been possible to compile a complete and precise morphological and anatomical matrix for characters of the rhizophyll. There are ongoing efforts to come up with such a compilation (Płachno et al., in preparation); however, the limited data available at this point already allow an initial discussion of the most obvious trends.

In the absence of a phylogenetic framework, Płachno et al. (2007) suggest a "basal position" of subgenus *Genlisea* within the genus due to the distribution pattern of digestive glands in the vesicular distal part of the trap leaves. According to the authors, the concentration of digestive hairs in dense ridges along the vascular bundles (as observed in members of subgenus *Genlisea*; Płachno et al., 2007; Reut, 1993) is a "primitive feature", whereas in subgenus *Tayloria*, the secretory hairs are distributed more or less equidistantly on the vesicle interior, with a tendency to form latitudinally arranged rows in some species (Płachno et al., 2007). Our data, however, are more congruent with a scattered distribution pattern of digestive glands in the traps as plesiomorphic character state in *Genlisea*. It appears that in subgenus *Genlisea*, the digestive hairs became accumulated and concentrated more equidistantly in ridges along the vascular bundle of the trap vesicle, most likely in order to create areas of increased enzymatic activity, and to enable rapid transport and use of nutrients gained from the prey after digestions and uptake through the secretory glands. A similar tendency towards concentration of digestive glands to certain regions of the trap leaves can be observed in various non-related carnivorous plants (displaying distinct trap types of the carnivorous syndrome), e.g. in the pitchers of *Cephalotus* Labill. (Cephalotaceae; Juniper et al., 1989; Lloyd, 1942).

The morphology of the trap leaves, especially the shape of the 2–3 celled retrorse detentive hairs of the interior part of the tubular, is in agreement with the resolved topology. The different types of detentive hairs distinguished on species level by Fromm-Trinta (1979, 1981) and Reut (1993) based on some minor interspecific differences, can be grouped into three distinct general types by the shape of the terminal cell.

In subgenus *Tayloria*, the detentive hairs of the tubular part (neck) of the rhizophylls are rather short and not distinctly curved upwards. The central cell of the hairs is widened towards a globose apex, the terminal cell is rounded or with a short mucronate tip (Fromm-Trinta, 1979; figures in Płachno et al., 2008). This type of detentive hairs seems to be confined to members of *Tayloria*, and was found in all three species recognized for the subgenus, as well as in all of the undescribed taxa used in this study (Płachno, unpublished data; Fleischmann, pers. obs.).

In subgenus *Genlisea*, two distinct types of detentive hairs have been observed in all South American species studied (Fromm-Trinta, 1979; Lloyd, 1942; Goebel, 1891; Reut, 1993), the detentive hairs are thin and long and distinctly continuously reflexed, as the elongated middle cell is strongly curved backwards, and usually the hairs of two subsequent rows even overlap for some part. The terminal cell is not distinctly set apart from the middle cell, and is more or less gradually tapering into an acute apex. An identical arrangement and shape of detentive hairs can be observed in *G. margaretae* (Fig. 1; Fischer et al., 2000) and *G. glandulosissima* and is most likely to occur in *G. pallida* as well (no material useful for DNA extraction available). Therefore, this type of detentive trap hairs can be assumed to represent a synapomorphic character of the monophyletic group comprising the African species of clade II and the Neotropical members of subgenus *Genlisea*.

A third type of detentive hair can be assigned to the monophyletic group of African species that have pedicels not reflexed in fruit (clade I). In members of this clade, a type of detentive hairs is found that differs from all other members of subgenus *Genlisea* in both the terminal and the basal cell. Terminal cells with cylindrical apex (the distal part being either globose or club-shaped (Reut, 1993)), as well as basal cells with a longitudinal furrow, and which occasionally exhibit a pusticulate surface (Reut, 1993) have so far only be observed in this group of African species, and have been recorded and illustrated for *G. hispidula*, *G. subglabra* and *G. africana* (Reut, 1993) and illustrated for *G. stapfii* in Fischer et al. (2000). Reut (1993) records a second type of detentive hair from the neck of the rhizophylls of *G. hispidula* and *G. subglabra* (but not for *G. africana*), which he refers to as "smooth awl-shaped type". This type is very similar to the detentive hairs found in Neotropical members of subgenus *Genlisea*, however, is not as distinctly curved. This hair type may represent an evolutionary link of the two major subgroups of subgenus *Genlisea*. However, many intermediate transition forms or developmental stages can be observed in *Genlisea* trap hairs, and hair morphology can vary notably between different regions of a single trap (Płachno, pers. com., Fleischmann, pers. obs.). Therefore,

the value of the detentive trap hair morphology for taxonomy and phylogenetic reconstruction remains arguable in *Genlisea* (cf. Taylor, 1991a), whereas in the sister genus *Utricularia*, trap anatomy and ultrastructure clearly support phylogeny and infrageneric taxonomic delimitations (Jobson et al., 2003; Müller et al., 2006; Taylor, 1989).

4.5. Flower morphology and palynology

Similar to the situation with trap data, no complete and detailed survey of pollen morphology and ultrastructure of all members of *Genlisea* is available at this point. Therefore, the following discussion is based on the literature and preliminary own observations.

Pollen in subgenus *Tayloria* is 4-zono-colporate, with a narrow constriction at the equator, and spiraperturate colpores have been found at least in *G. violacea* (Taylor, 1989). In contrast, pollen grains in subgenus *Genlisea* are more or less spherical, with three or four furrows distributed equidistantly along the equator, with a tendency to form syncolpi (Fromm-Trinta, 1979, 1981; Taylor, 1989).

In view of the incompleteness of the data, no conclusions on the ancestral pollen type in *Genlisea* can be drawn. In the sister genus *Utricularia*, there seems to be a strong evolutionary tendency to an increased number and size of apertures (Jobson et al., 2003; Lobreau-Callen et al., 1999; Taylor, 1989). Accepting *Tayloria* as the less derived sublineage in *Genlisea*, the initial data cited above would indicate a contrary trend for this genus. However, the different pollen grain shape in *Tayloria* is considered here to represent a synapomorphic character state, which developed from a common ancestral type, which is similar to the extant pollen in *Pinguicula*, the basal lineage of *Utricularia* (subgenus *Polypompholyx*, sensu Müller and Borsch, 2005) and *Genlisea* subgenus *Genlisea*. They all display a very similar type of spherical zonocolporate pollen with discrete endoapertures (bearing five to eight furrows in *Pinguicula* (Casper, 1966) and three furrows in members of *Utricularia* subgenus *Polypompholyx* (Lobreau-Callen et al., 1999; Huynh, 1968; Taylor, 1989)).

Palynological data support the three major phylogenetic clades *viz.* subgenera of the sister genus *Utricularia* (Jobson et al., 2003; Müller et al., 2006); likewise in *Genlisea*, both subgenera can be distinguished by pollen morphology. The distinct pollen types of both subgenera might reflect adaptation to different types of pollinators, a general trend observed in Lamiales (Minkin and Eshbaugh, 1989; Lobreau-Callen, 1980). This is supported by the difference in general flower display between the two subgenera: flowers in subgenus *Genlisea* have a lower corolla lip that forms a big gibbose palate arching upwards to close the tube (masked flower or "snap-dragon flower") (Fig. 1G). The spur is tapering towards an acute apex, and it is always sharply pointing downwards from the palate, i.e. forming a more or less right angle with the corolla opening from its base. The upper lip in subgenus *Genlisea* is always entire. Therefore, the bisymmetrical flowers of subgenus *Genlisea* closely resemble flowers of *Utricularia*. In contrast, in members of subgenus *Tayloria*, the palate is reduced to a small, distinct yellow round marking on the lower lip, and the palate is not firmly appressed to the upper lip (usually even a narrow slit between upper and lower lip is present). Moreover, the saccate spur is not curved from its base, but held horizontally and therefore forms a gradual elongation of the corolla tube. The bilabiate flowers of subgenus *Tayloria* represent a rather polysymmetric disc-like display type, resembling a salverform flower type (Fig. 1F). The upper corolla lip is entire in the basal *G. uncinata*, but distinctly bilobed in all other members of the subgenus (Figs. 3 and 4), the corolla tending towards polysymmetry in some species (most notably in the smaller forms of *G. violacea* and *G.* sp. 'Itacambira'). The straight spur of members of *Tayloria* is cylindrical, with rounded apex and often widened towards the tip (only narrowed and curved towards the apex in *G. uncinata*). In terms of overall morphology, *Tayloria* flowers are more reminiscent of flowers of the basal Lentibulariaceae genus *Pinguicula*.

4.6. Life history

A perennial growth form seems to represent the plesiomorphic habit in *Genlisea*, from which annuals have evolved at least three times independently in all major clades (Figs. 4 and 5). Within *Tayloria*, the first-branching *G. uncinata* and *G.* aff. *violacea* 'giant' are a large perennial montane plants (Taylor and Fromm-Trinta, 1983; Rivadavia, 2000, 2007; Rivadavia pers. obs.), the ancestors of which gave rise to the more derived annual members of the subgenus. Interestingly, the annual growth habit of *G.* sp. 'Itacambira', *G. violacea* and *G. lobata* seems to be exclusively induced by ecological conditions, as all members of this subgenus have been shown to be perennial under artificial conditions in cultivation (Rivadavia, 2002, 2007; Fleischmann, pers. obs.). The same can be assumed for the annual species among the Neotropical members of subgenus *Genlisea*, i.e. *G. filiformis*, *G.* aff. *filiformis* and *G. pygmaea*, respectively, which evolved from two distinct perennial ancestral lineages (Figs. 4 and 5). These three species can continue growth and flowering for more than one growing period if conditions are suitable to maintain growth (Rivadavia, 2007), and therefore can be considered facultative biennials or short-lived polycarpic species (Harper, 1977).

In contrast, the annual species of clade I, which grow in ephemeral wet habitats of inselberg outcrops and periodically dry swamps (Fischer et al., 2000), seem to be true monocarpic annuals. Even if artificially grown in permanently wet conditions, *G. barthlottii*, *G. stapfii* and *G. subviridis* fail to continue growth and inevitably die back after seed production (Fleischmann, pers. obs.). The African monocarpic annuals could represent an adaptation to ephemeral flush vegetation habitats on inselbergs and ferricretes, a highly seasonal habitat type which is only available for a short time every season, and which rapidly becomes desiccated during the dry season (e.g. Müller, 2007; Porembski and Barthlott, 1997). In contrast, the seasonal habitats of white oligotrophic sand plains of the Guianas and the Brazilian "cerrado", "campos rupestre" and coastal "restingas" vegetation, where the short-lived Neotropical facultative biennials among *Genlisea* occur, usually feature some localized wet niche spots that persist for a prolonged time into the dry season, like wetter depressions found along streams and seepages, which may support an extended growth and flowering period for short-lived therophytes (e.g. Castellani et al., 2001). Likewise, the "annual" carnivorous *Drosera sessilifolia* A. St.-Hil., which often can be encountered growing next to short-lived Neotropical *Genlisea* spp. in similar seasonal habitats, is reported to be a size-dependant facultative biennial (Nemoto and Libeiro, 2006).

A single member among the annuals of clade I is able to grow as a facultative perennial: although *Genlisea africana* is reported to be an annual element of seasonal wet flush vegetation of African inselbergs and ferricretes (Fischer et al., 2000), it was found growing as a perennial plant in at least a few permanently wet seepage areas in northern Zambia, sympatrically with the perennial species *G. glandulosissima* and *G. margaretae* (Fleischmann and Rivadavia, pers. obs.). The sister species of clade I, *G. hispidula* and *G. subglabra*, are perennial species of permanently wet marshes and seepage areas (Fischer et al., 2000; Taylor, 1991a).

Interestingly, among the perennial New World species of subgenus *Genlisea*, a geophytic life strategy has evolved. Plants from the highlands in north-eastern Goiás and Minas Gerais states of central Brazil (not included in the present taxon sampling), so far considered a large-flowered phenotype of *G. pygmaea* (but rather representing a distinct taxon related to *G. aurea*; Fleischmann et al., in preparation), survive a dry dormancy in seasonally wet, sandy

habitats by forming stolon-derived subterraneous tubers (Rivadavia, 2007; Carow and Fürst, 1991).

The rosetted habit was lost in a single more derived species of subgenus *Genlisea*, *G. repens*, which forms long, elongated rhizomes growing horizontally below the soil surface and producing both subterraneous rhizophylls and epigeous green leaves (e.g. Lloyd, 1942; Taylor, 1991a). This growth type is similar to the derived terrestrial species of *Utricularia*, which seem to have evolved several times from rosetted (generally annual) ancestors, as exemplified by, e.g. subgenus *Polypompholyx* (Taylor, 1989; Müller and Borsch, 2005).

4.7. Phylogeography

The genus *Genlisea* is likely to have originated in the Neotropics (Fig. 5), like its sister genus *Utricularia* (Jobson et al., 2003; Müller and Borsch, 2005). The origin can be assumed in the south-eastern Brazilian highlands, where the highest number of extant species (greatest α-diversity) can be found (Fromm-Trinta, 1979; Fig. 2). Subgenus *Tayloria* is also confined to this area (comprising the Brazilian states of Bahia, Minas Gerais, Espírito Santo and São Paulo), which coincidentally is simultaneously remarkable for hosting the largest number of species of the sister genus *Utricularia* (Taylor, 1989).

In contrast, subgenus *Genlisea* can be assumed to be of African origin. Out of all extant species of subgenus *Genlisea*, the two clades consisting exclusively of African species represent the basal lineages, one of them (clade II) gave rise to all Neotropical species.

The two main lineages (clade I and clade II + III) of subgenus *Genlisea* are therefore, likely to have evolved from a common ancestor on the African continent, as one of them consists entirely of species restricted to Africa (clade I), whereas in clade II, the basal lineage is composed of African species (with *G. margaretae* reaching Madagascar), from which a monophyletic group of species has branched whose extant members are exclusively found in South America (Figs. 4 and 5).

Given the disjunct trans-Atlantic distribution pattern of *Genlisea*, it seems most parsimonious that the genus evolved in the Western Gondwana area while South America was still joined with the African continent (as proposed by Płachno and Swiątek, 2009). However, this would imply an origin of Lentibulariaceae not later than the Late Cretaceous (in the Mid-Late Cretaceous (110–100 mya), northern South America and Africa began to drift away along a transform fault between Brazil and Guinea opening the central South Atlantic (Scotese et al., 1988), connections between these two continents, however, could have persisted via volcanic islands on mid-ocean ridges until about 95 mya (Raven and Axelrod, 1972)). This is too old an estimated age for a family that belongs to the rather young crown group of Lamiales (estimated age of crown Lamiales ca. 95 mya, of the stem group ca. 104 mya (Janssens et al., 2009)). So far, reliable relaxed-clock estimates for the age of Lentibulariaceae have been hampered by both the absence of useful fossil calibration points and the uncertainty with respect to the phylogenetic position of the family within Lamiales. A currently compiled large data matrix for Lamiales based on non-coding cp DNA regions may shed light on divergence times of the more derived lamialean families (Schäferhoff et al., in preparation).

At least 111 genera of angiosperms are known to display a tropical trans-Atlantic distribution between South America and Africa, including Madagascar (Thorne, 1973), and also five sections of the sister genus *Utricularia* (all of the latter having just a single member occurring on the African continent (Dörrstock et al., 1996)). These "outliers" have been explained by rather recent long-distance dispersal to Africa (Dörrstock et al., 1996; Renner, 2004; Taylor, 1989; Thorne, 1973). The case of *Genlisea,* however, is different, because no species co-occur in both Africa and South America. On the other hand, a more or less comparable α-diversity can be found on both continents.

To explain the extant distribution of *Genlisea*, two subsequent colonization events by long-distance dispersal in opposite directions have to be assumed. Presumably the common ancestral lineage for all African species (leading to subgenus *Genlisea*) has colonized the African continent by long-distance dispersal events from South America. After a radiation of subgenus *Genlisea* in Africa (extending to Madagascar) that gave rise to clades I and II, an ancestral member of clade II + III re-colonized South America, leading to a second radiation of Neotropical *Genlisea* (clade III).

Indeed, bidirectional trans-Atlantic dispersal of diaspores of many angiosperms can parsimoniously be linked to water currents between South America and Africa (Renner, 2004). Trans-Atlantic long-distance dispersal by water seems to be much more common than dispersal by birds or wind, and even small seeds such as those of Melastomataceae (a family of which many annual members share similar habitat needs with *Genlisea*) could have possibly crossed the Atlantic in soil attached to drifting vegetation ("floating islands"; Renner, 2004). Exceptional westerly winds are known to occur from north-eastern Brazil to northwest Africa, creating a putative migratory pathway for diaspores from the extant Neotropical center of diversity of *Genlisea* to its African range. Seed of *Genlisea* is minute (Fromm-Trinta, 1979; Taylor, 1991a), rather short-lived and not able to float for a prolonged time (Fleischmann, pers. obs.), and therefore not likely to be drifted in sea water directly. However, fast equatorial currents have been demonstrated to transport larger floating objects (with surfaces exposed to the wind) across the Atlantic in less than two weeks, and transport was probably even faster across the narrower Atlantic when a shorter distance between the African continent and South American had to be covered (Renner, 2004).

Following this assumption, the South American subcontinent, especially the center of diversity in the south-eastern Brazilian highlands, has been colonized by *Genlisea* twice independently (first a radiation of subgenus *Tayloria* and a subsequent re-colonization event by derived members of subgenus *Genlisea* via long-distance dispersal from Africa).

This contradicts the boreotropics hypothesis as explanation for the disjunct area of *Genlisea* as put forward by Jobson et al. (2003) (see Renner, 2004), which assumes an interchange of tropical biota between temperate North America and Eurasia during the Early Tertiary. The paraphyly of the African *Genlisea* species (giving rise to all Neotropical members of subgenus *Genlisea*; Figs. 4 and 5) is not in agreement with such a migration pattern, but favors trans-Atlantic long-distance dispersal as explanation for the extant disjunction in the range of *Genlisea*, like it had already been proposed by Thorne (1973).

The migratory pathways of *Genlisea* on the South American continent are difficult to reconstruct, and no conclusions can be drawn from the tree (Fig. 5) as to which of the two extant centers of diversity in the New World (north and south of the Amazon) might have been the origin of diversification. Presumably *Genlisea* had once been more widespread in the uplands of north-eastern South America to the north and the south of the Amazon basin, and the two extant centers of species endemism and diversity (in the Guiana Highlands and the highlands of south-eastern central Brazil) represent relict areas providing suitable habitats. The basal lineage of the New World *Genlisea* consists of two species inhabiting the Guianas (Amazonas and Bolívar state of Venezuela and adjacent Guyana), at least one of them (*G. guianensis*) has a few disjunct populations in the states of Bahia, Goiás, Minas Gerais and Mato Grosso of southern Brazil (Fromm-Trinta, 1981; Taylor, 1991a; Rivadavia, pers. obs.) and adjacent eastern Bolivia (Ritter and Crow, 2000). In the present study, the Brazilian plants have been shown to be sister to *G. guianensis* from Guiana populations, but they dif-

fer in several morphological characters and therefore may deserve to be recognized as distinct taxa (Fleischmann et al., in preparation). The next-branching lineages are either endemic to the central Brazilian highlands (*G. aurea*) or Venezuela (*G. glabra*, a high montane species of very narrow distribution range, restricted to a few tepui summits of the central Guiana Shield). The remaining members of this clade are widespread throughout the range of Neotropical *Genlisea*, and occur in both montane and lowland areas, with one single species, the annual *G. filiformis*, greatly extending the range of New World *Genlisea* to the north and west by reaching Central America, Cuba and Mexico (Fromm-Trinta, 1979; Olvera and Martínez, 2002; Taylor, 1991a).

4.8. Taxonomic implications

Based on the morphologically distinctive characters found between members of the three clades of subgenus *Genlisea*, these three lineages (clades I–III) are described at the taxonomical rank of sections here. The two sections proposed for the 12 African species are identical to the groups I and II + III, respectively, as suggested by Fischer et al. (2000), and can be clearly distinguished by the position of the capsule in fruit (pedicels recurved or upright). The monophyletic group of Neotropical members of subgenus *Genlisea* (including the type of the genus, *G. aurea*) becomes the autonymous type section.

Genlisea A. St.-Hil subgen. *Genlisea* sect. *Genlisea*.

Plantae pedicellis in fructu erectis, corollis unicoloribus luteis, violaceis vel albidis, plantae Neotropicae. Type: *Genlisea aurea* A. St.-Hil., Voy. Diam.: 429, 1833.

Plants with pedicel upright in fruit (a character shared with members of section *Africanae*); corolla without bicolored palate (a character shared with members of section *Recurvatae*); inflorescence covered with glandular or eglandular hairs or glabrous, from glabrous (rarely pubescent) base; ovary glabrous or covered with glandular and/or eglandular hairs; perennial or annual plants; leaves spathulate (or linear to lanceolate in *G. guianensis*); detentive hairs from the tubular neck of trap leaf interior strongly recurved, narrow with tapering acute apex (a character shared with members of section *Recurvatae*). Section *Genlisea* corresponds to clade III in Fig. 3.

Genlisea subgen. *Genlisea* sect. *Recurvatae* A. Fleischm., Kai Muell., Barthlott and Eb. Fisch. sect. nova.

Plantae pedicellis glandulosis in fructu recurvis, corollis unicoloribus violaceis vel albidis, plantae Africanae. Type: *Genlisea glandulosissima* R.E. Fries, Schwed. Rhod.-Kongo Exp. 1: 301, 1916.

Species included: *G. margaretae* Hutch. (syn. *G. recurva* Bosser), *G. glandulosissima* R.E. Fries, *G. pallida* Fromm-Trinta and P. Taylor.

Plants with pedicel recurved in fruit (a character shared with subgenus *Tayloria*); corolla without bicolored palate (a character shared with members of section *Genlisea*), usually with distinct constriction above the ovary; inflorescence glandular from the base; ovary densely glandular; perennial plants forming dense rosettes of multiple narrowly spathulate leaves; detentive hairs from the tubular neck of trap leaf interior strongly recurved, narrow with tapering acute apex (a character shared with members of section *Genlisea*).

The section name refers to the strongly recurved fruiting pedicels of the members of this section, which are easily detectable in fruiting specimens. The three members of this section are the only African species of *Genlisea* with recurved pedicels. In Neotropical *Genlisea* species, reflexed pedicels are confined to subgenus *Tayloria*. Section *Recurvatae* corresponds to clade II in Fig. 3.

Genlisea subgen. *Genlisea* sect. *Africanae* A. Fleischm., Kai Muell., Barthlott and Eb. Fisch. sect. nova.

Plantae pedicellis glabris vel glandulosis in fructu erectis, corollis violaceis vel albidis labiis inferioribus macula alba reticulata, plantae Africanae.

Type: *Genlisea africana* Oliv., J. Linn. Soc. Bot. 9: 145. 1865.

Species included: *G. africana* Oliv., *G. angolensis* R.D. Good, *G. barthlottii* Porembski, Eb. Fisch. and Gemmel, *G. hispidula* Stapf, *G. stapfii* A. Chev., *G. subglabra* Stapf, *G. subviridis* Hutch., *G. taylorii* Eb. Fisch., Porembski and Barthlott.

Plants with pedicel not reflexed in fruit, fruiting capsules held upright (a character shared with members of subgenus *Genlisea* section *Genlisea*); corolla pale mauve, violet or white, with distinctly white palate with darker reticulation, not constricted above the ovary; inflorescence glabrous from the base, but covered with glandular or eglandular hairs in the uppermost part in some species; ovary covered with glandular and/or eglandular hairs, or glabrous; perennial or annual plants forming more or less lax rosettes consisting of few broadly spathulate to obovate leaves; detentive hairs from the tubular neck of trap leaf interior not strongly recurved, with cylindrical apex, often two distinct types of detentive hairs.

The section name is based on the type species of this section, *G. africana*, which was the first African species to be described (Oliver, 1865), as well as on the geographic distribution of the members of this section, which is limited to the African continent. Section *Africanae* corresponds to clade I in Fig. 3.

5. Conclusions

Phylogenetic trees based on the plastid markers *trnK*/*matK*, *rps16* and *trnQ–rps16* are in agreement with the distribution of morphological characters such as flower-color and -shape, life history (annuals vs. perennials), and with biogeographic patterns. Subgenera *Genlisea* and *Tayloria* are clearly recovered by our molecular data, and are not only distinguished by the dehiscence of the capsules (Fromm-Trinta, 1977), but also in terms of trap morphology, pollen morphology and flower shape (most likely linked to different pollination syndromes).

Members of subgenus *Tayloria* share a number of character states with the basal lineage of Lentibulariaceae, *Pinguicula*, such as bivalvate capsules, tubular flowers with a straight spur but lacking a gibbose palate, an upper corolla lip that is crenate or bilobate, and predominantly violet to white flower colors. In contrast, subgenus *Genlisea* displays several parallels to derived members of the sister genus *Utricularia*, such as an entire upper corolla lip, "masked flowers" with a gibbose palate from the lower lip, and predominantly yellow flower-colors.

The indumentum of scape and capsule turned out to be a rather homoplastic character, but is useful for taxonomical delimitation on species level. Anatomical characters of the rhizophylls, especially the shape of the internal detentive hairs, are less useful for species delimitation (cf. Fischer et al., 2000; Taylor, 1991a) and their distribution shows only weak agreement with the clades found based on the molecular data.

Combining evidence from molecular, morphological, and biogeographical evidence, three distinct groups could be delimitated and be formally described as sections in subgenus *Genlisea*.

Finally, since only very few nodes in the trees reconstructed here receive low support, this study provides an exceptionally clear picture of the evolution in a genus, and therefore will also provide a valuable foundation for studies on genome size plasticity in *Genlisea* and beyond.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft, DFG Grant MU2875/2 to K.F.M. Support to A.F. was given by the graduate funding of the Universität Bayern e.V. Thanks to the Akademie der Wissenschaften und der Literatur, Mainz, for

continued support to the Carnivorous Plants working group at the Nees Institute. The contribution of Johann Greilhuber in ongoing studies on genome evolution in *Genlisea* is highly acknowledged. Eberhard Fischer is thanked for his collaboration on African *Genlisea* taxonomy, Vitor Miranda for information on Brazilian *Genlisea* species, and Susanne Renner for insightful comments on plant dispersal. The help of Bartosz Płachno in providing SEM images and information on *Genlisea* trap anatomy is kindly acknowledged. We thank Tanja Ernst for technical lab assistance; Eva Facher helped with SEM studies of seed and trap material. Vitor Batista, Carlos Rohrbacher, Paulo Gonella, Kamil Pasek, Markus Welge and Matthias Teichert are acknowledged for additional plant material from cultivated specimens. The curators of M are thanked for providing study accession to their collection. Thanks to the current and former staff of the Bonn Botanical Gardens (W. Höller and O. Kriesten in particular) for maintaining the valuable living plant collection of *Genlisea*.

References

Adamec, L., 2003. Zero water flows in the carnivorous genus *Genlisea*. Carniv. Pl. Newsl. 32, 46–48.

Adamec, L., 2007. Oxygen concentrations inside the traps of the carnivorous plants *Utricularia* and *Genlisea* (Lentibulariaceae). Ann. Bot. 100, 849–856.

Angiosperm Phylogeny Group. [A.P.G.], 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Lin. Soc. 161, 105–121.

Barthlott, W., Porembski, S., Fischer, E., Gemmel, B., 1998. First protozoa-trapping plant found. Nature 392, 447.

Barthlott, W., Porembski, S., Seine, R., Theisen, I., 2007. The Curious World of Carnivorous Plants: A Comprehensive Guide to their Biology. Timber Press, Portland.

Benjamin, L., 1847. Lentibulariaceae. In: Martius, C.P.F. de, Flora Brasiliensis 10. Munich.

Bosser, J., 1958. Sur deux nouvelles Lentibulariacées de Madagascar. Naturaliste Malgache 10, 21–23.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Kallersjo, M., Barkhordarian, E., 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol. Phylogenet. Evol. 24, 274–301.

Calviño, C.I., Downie, S.R., 2007. Circumscription and phylogeny of Apiaceae subfamily *Saniculoideae* based on chloroplast DNA sequences. Mol. Phylogenet. Evol. 44, 175–191.

Carow, T., Fürst, R., 1991. Fleischfressende Pflanzen. Artenübersicht, Kultur, Vermehrung. Verlag Thomas Carow, Nüdlingen.

Casper, S.J., 1962. "Systematisch maßgebende" Merkmale für die Einordnung der Lentibulariaceae in das System. Österr. Bot. Zeitschr. 1, 108–131.

Casper, S.J., 1966. Monographie der Gattung *Pinguicula* L. Bibliotheca Botanica 127/128, 1–225.

Castellani, T.T., Scherer, K.Z., de Souza, P.G., 2001. Population ecology of *Paepalanthus polyanthus* (Bong.) Kunth: demography and life history of a sand dune monocarpic plant. Rev. Bras. Bot. 24, 122–134.

Cieslack, T., Polepalli, J.S., White, A., Müller, K., Borsch, T., Barthlott, W., Steiger, J., Marchand, A., Legendre, L., 2005. Phylogenetic analysis of *Pinguicula* (Lentibulariaceae): chloroplast DNA sequences and morphology support several geographically distinct radiations. Am. J. Bot. 92, 1723–1736.

Darwin, C., 1875. Insectivorous Plants. Murray, London.

Dörrstock, S., Seine, R., Porembski, S., Barthlott, W., 1996. First record of the American *Utricularia juncea* (Lentibulariaceae) from Africa. Kew Bull. 51, 579–583.

Fischer, E., Porembski, S., Barthlott, W., 2000. Revision of the genus *Genlisea* (Lentibulariaceae) in Africa and Madagascar with notes on ecology and phytogeography. Nordic J. Bot. 20, 291–318.

Fromm-Trinta, E., 1977. *Tayloria* Fromm-Trinta – Nova Seção do gênero *Genlisea* St.-Hil. (Lentibulariaceae). Bol. Mus. Nac. Bot. (Rio. J.) 44, 1–4.

Fromm-Trinta, E., 1979. Revisão das espécies do gênero *Genlisea* St.-Hil (Lentibulariaceae) das regiões sudeste e sul do Brasil. Rodriguésia 31 (49), 17–139.

Fromm-Trinta, E., 1981. Revisão do gênero *Genlisea* St.-Hil. (Lentibulariaceae) no Brasil. Bol. Mus. Nac. Bot. (Rio. J.) 61, 1–21.

Fromm-Trinta, E., 1984. Genliseas Americanas. Sellowia 36, 55–62.

Goebel, K., 1891. Pflanzenbiologische Schilderungen 2. Marburg, pp. 121–127.

Greilhuber, J., Borsch, T., Müller, K., Worberg, A., Porembski, S., Barthlott, W., 2006. Smallest angiosperm genomes found in Lentibulariaceae, with chromosomes of bacterial size. Plant Biol. 8, 770–777.

Harper, J.L., 1977. Population Biology of Plants. Academic Press, London.

Heslop-Harrison, Y., 1975. Enzyme release in carnivorous plants. In: Dingle, J.T., Dean, R.T. (Eds.), Lysozymes in Biology and Pathology, vol. 4. North Holland Publishing Company, Amsterdam, pp. 525–578.

Hilu, K.W., Borsch, T., Müller, K.F., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M.P., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A.B., Rohwer, Campbell, C.S., Chatrou, L.W., 2003. Angiosperm phylogeny based on matK sequence information. Am. J. Bot. 90, 1758–1776.

Hutchinson, J.A., 1946. A Botanist in Southern Africa, London, pp. 528–529.

Huynh, K.-L., 1968. Étude de la morphologie du pollen du genre *Utricularia* L. Pollen Spores 10, 11–55.

Janssens, S.B., Knox, E.B., Huysmans, S., Smets, E.F., Merckx, V.S.F.T., 2009. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: result of a global climate change. Mol. Phylogenet. Evol. 52, 806–824.

Jobson, R.W., Albert, V.A., 2002. Molecular rates parallel diversification contrasts between carnivorous plant sister lineages. Cladistics 18, 127–136.

Jobson, R.W., Playford, J., Cameron, K.M., Albert, V.A., 2003. Molecular phylogenetics of Lentibulariaceae inferred from plastid rps16 intron and trnL-F DNA sequences: implications for character evolution and biogeography. Syst. Bot. 28, 157–171.

Johnson, L.A., Soltis, D.E., 1995. Phylogenetic inference in Saxifragaceae s.str. and *Gilia* (Polemoniaceae) using matK sequences. Ann. Mo. Bot. Gard. 82, 149–175.

Juniper, B.E., 1986. The path to plant carnivory. In: Juniper, B.E., Southwood, R. (Eds.), Insects and the Plant Surface. Echvard Annald, London, pp. 195–218.

Juniper, B.E., Robins, R.J., Joel, D.M., 1989. The Carnivorous Plants. Academic press, London.

Katoh, K., Toh, H., 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics 9, 212.

Lloyd, F.E., 1942. Carnivorous Plants. Chronica botanica Co., Massachusetts, Waltham.

Lobreau-Callen, D., 1980. Is adaptation to parasitism and saprophytism necessarily linked with simplification of the exine ultrastructure. Abstracts of Fifth International Palynological Conference, Cambridge, 230.

Lobreau-Callen, D., Jérémie, J., Suarez-Cervera, M., 1999. Morphologie et ultrastructure du pollen dans le genre *Utricularia* L. (Lentibulariaceae). Can. J. Bot. 77, 744–767.

Luetzelburg, von P., 1923. Est. Bot. Nordeste 3, 223.

Macherey-Nagel, 2007. NuclepSpin®Plant: Genomic DNA from Plant User manual, June 2007/Rev. 6. Macherey-Nagel GmbH & Co. KG, Düren.

Maddison, W.P., Maddison, D.R., 2009. Mesquite: a modular system for evolutionary analysis. Version 2.71. Available from: <www.mesquiteproject.org>.

Minkin, J.P., Eshbaugh, W.H., 1989. Pollen morphology of the Orobanchaceae and rhinanthoid Scrophulariaceae. Grana 28, 1–18.

Morrison, D.A., 2007. Increasing the efficiency of searches for the maximum likelihood tree in phylogenetic analysis of up to 150 nucleotide sequences. Syst. Biol. 56, 988–1010.

Müller, J.V., 2007. Herbaceous vegetation of seasonally wet habitats on inselbergs and lateritic crusts in West and Central Africa. Folia Geobot. 42, 29–61.

Müller, K.F., Quandt, D., Müller, J., Neinhuis, C., 2005. PhyDE® 0.995: Phylogenetic Data Editor. Available from: <www.phyde.de>.

Müller, K.F., 2004. PRAP – computation of Bremer support for large data sets. Mol. Phylogenet. Evol. 31, 780–782.

Müller, K.F., 2005a. SeqState – primer design and sequence statistics for phylogenetic DNA data sets. Appl. Bioinformatics 4, 65–69.

Müller, K.F., 2005b. The efficiency of different search strategies in estimating parsimony jackknife, bootstrap, and Bremer support. BMC Evol. Biol. 5, 58.

Müller, K.F., Borsch, T., 2005. Phylogenetics of *Utricularia* (Lentibulariaceae) and molecular evolution of the *trnK* intron in a lineage with high substitutional rates. Plant Syst. Evol. 250, 39–67.

Müller, K.F., Borsch, T., Legendre, L., Fischer, E., Porembski, S., Barthlott, W., 2001. The evolution of carnivory in the Lamiales: evidence from *matK* and adjacent noncoding regions. Botany. Abstracts. Botanical Society of America, 129.

Müller, K.F., Borsch, T., Legendre, L., Porembski, S., Theisen, I., Barthlott, W., 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. Plant Biol. 6, 477–490.

Müller, K.F., Borsch, T., Legendre, L., Porembski, S., Barthlott, W., 2006. Recent progress in understanding the evolution of Lentibulariaceae. Plant Biol. 8, 748–757.

Nemoto, M., Libeiro, J.F., 2006. Factors determining the habitat of *Drosera sessilifolia* in the humid zone of the Brazilian Cerrado. Ecol. Res. 21, 150–156.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Oliver, D., 1865. On the Lentibulariaceae collected in Angola by Dr. Welwitsch, with an enumeration of the African species. J. Linn. Soc. Lond. Bot. 9, 145–146.

Olvera, G.M., Martínez, S.E., 2002. Primer registro de *Genlisea* (Lentibulariaceae) para México. Acta Bot. Mex. 59, 71–73.

Oxelman, B., Lidén, M., Berglund, D., 1997. Chloroplast *rps16* intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). Plant Syst. Evol. 206, 393–410.

Płachno, B.J., Faber, J., Jankun, A., 2005a. Cuticular discontinuities in glandular hairs of *Genlisea* A. St.-Hil. in relation to their functions. Acta Bot. Gall. 152, 125–130.

Płachno, B.J., Adamus, K., Faber, J., Kozlowski, J., 2005b. Feeding behaviour of carnivorous *Genlisea* plants in the laboratory. Acta Bot. Gall. 152, 159–164.

Płachno, B.J., Kozieradzka-Kiszkurno, M., Swiatek, P., 2007. Functional utrastructure of *Genlisea* (Lentibulariaceae) digestive hairs. Ann. Bot. 100, 195–203.

Płachno, B.J., Kozieradzka-Kiszkurno, M., Swiatek, P., Darnowski, D.W., 2008. Prey attraction in carnivorous *Genlisea* (Lentibulariaceae). Acta Biol. Cracov. Ser. Bot. 50, 87–94.

Plachno, B.J., Wolowski, K., 2008. Algae commensal community in *Genlisea* traps. Acta Soc. Bot. Pol. 77, 77–86.

Płachno, B.J., Swiątek, P., 2009. Functional anatomy of the ovule in *Genlisea* with remarks on ovule evolution in Lentibulariaceae. Protoplasma 236, 39–48.

Porembski, S., Barthlott, W., 1997. Seasonal dynamics of plant diversity on inselbergs in the Ivory Coast (West Africa). Botanica Acta 110, 466–472.
Posada, D., 2008. JModelTest: Phylogenetic Model Averaging. Mol. Biol. Evol. 25, 1253–1256.
Raven, P.H., Axelrod, D.I., 1972. Plant tectonics and Australasian paleogeography. Science 176, 1379–1386.
Renner, S.S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. Int. J. Plant Sci. 165 (Suppl. 4), S23–S33.
Reut, M.S., 1993. Trap structure of the carnivorous plant *Genlisea* (Lentibulariaceae). Bot. Helv. 103, 101–111.
Ritter, N.P., Crow, G.E., 2000. Notes on the Lentibulariaceae in Bolivia: a new genus record (*Genlisea*) for the country, with two additional species records in the genus *Utricularia*. Rhodora 102, 217–224.
Rivadavia, F., 2000. The giant *Genlisea uncinata* P. Taylor & Fromm-Trinta. Carniv. Pl. Newsl. 29, 83–86.
Rivadavia, F., 2002. *Genlisea lobata* Fromm-Trinta. Carniv. Pl. Newsl. 31, 116–118.
Rivadavia, F., 2007. A *Genlisea* myth is confirmed. Carniv. Pl. Newsl. 36, 122–125.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Saint-Hilaire, A., 1833. Voyage Dans le District des Diamans du Brésil. Librairie-Gide, Paris.
Scotese, C.R., Gahagan, L.M., Larson, R.L., 1988. Plate tectonic reconstructions of the Cretaceous and Cenozoic ocean basins. Tectonophysics 155, 27–48.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.
Stopp, K., 1958. Notiz über die Dehiszenzweise der Kapselfrüchte von *Genlisea hispidula* Stapf. Beitr. Biol. Pflanz. 34, 126–142.
Stöver, B., Müller, K.F., 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11, 7.
Studnička, M., 1996. Several ecophysiological observations in *Genlisea*. Carniv. Pl. Newsl. 25, 14–16.
Studnička, M., 2003a. *Genlisea* traps – a new piece of knowledge. Carniv. Pl. Newsl. 32, 36–39.
Studnička, M., 2003b. Further problem in *Genlisea* trap untangled? Carniv. Pl. Newsl. 32, 40–45.
Studnička, M., 2003c. Observations on life strategies of *Genlisea*, *Heliamphora* and *Utricularia* in natural habitats. Carniv. Pl. Newsl. 32, 57–61.
Swofford, D.L., 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.
Sylvén, N., 1908. Die Genliseen und Utricularien des Regnell'schen Herbariums. Arkiv för Botanik 8 (6), 1–48.
Taylor, P., 1967. Lentibulariaceae. In: Maguire, B., et al. (Eds.), Botany of the Guayana Highland 7, vol. 17. Mem. New York Bot. Gard., pp. 201–228.
Taylor, P., 1989. The genus *Utricularia*: a taxonomic monograph. London.
Taylor, P., 1991a. The genus *Genlisea*. Carniv. Pl. Newsl. 20, 20–26.
Taylor, P., 1991b. A revised World list of the genus *Genlisea* St.Hil. Carniv. Pl. Newsl. 20, 59.
Taylor, P., Fromm-Trinta, E., 1983. Uma nova espécie para o gênero *Genlisea* St.-Hil. sect. *Tayloria* (Lentibulariaceae): *Genlisea uncinata* P. Taylor & Fromm-Trinta. Bradea 3, 365–368.
Thorne, R.F., 1973. Floristic relationships between tropical Africa and tropical America. In: Meggers, B.J., Duckworth, W.D. (Eds.), Tropical Forest Ecosystems in Africa and South America: A Comparative Review. Smithsonian Institution, Washington, pp. 27–47.
Wang, L.S., Leebens-Mack, J., Wall, P.K., Beckmann, K., dePamphilis, C.W., Warnow, T., in press. The impact of multiple protein sequence alignment on phylogenetic estimation. IEEE/ACM Transactions on Computational Biology and Bioinformatics, doi:10.1109/TCBB.2009.68.

A revision of *Genlisea* subgenus *Tayloria* (Lentibulariaceae)

Article III

Fleischmann, A., Rivadavia, F., Gonella, P.M., Heubl, G., 2011.
Phytotaxa 33: 1-40.

Phytotaxa 33: 1–40 (2011)
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

A revision of *Genlisea* subgenus *Tayloria* (Lentibulariaceae)

ANDREAS FLEISCHMANN[1*], FERNANDO RIVADAVIA[2], PAULO MINATEL GONELLA[3] & GÜNTHER HEUBL[1]

[1]*Systematic Botany and Mycology, Department of Biology, University of Munich, Menzinger Strasse 67, D-80638 Munich, Germany. E-mail: fleischmann@lrz.uni-muenchen.de*
[2]*1 Daniel Burnham Ct., San Francisco, CA 94109, USA*
[3]*Laboratório de Sistemática Vegetal, Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Cx. Postal 05508–900, São Paulo, SP, Brazil*
**author for correspondence*

Abstract

Five new species of *Genlisea* are described from Brazil, and all eight species of *Genlisea* subgenus *Tayloria* are described and illustrated, including remarks on ecology, biogeography, and habitat. Distribution maps, line drawings, photographs of the corolla, and SEM microphotographs of the seeds are presented for all species, and an identification key is provided for the subgenus.

Resumo

Cinco novas espécies de *Genlisea* são descritas para o Brasil e todas as oito espécies de *Genlisea* subgênero *Tayloria* são descritas e ilustradas, incluindo observações sobre ecologia, biogeografia e habitat. São apresentados mapas de distribuição, ilustrações, fotografias da corola e microfotografias SEM das sementes para todas as espécies, além de uma chave de identificação para o subgênero.

Key words: Brazil, Chapada Diamantina, new species, Serra do Espinhaço

Introduction

The sandstone massifs of the Espinhaço Mountain Range (Cadeia do Espinhaço or "Backbone Range"), of Minas Gerais and Bahia states in eastern Brazil form a large north to south ranging mountain chain (see Fig. 1), usually over 800 m elevation, rising to over 2000 m in some areas (Rapini *et al*. 2002). It is made up by quartzitic rocks of Pre-Cambrian age (Harley 1988), which are covered by shallow, sandy, nutrient poor, acidic soils. The area hosts a unique flora, especially in the dominating *campo rupestre* ("rocky fields") vegetation type, which is found on the sandstone based soils in areas above (800–)900 m elevation (Harley 1988, Giulietti & Pirani 1988, Giulietti *et al*. 1987, 1997, Benites *et al.* 2007).

The Espinhaço Range is divided into two main parts: the northern section—the Chapada Diamantina—lies in the state of Bahia, the southern section—the Serra do Espinhaço—in Minas Gerais. The area in between (less than 200 km wide) is covered with a dry *cerrado* and *caatinga* shrubland vegetation and includes the Rio de Contas Basin with richer and deeper soils (Harley 1988), distinct from the specialized *campo rupestre* vegetation that occurs on the poor soils of the higher elevated areas of the Espinhaço Range. However, to the west of this basin lies a continuous series of fragmented sandstone foothills (reaching the 800–1000 m elevation range), that geologically connects the Serra do Espinhaço to the Chapada Diamantina, and possibly connected both areas floristically in the past during cooler periods (Antonelli *et al*. 2010).

Accepted by W. Baker: 21 Oct. 2011; published: 28 Nov. 2011

Other sections of the Serra do Espinhaço in Minas Gerais are separated by similar areas of lower elevation covered with *cerrado* vegetation, most notably between the northern Diamantina Plateau and the highlands extending from Itacambira to Grão Mogol (Fig. 1). These areas display edaphic and climatic conditions

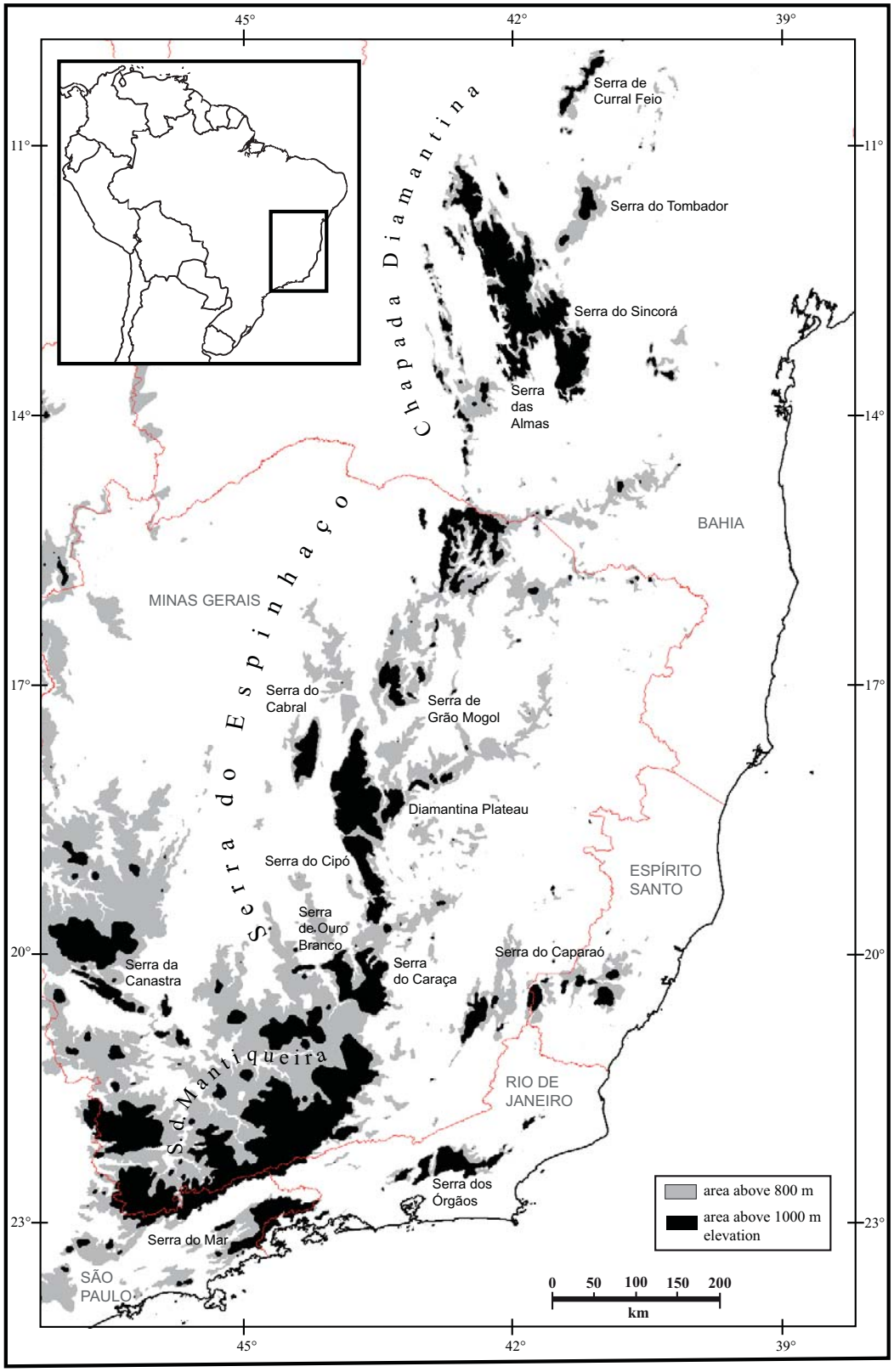


FIGURE 1. Map of eastern Brazil, illustrating the Espinhaço Mountain Range (including the Chapada Diamantina), showing the most important mountain massifs of the range, and the adjacent costal highlands and the Serra do Caparaó. Names of mountain ranges and massifs ("Serras") following Giulietti *et al*. (1997) and Harley (1988).

contrasting to those of the higher elevation areas, and therefore can also act as a barrier for migration of endemic mountain flora and *campo rupestre* plants (Harley 1988, Echternacht *et al*. 2011).

The flora of the Espinhaço Range shows a remarkably high degree of endemism (Harley 1988, Giulietti & Pirani 1988, Rapini *et al*. 2002, Alves *et al.* 2007, Echternacht *et al*. 2011), often on a small local scale, especially in the mountain ranges ("Serras") known as Serra do Cipó, Diamantina Plateau, Serra do Cabral and Serra de Grão Mogol, and in the Chapada Diamantina (see Fig. 1). The Serra do Cipó, which has been known as the "Serra da Lapa" to 18th century naturalists, has ever since been intensively explored by botanists (Giulietti *et al*. 1987); nevertheless, new taxa are still continuously described from that area (eg., Rivadavia & Gonella 2011).

The genus *Genlisea* Saint-Hilaire (1833: 428) belongs to the carnivorous plant family Lentibulariaceae of the angiosperm order Lamiales (Angiosperm Phylogeny Group 2009). It occurs in South and Central America and Africa, including Madagascar, and comprises 22 species previous to the present study (Fleischmann *et al*. 2010). The Brazilian endemic *Genlisea* subgenus *Tayloria* (Fromm-Trinta 1977: 1) Fischer, Porembski & Barthlott (2000: 293) has been proposed by Fromm-Trinta (1977) based on capsule dehiscence. Three species have so far been recognized therein (Taylor 1991): the type of the section, *G. violacea* Saint-Hilaire (1833: 431), and two additional species that were described 150 years later: in 1983, P. Taylor and Fromm-Trinta described the distinctive *G. uncinata* Taylor & Fromm (1983: 365) from Bahia state, and in 1989, Fromm-Trinta described *G. lobata* Fromm (1989: 152) from eastern Minas Gerais, close to the border of Espírito Santo. The earlier described *G. biloba* Benjamin (1847: 254), *G. reflexa* Benjamin (1847: 254) and *G. cylindrica* Sylvén (1909: 4) were altogether synonymized with *G. violacea* by Peter Taylor in 1976 on the respective specimen annotations, and this opinion was followed in subsequent treatments (Fromm-Trinta 1979, Taylor 1991).

However, in the studies leading up to this work, certain herbarium specimens could not be assigned to any of the three known species of *G.* subgenus *Tayloria*. These are here assigned to five newly described species.

Members of *G.* subgenus *Tayloria* can be distinguished from *G.* subgenus *Genlisea* by their longitudinally bivalvate capsule dehiscence (Fromm-Trinta 1977), flower morphology (Fleischmann *et al*. 2010), and in further morphological characters, such as a spur that is widely divergent from the lower lip of the corolla and paralleling the pedicel (parallel with corolla lower lip in *G.* subgenus *Genlisea*).

The following characters are equally found in all members of *G.* subgenus *Tayloria*, and are therefore not repeated in the descriptions of each species: The scape indumentum consists of glandular capitate hairs (stalk unicellular and multicellular oblong head) and/or eglandular hairs (simple bicellular or unicellular hairs). In contrast to *G.* subgenus *Genlisea*, the hairs found in *G.* subgenus *Tayloria* are not septate. Bracts and bracteoles are basifix in all species, and not connate at the base. The inflorescence of all *Genlisea* species is a raceme, or (less frequently) a double raceme. Like in all members of Lentibulariaceae, *Genlisea* flowers possess two stamens, the filaments are curved inwards, and the anthers are bithecate. The ovary is superior, the stigma is bilabiate (lower lobe smaller than enlarged orbiculate upper lobe) and persistent in fruit. The fruit is a dry capsule, longitudinally bivalvate, and many-seeded. Herbarized plants are usually drying dark brown or black.

All *Genlisea* species are distinctly heterophyllous, producing green, photosynthetic, epiterrestrial leaves, and tubular achlorophyllous, subterraneous leaves (rhizophylls). The latter replace the entirely absent roots, and beyond that act as carnivorous "eel-traps" in order to catch small soil organisms. The overall design of these inverted Y-shaped organs is more or less identical in all members of the genus, however a few anatomical differences in trap hairs and digestive glands are found between members of the two subgenera (Płachno *et al*. 2005), and between some species (Reut 1993, Fromm-Trinta 1979). These microscopic characters however are very constant in all members of *G.* subgenus *Tayloria*, and thus are not considered here, as they are of low taxonomic value in this group. Therefore, all leaf characters (lamina, petiole) and measurements given in the present work are referred to the epiterrestrial, green, photosynthetic leaves.

Seed morphology, especially testa structure, proved to be a valuable character for species delimitation in the sister genus *Utricularia* (Taylor 1989). Whereas seed shape and testa sculpture seems to be rather

consistent in subgenus *Genlisea*, it is apparently more or less species specific in *G.* subgenus *Tayloria* (Taylor & Fromm-Trinta 1983, Fromm-Trinta 1989). During herbarium work and observations made on living plants in the wild, several new species have been revealed, which are described and illustrated here. *Genlisea oligophylla* from Minas Gerais state has been both treated under *G. uncinata* and *G. violacea* in previous flora treatments (Fromm-Trinta 1996), whereas the remaining taxa have been assigned to *G. violacea* in the past (Fromm-Trinta 1979, Harley & Mayo 1980, Fromm-Trinta 1996, Fromm-Trinta 2004), or were hitherto unknown to science.

Material and Methods

Herbarium specimens were personally studied and annotated by at least one of the authors in the following herbaria: B, BHCB, ESA, HB, K, M, OUPR, RB, SPF, UEC. Herbarium acronyms follow *Index Herbariorum* (http://sweetgum.nybg.org/ih/).

For SEM analysis, seeds were taken from herbarium specimens from SPF or M, gold-coated by a sputter-coater (BAL-TEC, Liechtenstein) and analysed with a scanning electron microscope (438VP, LEO, Germany).

Distribution maps were prepared using georeferenced location data obtained from herbarium records (extrapolated or approximated for some old or vaguely specified localities) or own field observations. The underlying maps were generated using DIVA-GIS (Hijmans *et al*. 2005), based on the spatial cartographic data provided by the Brazilian Institute of Geography and Statistics (IBGE; http://www.ibge.gov.br). Names of mountain ranges and massifs used here follow Harley (1988) and Giulietti *et al*. (1997).

A key to the species of *Genlisea* subgenus *Tayloria*

[0. Capsule opening with bivalvate dehiscence, corolla lilac, lavender or white, spur divergent from corolla lower lip .. .. —> § *Tayloria*
- Capsule opening with circumscissile dehiscence, corolla yellow, lilac, bluish or white, with gibbous palate near throat, spur paralleling the lower lip ..... —> § *Genlisea*]

1. Scape thick (more than 1 mm in diam. above the middle in dried specimens), succulent. Upper lip of the corolla longer than (equalling) 5 mm 2
- Scape thin (less than or equalling 1 mm in diam. in dried specimens, but base sometimes to 1.5 mm wide), often filiform. Upper lip of the corolla shorter than 5 mm 4

2. Scapes with a dense indumentum of glandular capitate hairs only. Leaves many, present in flowering plants, arranged in a dense rosette. Fruiting pedicels recurved (apical part of the fruiting pedicel straight, paralleling the scape). Upper lip of the corolla deeply bilobate, subequal to lower lip. Spur equalling (slightly longer than) the corolla upper lip. Corolla deep violet. [Minas Gerais only] ..... *G. metallica*
- Scapes with a dense indumentum of both eglandular simple hairs and glandular hairs. Leaves few, not present in flowering plants (or only single leaves arranged near the base of the scape). Fruiting pedicels circinate (apical part of the fruiting pedicels strongly curved inwards, not paralleling the scape). Upper lip of the corolla entire or upper third bilobate, shorter than the lower lip. Spur distinctly longer than the upper corolla lip. Corolla pale lilac to lavender 3

3. Upper lip entire. Spur longer than lower corolla lip, with apex uncinate (rarely apex straight in single flowers). Pedicel shorter than (equalling) the spur, to 8.5 mm long, strongly circinate in fruit. Corolla without darker venation. Seed papillate (magnifier!). [Bahia only] . ..... *G. uncinata*
- Upper lip bilobate (rarely single flowers of a scape with lobes of the upper lip fused). Spur shorter than (equalling) lower corolla lip, with apex straight. Pedicel distinctly longer than the spur, to 10 mm long, laxly circinate in fruit. Corolla with dark purple veins. Seed not papillate, margins winged (magnifier!). [Minas Gerais only] .... *G. oligophylla*

4. Spur about 1.5–2.0 times longer than the upper corolla lip, or spur more than 5 mm long 5
- Spur shorter than (equalling, or just slightly exceeding) the upper corolla lip, or spur shorter than (equalling) 5 mm. 9

5. Flowering scapes usually longer than (equalling) 25 cm 6
- Flowering scapes shorter than 25 cm..... 7

6. Upper corolla lip much shorter than lower lip (lower lip from tip to base 3–4 times longer than upper lip). Fruiting pedicels recurved but older ones turned upwards from the middle. Scapes with indumentum of glandular capitate and shorter eglandular simple hairs. Leaves 10–40 mm × (3–)8–19 mm, sometimes with sparse cover of microscopic glandular hairs. Plants of perennially wet swamps. [northern Minas Gerais only] ..... *G. flexuosa*
- Upper corolla lip subequal to (slightly shorter than) lower lip (lower lip from tip to base 2.0–2.5 times longer than upper lip). Fruiting pedicels recurved and pointing downwards (rarely gradually arcuated more than 360° and then pointing upwards). Scapes with indumentum of glandular capitate hairs and equally long eglandular simple hairs, or exclusively glandular capitate hairs. Leaves (1.5–)3.0–12.0 mm × (0.5–)1.5–7.0 mm, always glabrous. Annual plants of periodically wet areas. [Minas Gerais, São Paulo] ..... *G. violacea*

7. Corolla white (pale lilac), spur dark purple. Corolla lobes conspicuously emarginate. Fruiting pedicels sharply reflexed (bent downwards abruptly) from the base. Spur narrowly conical, never widened towards the apex. Scapes with sparse indumentum of eglandular simple hairs and fewer glandular hairs. [Espírito Santo and eastern Minas Gerais] ..... *G. lobata*
- Corolla and spur of the same colour, pale lilac to dark violet, rarely white. Corolla lobes entire, or shallowly retuse. Fruiting pedicels recurved (curved downwards arcuated (arching) from the base). Spur saccate, narrowly conical or tubular, narrowed or widened towards the apex. Scapes with dense indumentum of eglandular simple hairs and glandular capitate hairs, with dense indumentum of glandular capitate hairs only, or with sparse indumentum of exclusively glandular capitate hairs. [Minas Gerais, São Paulo, Bahia] 8

8. Corolla length shorter than 7 mm. Sepals and bracts subglabrous. Scapes with sparse indumentum of glandular capitate hairs only. [Minas Gerais only]..... *G. nebulicola*
- Corolla length longer than (equalling) 7 mm. Sepals and bracts with glandular capitate hairs (and usually also eglandular simple hairs). Scapes covered with glandular hairs only, or both glandular capitate hairs and eglandular hairs 9

9. Corolla lower lip 6–8 mm wide. Corolla with open throat, without gibbous marking at the base of the palate (but small greenish-yellow area). Corolla upper lip 2.0–2.5 mm long. Spur 2.0–3.0(–4.0) mm long, saccate or shortly conical. [Bahia only] ..... *G. exhibitionista*
- Corolla lower lip (6.5–)10.0–15.0 mm wide. Corolla with pronounced gibbous marking consisting of two yellow rims at the base of the palate. Corolla upper lip 3.0–5.0 mm long. Spur (2.3–)3.0–5.0(–6.0) mm long, cylindrical, usually widened towards the apex. [Minas Gerais, São Paulo] ..... *G. violacea*

Species descriptions

The species are arranged in order of affinity, with the three large perennial species related to *G. uncinata* first, followed by the species related to *G. violacea*.

1. *Genlisea metallica* Rivadavia & A.Fleischm., *sp. nov.* (Figs. 2, 4, 12A,B, 13E,F, 14)

Species a Genlisea uncinata et Genlisea oligophylla scapis pilis glanduligeris tantum obsitis, pilis eglanduligeris omnio destitutis, et corollae lobis subequalibus diagnoscenda.

Type:—BRAZIL. Minas Gerais: Município de Itacambira, a alguns km de Itacambira pela estrada para Montes Claros, alto da Serra, ca.1300 m, 29 July 2002, *F. Rivadavia & R. Gibson 1366* (holotype SPF!, isotypes BHCB!, M!, NY!).

Rosetted perennial herb, to 30 cm tall, forming short thickened underground stems; rosette dense and compact, comprising 15–40 leaves. *Leaves* spathulate, yellowish green, up to 25 mm long; petiole 1–15 mm long and up to 3 mm wide, grading into the lamina; lamina spathulate to broadly obovate, 10–20 mm × 8 mm.

Inflorescence a raceme, with up to 20 flowers per scape; scapes, bracts, bracteoles and calyx densely covered with indumentum of glandular capitate hairs 0.2–0.3 mm long. *Scapes* 1–3(–6), erect, succulent, self-supporting, up to 300 mm long, terete, diam. 1.0–2.0 mm, occasionally bifurcate or multiple-branched, lower part of the scape with dispersed sterile bracts. *Bracts* narrowly lanceolate to narrowly oblanceolate, 2.5–4.0 mm long and 0.8–1.5 mm wide, from gibbous base, with apex retuse or emarginate, rarely obtuse. *Bracteoles* subulate to narrowly lanceolate, 1.5–2.0 mm long and 0.3–0.5 mm wide, with apex obtuse or emarginate. *Pedicel* terete, ca. 0.5 mm in diam., 5–10 mm long and erect at anthesis, 10–15 mm long and recurved in fruit. *Sepals* 5, oblanceolate to obovate, 4–8 mm × 0.8–1.3 mm. *Corolla* bilabiate, to 13 mm long, deep violet (often with a velvety or metallic shine in living plants, caused by papillae on the corolla upper surface), with a marking on the palate consisting of two yellow ridges surrounded by a paler, white to bluish area; upper lip cuneate to very broadly obovate, 5–8 mm long and up to 8 mm wide, divided to the half into two widely divergent lobes, lobes 3.0–3.5 mm wide, with apex rounded or shallowly retuse; lower lip to transversely elliptic to broadly obtrullate in outline, trilobate, up to 11 mm wide and 8 mm long, lobes subequal, median one 5 mm wide, lateral lobes 4.0–4.5 mm wide; spur up to 5 mm long, equalling (or slightly longer as) the lower corolla lip, cylindrical, straight or slightly curved downwards near the apex, 2 mm in diam., with apex obtuse; corolla margins glabrous, corolla lower surface and spur covered with capitate glands. *Capsule* globose, 2.0–3.5 mm in diameter, covered with capitate glandular hairs, opening septicidally. *Seeds* ellipsoid to prismatic, about two times wider than high, 300–600 µm long, the testa cells isodiametric to slightly elongate, with the anticlinal walls much raised, with veriform transverse ridges, and the periclinal walls slightly concave, minutely verrucose.

Distribution:—Brazil, endemic to Minas Gerais, so far only known from a small population in wet *campo rupestre* near the town of Itacambira in the Serra do Grão Mogol, and from a single collection made on the Pico Itambé. Rare, in localized populations.

Habitat:—At the type location, *G. metallica* grows in a seasonal seepage area, in shallow accumulations of quartzitic sand over or between rocks. It grows near other species of *Genlisea*, but not sympatric with any.

Etymology:—The epithet *metallica* refers to the metallic sheen of the dark violet flowers, caused by microscopic papillae on the upper surface of the corolla.

Conservation Status:—Critically Endangered (CR), according to the criteria of IUCN (2001), as only two small and localized populations are currently known. The *locus classicus* at Itacambira faces potential threat by *Eucalyptus* plantations and cattle ranching (although that place is considered an Environment Protection Area (APA)—as any river and headwater in Brazil is), and thus we strongly recommend to create a government protected area that encompasses the common type location of *G. metallica* and *G. flexuosa* and surrounding highlands, which are rich in numerous other endemic plants species. For example, the same area represents the *locus classicus* of *Drosera grantsaui* Rivadavia (2003: 82) and *D.* × *fontinalis* Rivadavia (2009: 121).

Notes:—*Genlisea metallica* has a very distinctive dark violet flower colour, with a velvety or metallic shine in live specimens, which is caused by small papillae of the corolla epidermis. The stiff and erect, thick scapes covered exclusively by glandular hairs distinguish this species from *G. flexuosa* and *G. violacea*, which both have very slender scapes with a much more sparse indumentum of glandular, and often eglandular hairs. The scapes of *G. metallica* are so densely covered by glandular capitate hairs, that even dried herbarium specimens still remain somewhat viscid.

In contrast to all other species of *Genlisea* subgenus *Tayloria*, *G. metallica* tends to have a much more compact rosette of many overlapping leaves. To survive the dry season, *G. metallica* dies back to turnip-like organs formed by short, succulent vertical underground stems at the base of the flower scapes (Fig. 14). At its type location, *G. metallica* grows within a short distance, but not sympatric with, *G. aurea* Saint-Hilaire (1833: 429), *G. filiformis* Saint-Hilaire (1833: 430), *G. repens* Benjamin (1847: 253), *G. violacea*, *G. oligophylla* and *G. flexuosa*, making Itacambira the place with the highest number of species known throughout the range of *Genlisea*. Moreover, no other area is currently known where more than two species of *G.* subgenus *Tayloria* co-occur (see Figs. 2, 3). This high alpha-diversity observed in *Genlisea* at Itacambira is in contrast with many

other flora elements of the Espinhaço *campo rupestre* vegetation, which show a lower rate of endemism in the northern part of the Serra do Espinhaço of Minas Gerais (eg. Rapini *et al*. 2002, Echternacht *et al*. 2011). *Genlisea metallica* was referred to as *G.* sp. 'Itacambira' in the phylogenetic reconstructions of Fleischmann *et al.* (2010).

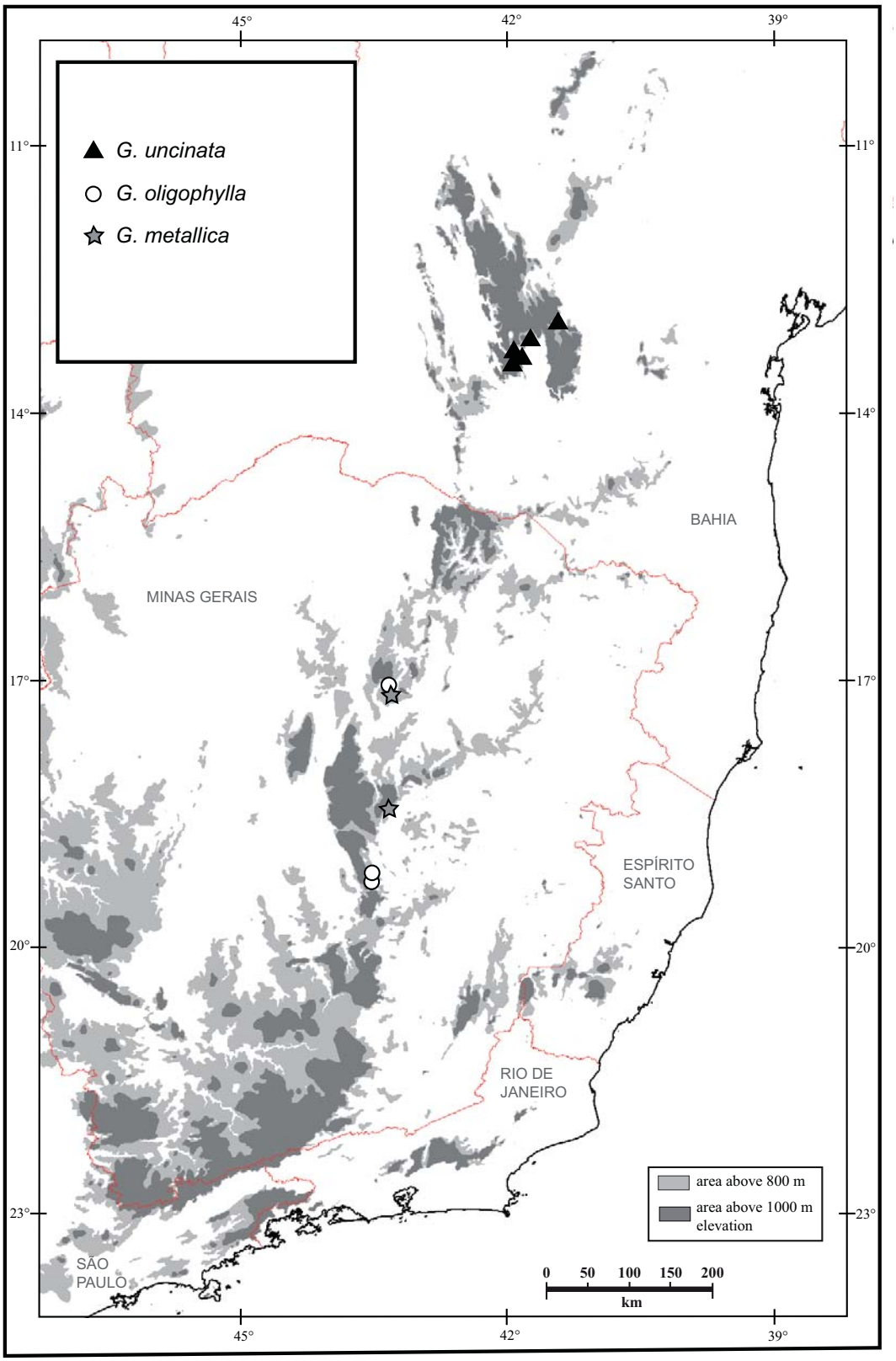


FIGURE 2. Distribution map of the *Genlisea* subgenus *Tayloria* species of affinity to *G. uncinata* (large perennial species with thick scapes).

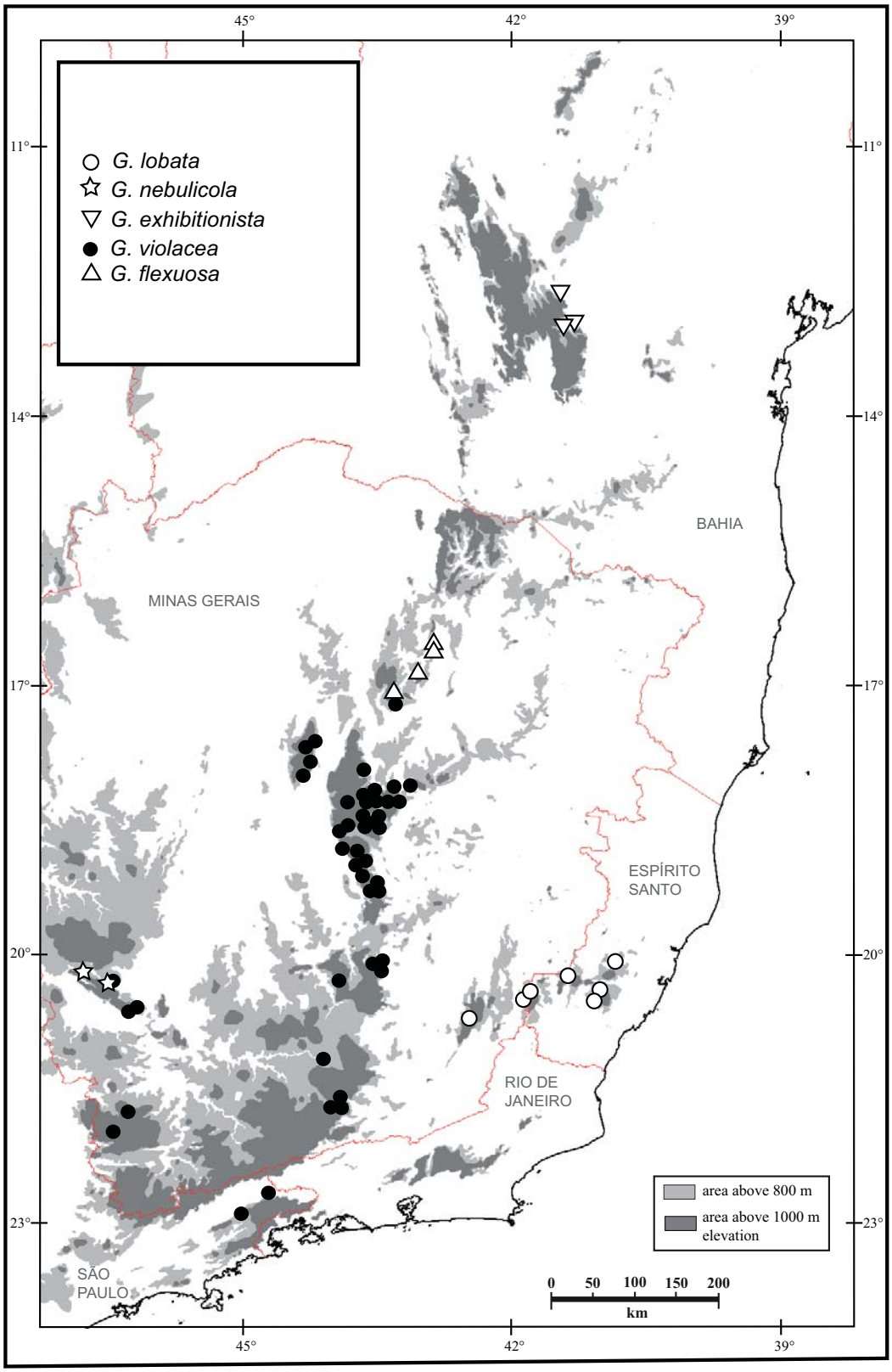


FIGURE 3. Distribution map of the *Genlisea* subgenus *Tayloria* species of affinity to *G. violacea.*

Additional specimens examined (paratypes):—BRAZIL. Minas Gerais: Município de Itacambira, estrada para Montes Claros, ca.1300 m, 14 July 1999, *F. Rivadavia & F. Pinheiro 1139* (SPF); 22 April 2010, *P.M. Gonella et al. 293* (SPF). Município de Santo Antonio do Itambé, descida do Morro do Pico Itambé, ca. 1000 m, [the elevation given as 3068 m is clearly wrong], 06 April 1982, *I. Rossi et al. CFCR 3093A* (K, SPF).

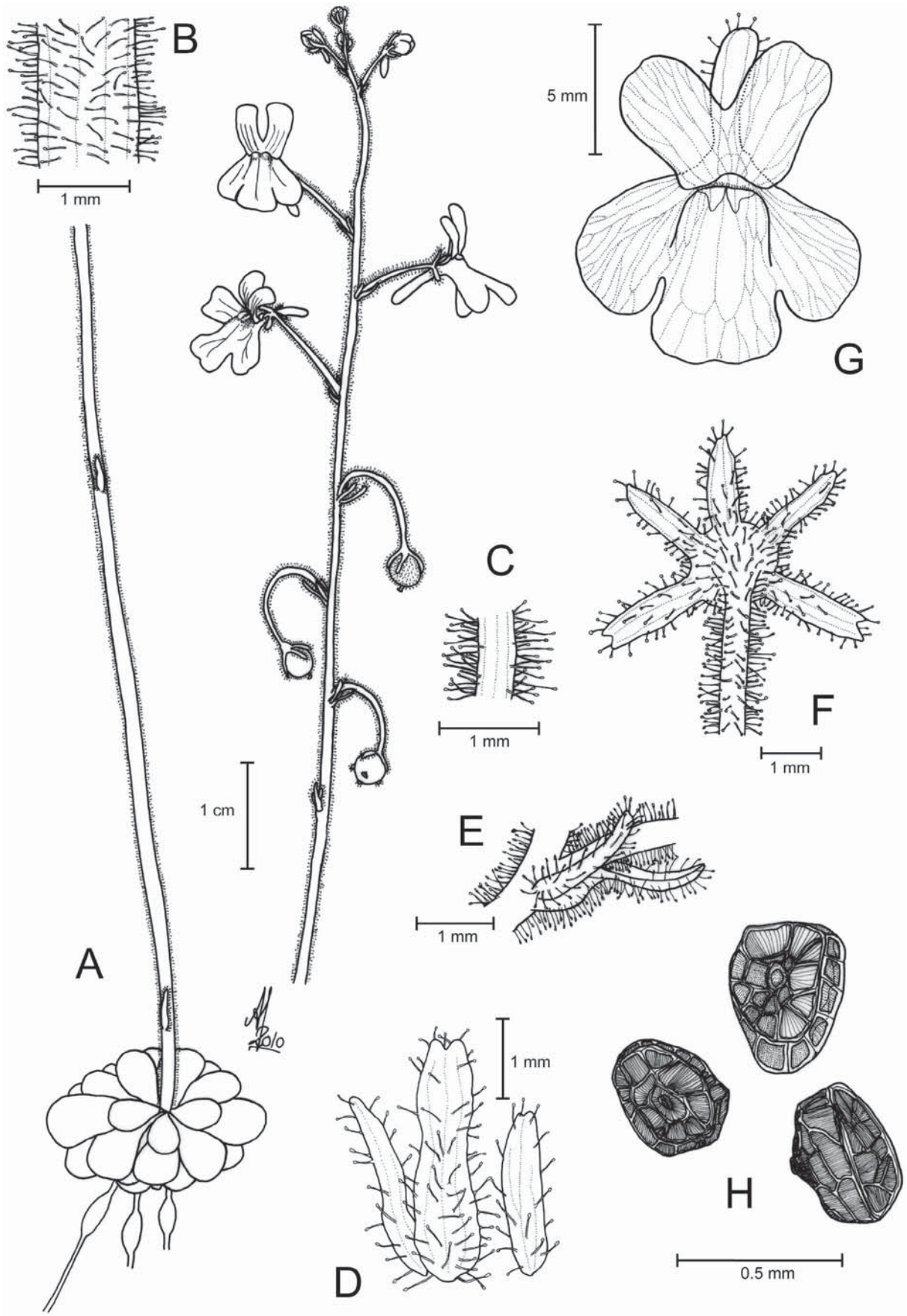


FIGURE 4. *Genlisea metallica* (all from *P.M. Gonella et al. 293*). **A,** habit. **B,** section of the scape. **C,** section of the pedicel. **D,** bract and bracteoles. **E,** insertion of bract and bracteoles to the peduncle. **F,** calyx. **G,** corolla. **H,** seeds. Drawing by A. Fleischmann.

2. *Genlisea oligophylla* Rivadavia & A.Fleischm., *sp. nov.* (Figs. 2, 5, 12C,D, 13C,D)

Genliseae uncinatae similis sed corollae labio superiore bilobato, corolla reticulato-venosa, calcari apicem versus non uncinato, calcari corollae labio inferiore paulo breviore vel subaequante, pedicello quam calcar longiore, et seminibus prismaticis alatis differt.

Type:—BRAZIL. Minas Gerais: Município de Santana do Riacho, bifurcação da estrada para Conceição do Mato Dentro e Morro do Pilar, ca. 1450 m, 23 February 1996, *F. Rivadavia & J. Mullins 535* (holotype SPF!, isotypes BHCB!, M!).

Robust perennial herb, up to 50 cm tall. *Leaves* few, spathulate, forming a lax rosette; petioles subterraneous, lamina orbiculate to transverse broadly obovate; leaves usually not present at anthesis. *Inflorescence* a lax raceme, with up to 25 flowers; scape, bracts, bracteoles, pedicels and calyx densely covered with glandular capitate hairs up to 0.8 mm long and fewer eglandular simple hairs of equal length; scape indumentum denser towards the apex; *Scapes* solitary, erect, succulent, self-supporting, terete, ca. 2–3 mm in diam., up to 50 cm long, rarely bifurcate or branched, lower part of the scape with dispersed sterile bracts.

Bracts lanceolate, up to 2.5 mm long and 0.8 mm wide, with gibbous base. *Bracteoles* subulate, 1–2 mm long, 0.3 mm wide. *Pedicels* up to 10 mm long at anthesis, ca. 0.5 mm in diam., terete, glandular, erect at anthesis, enlarged and circinately curved downwards in fruit.

Sepals 5, lanceolate to oblong, ca. 1 mm wide, 2.0–2.5 mm long. *Corolla* up to 18 mm long, pale lavender to lilac-bluish, with dark purple reticulate venation along the nerves, base of the lower lip whitish with two yellow spots on the two vertical ridges of the palate; upper corolla lip very broadly obovate, up to 5 mm long and 9 mm wide, deeply bilobate to the middle (rarely only shallowly truncate), lobes divergent, each lobe ca. 4 mm wide, oblong to rectangular in outline, with apex retuse; lower corolla lip 3-lobate, the entire limb broadly obtrullate to broadly oblong in outline, up to 10 mm long and 14 mm wide, lobes subequal in size, the median lobe ca. 6 mm wide, lateral lobes ca. 5 mm wide, lobe margins entire or slightly crenulate; spur narrowly cylindrical, straight, about as long as or slightly shorter than the lower lip of the corolla, 6–8 mm long, ca. 1 mm in diameter, with apex obtuse or subacute; back of the corolla, margins of the corolla lobes and spur sparsely covered with glandular capitate hairs. *Capsule* globose, 2–4 mm in diameter, glandular. *Seeds* prismatic, 4–6-angled, 300–350 µm long and 250–300 µm wide, ca. 100 µm high, broadly winged on the angles, the testa cells elongate (to isodiametric on the distal surface), with the anticlinal walls much raised, straight, and the periclinal walls tabular, microscopically rugose.

Distribution:—Brazil, endemic to Minas Gerais, so far only known from a few localities in the Serra do Cipó and from a single population near the town of Itacambira in the Serra de Grão Mogol.

Habitat:—Moist drainage sites, in humid and peat-based sandy soils, between tall grasses, growing in close proximity to other *Genlisea s*pp. (including *G. flexuosa*, *G. metallica*, and *G. violacea*), *Drosera* spp. and *Utricularia* spp.

Etymology:—The specific epithet *oligophylla* refers to the few photosynthetic leaves of this taxon (often single or no leaves present during flower anthesis).

Conservation Status:—Critically Endangered (CR). A few very small populations (often single individuals) occur inside protected areas of the Serra do Cipó National Park, however any of the populations outside borders of this park are under high threat of destruction by cattle farming, *Eucalyptus* plantations, and mining activity.

Notes:—*Genlisea oligophylla* shares with *G. uncinata* the thick, erect succulent scapes, which in both species seem to represent the main photosynthetic organ during anthesis and fructification, as leaves are infrequent or absent in flowering specimens of both taxa (pers. obs.; Rivadavia 2000). *Genlisea oligophylla* differs from *G. uncinata* in its upper corolla lip, which is always divided into two distinct lobes in the upper part (entire, or apex shallowly retuse in *G. uncinata*), the distinctive dark purple venation of the lavender corolla (of uniform lilac colour in *G. uncinata*), a spur which is not curved hook-like, and which is slightly shorter than or as long as the corolla lower lip (curved, or rarely not curved in some exceptional specimens of *G. uncinata*, but always distinctly longer than the lower lip). Furthermore, *G. oligophylla* differs in having

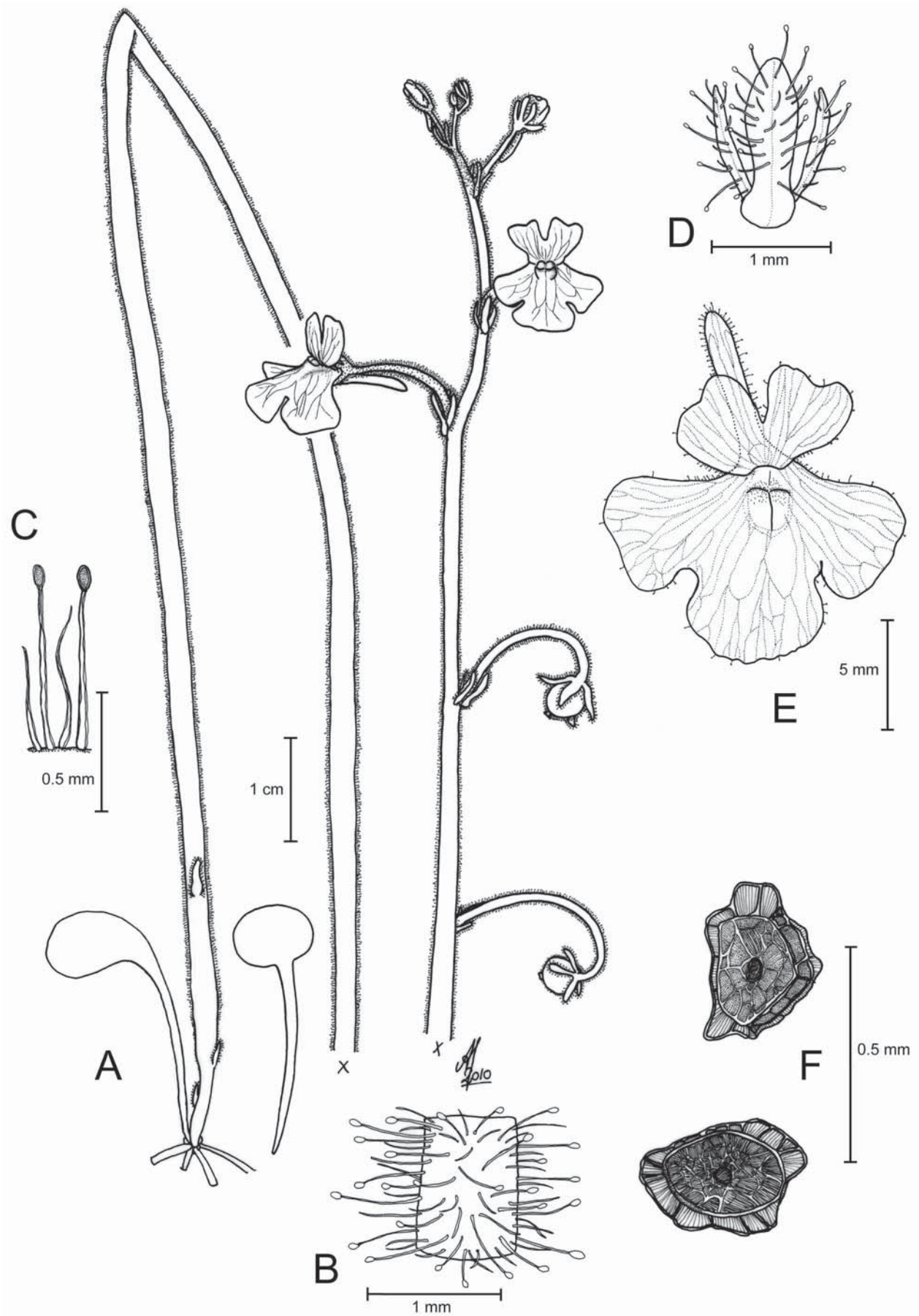


FIGURE 5. *Genlisea oligophylla* (all from *P.M. Gonella et al. 227*). **A,** habit, one leaf detached. **B,** section of the scape. **C,** scape indumentum. **D,** bract and bracteoles. **E,** corolla. **F,** seeds. Drawing by A. Fleischmann.

pedicels at anthesis longer than the spur (shorter than the spur in *G. uncinata*), which are enlarged and widely circinate in fruit (not much enlarged and strongly circinate in fruit in *G. uncinata*), and winged prismatic seed (papillate ellipsoid seed in *G. uncinata*).

Fromm-Trinta (1996) cited *G. uncinata* for the Serra do Cipó, but the respective specimens she studied actually represent *G. oligophylla*. Curiously, the seed shape illustrated for those Cipó specimens is papillate, which suggests that she may not have seen the actual seeds and simply based her drawings on seed belonging to *G. uncinata* from Bahia. Unfortunately, no voucher specimens are given for the source of the drawing. However, no specimens of *G. oligophylla* examined for the present study did show papillate seed, including those cited by Fromm-Trinta (1996).

Genlisea uncinata and *G. oligophylla* are geographically widely separated, with *G. oligophylla* currently only known to occur in the central sections of the Serra do Espinhaço in Minas Gerais, South-Eastern Brazil, ca. 500 km distant from the closest known populations of *G. uncinata*, which is endemic to the Chapada Diamantina mountain range of central Bahia state, North-Eastern Brazil. The higher elevation *campo rupestre* habitats of these two species are separated by lower elevation areas covered with dry *cerrado* and *caatinga* vegetation, most notably the fragmented sandstone foothills which lie between the Chapada Diamantina and Serra do Espinhaço. Today, these intermediate areas represent a climatic and edaphic barrier for the exchange of montane flora from north to south or vice versa. Harley (1988) postulates that this barrier probably already acted as such in the past, when the area could have been drier or covered by forest. However, *campo rupestre* vegetation may have occupied these foothills during cooler periods, which then may have served as stepping stones for species migration in either direction, and therefore might explain extant species disjunctions in the floras of the two highlands of the Espinhaço Mountain Range (Antonelli *et al*. 2010).

Additional specimens examined (paratypes):—BRAZIL. Minas Gerais: Município de Itacambira, estrada para Montes Claros, ca.1285 m, 05 March 1997, *F. Rivadavia 609* (SPF). Município de Santana do Riacho, Serra do Cipó, 1938, *J. Badini & H.L. Mello Barreto s.n.* (OUPR 2111); km 135 da rodovia Belo Horizonte Conceição do Mato Dentro, Parque Nacional da Serra do Cipó, 20 May 1989, *A.M. Giulietti & J.R. Pirani CFSC 11442* (SPF); Serra do Cipó, bifurcação da estrada para Conceição do Mato Dentro e Morro do Pilar, ca.1450 m, 04 April 2003, *F. Rivadavia 1549* (SPF); atual km 142 (antigo km 149), ca. 1350 m, 17 May 2008, *P.M. Gonella et al. 119* (SPF); 07 April 2009, *P.M. Gonella et al. 227* (SPF). Município de Conceição do Mato Dentro, ca. 70 km NE of Belo Horizonte, Serra do Cipó km 149, estrada do Pilar, 03 November 1938, *H.L. Mello Barreto 8915* (BHCB, F (not seen, K photo!)) [the duplicate at BHCB consists of a single sterile scape of *G. oligophylla*, and eight much more slender scapes of *G. violacea*]. Município de Santa Luzia, ca. 70 km NE of Belo Horizonte, Serra do Cipó km 137, estrada de Conceição, 04 February 1938, *H.L. Mello Barreto 8968* (K photo!).

3. *Genlisea uncinata* P.Taylor & Fromm (Figs. 2, 6, 12E,F, 13A,B)

Type:—BRAZIL. Bahia: Serra do Sincorá, ca. 15km NW of Mucugê on the road to Guiné and Palmeiras, 1300–1500m, 19 March 1988, *R.M. Harley et al. 20980* (holotype CEPEC!, isotypes K!, R, NY, U image!, P).

Robust perennial herb, to 80 cm tall. *Leaves* few, especially during anthesis, slightly succulent, spathulate; petiole narrowly cuneate, 1–5 cm long; lamina obovate to transversely elliptical, up to 15 mm long and 6 mm wide. *Inflorescence* a dense raceme, multi-flowered (up to 21 flowers; Taylor & Fromm-Trinta 1983); scape, bracts, bracteoles and pedicels densely covered by glandular capitate and fewer eglandular simple hairs to 0.8 mm long; indumentum denser towards the apex of the scape; calyx and ovary covered by glandular capitate hairs only. *Scapes* solitary, thick, succulent, terete, 23–80 cm long, up to 3.5 mm in diam. at the base, erect, simple, rarely bifurcate or branched, covered with numerous sterile gibbous bracts dispersed along the whole length. *Bracts* ovate, 2.0–3.3 mm long, 0.5–1.3 mm wide, with gibbous base, glandular. *Bracteoles* lanceolate, 1.3–1.5 mm long, 0.2–0.3 mm wide, glandular. *Pedicels* terete, 6.0–8.5 mm long, up to 1 mm in diameter, erect at anthesis, circinate and pointing downwards in fruit. *Sepals* 5, subequal, ovate, elliptical or oblong,

2.0–2.5 mm long, up to 1 mm wide, the lowermost two slightly curved, densely covered exclusively with glandular capitate hairs. *Corolla* bilabiate, up to 20 mm long, pale lavender to lilac-bluish, base of the lower lip with two yellow marks on the two vertical ridges of the palate; upper lip entire, semicircular to very broadly ovate, ca. 6 mm long and 6–9 mm wide, apex obtuse or slightly emarginate; lower lip 3-lobate, the entire limb approximately semicircular to broadly obtrullate in outline, 7–9 mm long and 13–17 mm wide, lobes subequal, the median one slightly larger, lobe margins rounded or slightly undulate; spur conical, from slightly constricted base, with apex uncinate (rarely in some exceptional specimens with apex straight), longer than the pedicel and longer than the lower corolla lip, up to 11 mm long and to 3 mm at its greatest width; back of the corolla, margins of the corolla lobes and spur densely covered with glandular capitate hairs. *Capsule* globose, ca. 3.0–4.5 mm in diameter, longitudinally bivalvate, densely covered with glandular capitate hairs. *Seeds* ellipsoid, 350–600 µm long, 250–350 µm wide, 150–200 µm high (all dimensions including papillae), testa papillose, with membranous flat papillae up to 130 µm long, papillae with striate reticulation.

Distribution:—Brazil, endemic to Bahia, where it is known from the high elevation sandstone massifs of the Serra das Almas, Serra do Barbado, Serra da Mesa and Serra do Sincorá in the Chapada Diamantina Mountain Range. Fromm-Trinta's (1996) erroneous report of *G. uncinata* for Minas Gerais, from the Serra do Cipó, is based on two specimens representing *G. oligophylla* (see under that species).

Habitat:—High montane to subalpine, (1300–)1500–2033 m. Mostly growing among tall grasses in moist or damp peaty or quartzitic soils, but also growing in wetter organic soil on seepage sites and along streamsides at higher elevations. Many of the high elevated mountain tops of the Chapada Diamantina Range are often cloud-covered at night even during the dry winter, and thus nocturnal condensation is a major source of water in the dry season for numerous plants of the *campos rupestres* (Harley & Simmons 1986). Even in late July (the dry season), the soil in the high montane habitats of *G. uncinata* was found to be relatively moist (Rivadavia 2000).

The higher elevated areas of the Chapada Diamantina may receive two peaks of rainfall per year: the first main rainy season is concentrated between November and January (sometimes extending to April), and a second shorter and less regular one from May to June (occasionally to July) (Zappi *et al*. 2003). This additional precipitation during the dry season is concentrated on the high mountain tops, and occurs when the temperatures suddenly drop at night. Together with the dew from condensation, it allows evergreen vegetation (with xeromorphic adaptations) to occur in the high montane areas (Zappi *et al*. 2003). These are the perennially moist areas of *campo rupestre* vegetation, where *G. uncinata* predominantly occurs. But it can also be found on high mountain passes, where wind is funnelled from one side of the highlands to the other, and thus more humidity accumulates and condensates along these natural corridors. At these lower elevations, *G. uncinata* plants were often observed growing localized in the lee of large rocks, and in pockets of soil on wet vertical cliffs, whereas the plants are more common and widespread in the *campos rupestres* on the summit areas.

Conservation Status:—Near Threatened (NT). *Genlisea uncinata* is locally endemic (although thought to be more widespread on other summits throughout the Chapada Diamantina), but most of the known locations are situated on remote mountain tops of the Chapada Diamantina, thus are considered relatively well preserved, although not all are protected within National Parks.

Notes:—The large *Genlisea uncinata* is not likely to be confused with any other species. It is the only species of *G.* subgenus *Tayloria* with a spur longer than the pedicel at anthesis, and with an entire upper lip (although an undivided upper lip can be found in exceptional single flowers of some specimens of *G. oligophylla*. For differences between the closely related species *G. uncinata* and *G. oligophylla*, see under the latter species). Moreover, the papillate seeds of *G. uncinata* are very distinctive, and do not correspond with the seed morphology of any other species of *Genlisea.* The thick, succulent, tall erect scape of *G. uncinata* is also very diagnostic, and further only found in *G. oligophylla* and (to a lesser degree) in *G. metallica*. *Genlisea uncinata* bears the tallest inflorescences of all *Genlisea* species, with scapes up to 80 cm tall (up to 120 cm in cultivation; Rivadavia 2000), and as thick as a pencil near the base (Rivadavia 2000).

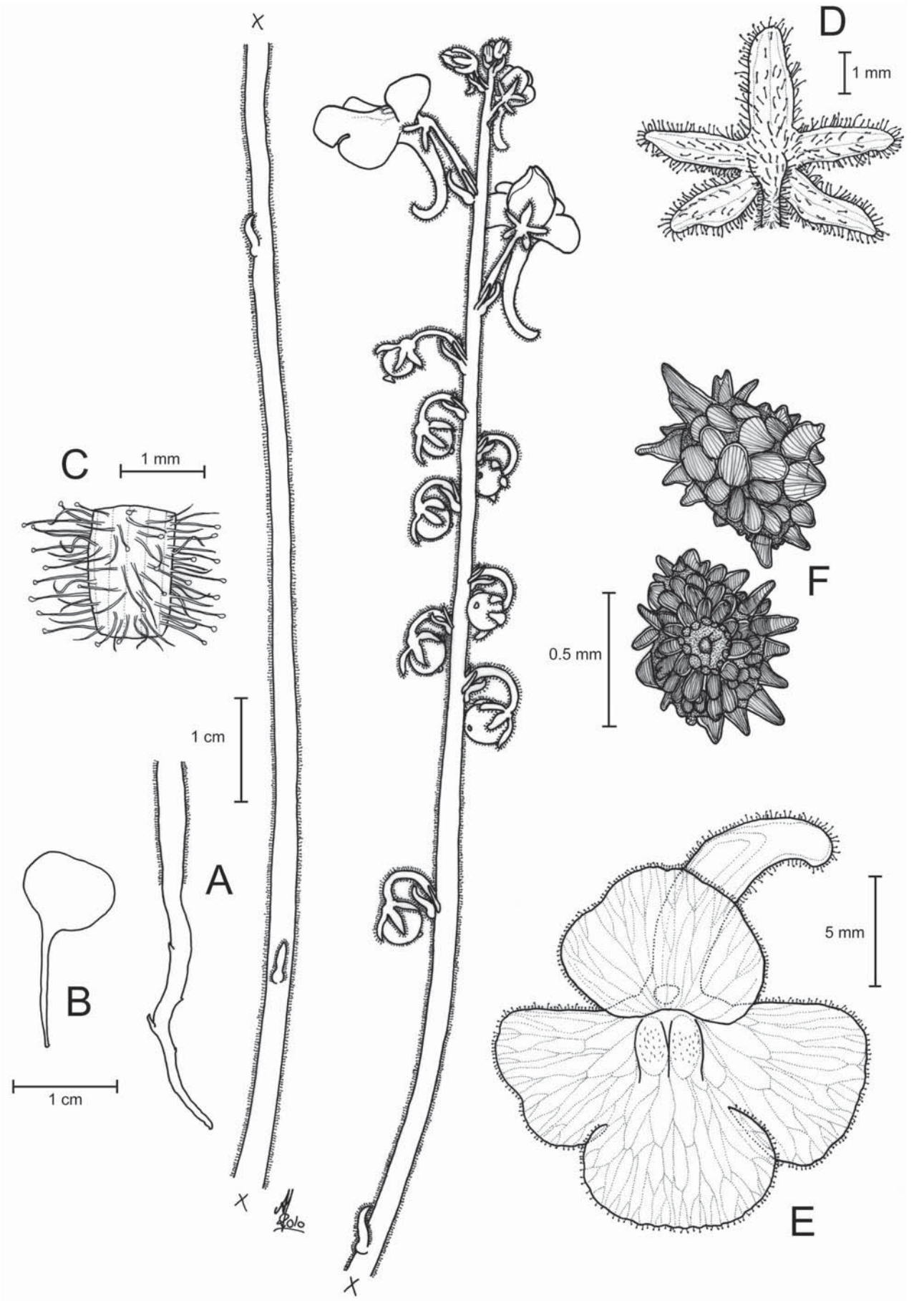


FIGURE 6. *Genlisea uncinata*. **A,** habit (*Harley et al. 20980*). **B,** leaf. **C,** section of pedicel. **D,** calyx. **E,** corolla. **F,** seeds (**B**–**C** from *F. Rivadavia 471*). Drawing by A. Fleischmann.

In *G. uncinata*, as well as in the related *G. oligophylla*, the leaves and the thick succulent inflorescences originate from a short vertical subterraneous stem, ca. 1–5 cm below ground. This is considered an adaptation to anchor and give support to the thick and tall inflorescence scapes (Rivadavia 2000). Moreover, the underground stem most likely also helps the plants to survive fires, as the *campo rupestre* vegetation within of the Chapada Diamantina frequently experiences seasonal burning (Funch *et al*. 2009).

The strongly circinate, short fruiting pedicels of *G. uncinata* (usually curved inwards forming a circle of almost 360°) are not found in any other New World member of the genus. A homologous pedicel circination is only observed in the three African members of *Genlisea* section *Recurvatae* Fleischmann *et al.* (2010: 781).

Genlisea uncinata has been revealed to represent the most basal branch of *G.* subgenus *Tayloria* (Fleischmann *et al*. 2010). Interestingly, it shares floral characters of both subgenera: the entire upper corolla lip of *G.* subgenus *Genlisea* (an undivided upper corolla lip is found in all members of *G.* subgenus *Genlisea*, whereas members of *G.* subgenus *Tayloria* have an upper lip that is bilobate), and a slightly expressed gibbous mask of the lower lip palate, which is not found in any other member of *G.* subgenus *Tayloria*. But the overall flower design (spreading spur) and corolla colouration fits the typical pattern of *G.* subgenus *Tayloria*, as well as the defining capsule dehiscence.

Specimens examined:—BRAZIL. Bahia: Município de Abaíra, Campo do Cigano, 1780 m, 12 February 1992, *R.M. Harley et al. H52003* (K, SPF); Riacho da Taquara, 1650–1800 m, 24 February 1992, *E. Nic Lughadha et al. H52162* (K, SPF); Garimpo do Bicota, 1530 m, 24 March 1992, *B. Stannard & T. Silva H52806* (K, SPF); Catolés, Serra do Barbado, 1750–2033 m, caminho da forquilha da Serra, 26 February 1994, *P.T. Sano et al.* CFCR 14625 (SPF); Catolés, subida do Morro do Barbado pela Serra do Guarda Mor, 1700–1950 m, 28 July 1995, *F. Rivadavia 487* (SPF); Serra dos Cristais, 1960 m, 08 June 1994, *W. Ganev 3335* (K, SPF); Catolés, Serra dos Cristais, 1600 m, 20 May 1999, *V.C. Souza et al. 22881* (ESA); trilha para o Campo da Mutuca, Serra da Mesa, vila de Catolés, ca. 1540 m, 15 July 2005, *F. Rivadavia et al. 2026* (SPF); trilha para o Pico do Barbado e Campo da Mutuca, 1540 m, 28 April 2010, *P.M. Gonella & A. Fleischmann 327* (SPF). Município de Mucugê, no alto da serra ao norte da cidade, 1300–1450 m, 24 July 1995, *F. Rivadavia 471* (SPF).

4. *Genlisea flexuosa* Rivadavia, A.Fleischm. & Gonella, *sp. nov.* (Figs. 3, 7, 12G,H, 13G,H)

Genliseae violaceae similis sed foliis majoribus, scapis longioribus et flexuosis prostratis, calcari labio superiore 1.5–2.0-plo longiore, labio superiore labio inferiore multo breviore, pedicellis fructigeris deorsum recurvatis medio sursum flexis.

Type:—BRAZIL. Minas Gerais: Município de Itacambira, a alguns km de Itacambira pela estrada para Montes Claros, 1300 m, 12 February 2011, *P.M. Gonella et al. 394* (holotype SPF!, isotypes BHCB!, M!, NY!).

Rosetted perennial herb, to 60 cm tall; rosette lax, comprising ca. 8–20 spathulate leaves; rosette diameter up to 75 mm. *Leaves* spathulate, 10–40 mm long; petiole (4–)9–25 mm long and up to 18 mm wide, gradually widened into the lamina; lamina obovate to oblongate, (3–)10–30 mm × (3–)8–19 mm, glabrous or adaxial surface with sparse glandular hairs up to 0.2 mm long. *Inflorescence* a lax raceme, many-flowered, with up to 30 flowers, occasionally bifurcate or multiple branched; scape, bracts, bracteoles and calyx densely covered by an indumentum consisting of glandular capitate hairs 0.2–0.3 mm long and numerous simple eglandular hairs 0.1–0.3 mm long. *Scapes* 1–2(–4), 100–600 mm long, terete, diam. ca. 0.5 mm (to 1.5 mm near the base), long and flexible and supported by nearby grasses and herbs. *Bracts* narrowly obovate to linear-triangular, up to 20 mm long and 0.5 mm wide. *Bracteoles* subulate, up to 15 mm long and 0.2 mm wide. *Pedicels* terete, ca. 10–15 mm and erect at anthesis, curving downwards after anthesis for fructification, but curved upwards again and often twisted around nearby grasses and herbs when capsules split open; adaxial side of pedicels covered with both glandular and shorter eglandular hairs, abaxial side with glandular hairs only. *Sepals* 5, lanceolate to obovate, 4–8 mm × 0.8–1.3 mm. *Corolla* up to 17 mm long, pale lavender to

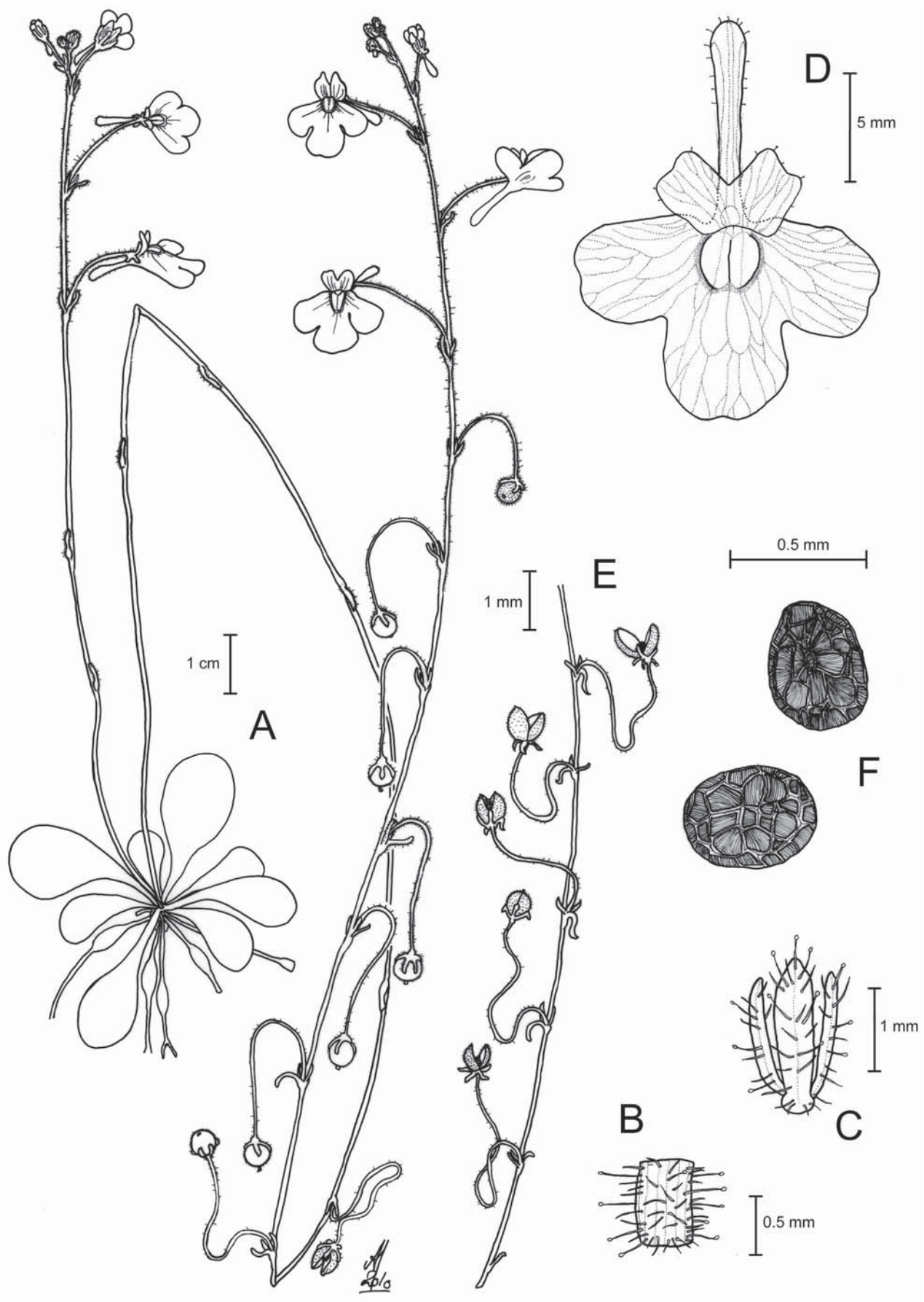


FIGURE 7. *Genlisea flexuosa* (all from *P.M. Gonella et al. 292*). **A,** habit. **B,** section of the scape. **C,** bract and bracteoles. **D,** corolla. **E,** mature infructescence. **F,** seeds. Drawing by A. Fleischmann.

lilac-bluish, with two yellow ridges forming a marking of ca. 3 × 3 mm at the base of the lower lip, the yellow marking surrounded by a darker purple or bluish line, and the upper lip often with darker purple streaks along the nerves; upper corolla lip broadly oblong, up to 5 mm long and 7 mm wide, upper third deeply divided into two divergent lobes, each lobe ca. 3 mm wide, with apex retuse or rounded; lower corolla lip up to 10 mm long and 15 mm wide, semicircular to broadly obtrullate in outline, trilobate, lobes subequal in size, median lobe ca. 6 mm wide, lateral lobes ca. 5 mm wide, apex of each lobe slightly retuse; spur cylindrical, straight (rarely slightly curved downwards near the apex), widening towards the tip, much longer than the upper lip of the corolla, 5–8 mm long, 1.5–2.0 mm in diameter, with apex obtuse. *Capsule* globose to broadly ovoid, 3.0–3.5 mm in diameter, opening septicidally. *Seeds* globose or broadly ellipsoid to slightly prismatic, 0.3–0.6 mm long, testa reticulate, testa cells large, polygonal, isodiametric, with the anticlinal boundaries straight and much raised, and the periclinal walls tabular, with minutely verrucose broad longitudinally striate bands (continuing on the anticlines).

Distribution:—Brazil, Minas Gerais, endemic to the highlands of Itacambira, Botumirim and Grão Mogol.

Habitat:—Wet seepage sites, swamps, and damp areas in montane regions (ca. 700–1400 m), on peat-based soils, quartzitic sand or among rocks, in open places, among tall grasses and sparse shrubs. Often growing together with other perennial carnivorous plants (*Utricularia* spp., *Drosera* spp., as well as *Genlisea repens* and *G. aurea*).

Etymology:—The specific epithets refers to the long and flexuous scapes, but also to the fact that the recurved fruiting pedicels reflex upwardly for some part at maturity.

Conservation Status:—Near Threatened (NT). *Genlisea flexuosa* is somewhat widespread in northern Minas Gerais, but restricted to localized wetlands. Except those populations that fall within the borders of the State Park of Grão Mogol, these areas are not protected. *Campo rupestre* habitats are quite sensitive to habitat destruction, and many locations in Serra do Espinhaço are under threat of habitat loss by mining activity.

Notes:—*Genlisea flexuosa* resembles a robust specimen of *G. violacea*, as it is larger in all parts, except for the flower size. It can be distinguished from *G. violacea* in vegetative state by the much larger spathulate leaves, which are arranged in a lax rosette. When in flower, *G. flexuosa* is distinguished by the usually taller scapes with more numerous flowers, and the proportion of the spur to the corolla upper lip: in *G. flexuosa*, the spur is distinctly longer (about twice as long) as the upper corolla lip, whereas in the quite variable *G. violacea*, the spur is about as long as the corolla upper lip or slightly shorter (rarely slightly longer). Moreover, the upper lip of *G. flexuosa* is much smaller in relation to the lower lip (size ratio ca. 1:3–4), and both form an acute to right (or slightly obtuse) angle. In *G. violacea*, the upper corolla lip has about 0.4 to half the size of the lower lip, and both join at an obtuse to straight angle. Size ratios of the corolla are already consistent in freshly opened flowers, whereas the angle between the corolla lips is correctly expressed in fully mature corollae only.

Both species also differ in life strategy and habitat preferences: the perennial *G. flexuosa* prefers generally wetter habitats (swamps, bogs and perennial seepages) comparing to the annual *G. violacea*, which occurs on seasonal dripping moss-covered rocks or walls, or periodically wet or moist sandy soils.

At the northernmost range of *G. violacea*, especially in the Serra do Cabral, Serra de Grão Mogol, in the Parque Estadual do Rio Preto, and between the towns of Diamantina and Gouveia, rare populations of very large and robust specimens of *G. violacea* can be found (see under *G. violacea* "specimens examined"). These large plants share several characters with *G. flexuosa*, such as long spurs that much exceed the upper corolla lip, and very long scapes. However, they lack other characteristics typical of *G. flexuosa*, such as the large rosettes and long, broadly spathulate leaves, or the scape indumentum of glandular and shorter eglandular hairs. Otherwise, they resemble *G. violacea* in all vegetative parts, and do not form the long flexuous scapes with upwardly recurved, twining fruiting pedicels. Like the perennial *G. flexuosa*, which inhabits grass-covered areas of perennially wet swamps, these large plants are mostly confined to wet, peaty habitats, in contrast to the annual *G. violacea*, which inhabits seasonally wet, open habitats. The distinct characters of *G. flexuosa* and the respective large *G. violacea* plants are maintained stable in cultivation, if kept under identical

growing conditions, thus ecologically induced variation can be excluded. As all morphological characters of the robust plants in question (except scape or spur lengths), conform to typical *G. violacea*, we here assign them to *G. violacea*. However, considering the fact that these large plants are found in areas up to 180 km south of where the ranges of *G. violacea* and *G. flexuosa* overlap, we cannot exclude a potential hybrid origin. Further evidence that *G. flexuosa* and *G. violacea* represent distinct taxa is the fact that both species have been observed to occur in close proximity, but in ecologically separated habitats (eg. near the town of Itacambira).

Genlisea flexuosa frequently and abundantly reproduces asexually by small plantlets budding from the apical, helically twisted part of the trap leaves. This mode of vegetative reproduction was also observed—to a lesser degree—in many other species of *Genlisea* by the authors of the present work, and is known to occur less frequently in cultivated plants of all members of *G.* subgenus *Tayloria*. *Genlisea flexuosa* was referred to as *G.* aff. *violacea* 'giant' in the phylogenetic reconstructions published in Fleischmann *et al.* (2010), and as *G. violacea* in Fromm-Trinta (2004).

Interestingly, plants of *G. flexuosa* from Itacambira and Botumirim bear small glandular capitate hairs sparsely scattered on the adaxial surface of the lamina. Glandular leaves have not been observed in any other species of *Genlisea* so far, all of which have entirely glabrous leaves, although some species (most notably *G. aurea*) are known to prolifically produce mucus from their rosette leaves.

Genlisea flexuosa is the only member of *G.* subgenus *Tayloria* with generally climbing inflorescences: the scapes are thin, long and flexible, and self-supporting only before anthesis. During anthesis, the scapes lean for support on nearby grasses and herbs. The pattern of the fruiting pedicel reflexion of *G. flexuosa* is unique among *Genlisea*: like in all members of *G.* subgenus *Tayloria*, the pedicels of pollinated flowers bend down after anthesis as soon as the capsule develops, so that the fruiting capsules will face the ground. In *G. flexuosa*, however, the ripe capsules are not pointing downwards when these split open, but face upwards as the pedicel curves or twists again at about half of its length when the seed capsules mature (Fig. 7E). Often, the apical part of the pedicel is twisted several times, twining around nearby grasses. Thus, the scape of the infructescence becomes anchored in the surrounding vegetation, and the ripe capsules are well exposed above the ground when they open. In the other species of *G.* subgenus *Tayloria* (most notably *G. violacea* and *G. lobata*), exceptional specimens sometimes have fruiting pedicels gradually arcuated more than 360°, and then pointing upwardly (Fig. 11A). However these are never abruptly bent upwards at about half of their length, as in *G. flexuosa*, but are continuously arcuately curved from the base.

Near the town of Itacambira, *G. flexuosa* can be found growing in close proximity to *G. metallica*, and a putative hybrid between both species was encountered by two of the authors, AF and PMG (*P.M. Gonella et al. 294*). The possible hybrid plant was growing sympatrically with *G. flexuosa* in a swampy area, and shared intermediate vegetative and floral characters of both parent species.

Additional specimens examined (paratypes):—BRAZIL. Minas Gerais: Município de Itacambira, ao lado da estrada para Itacambira, 16 December 1994, *F. Rivadavia 312* (SPF); a alguns km de Itacambira pela estrada para Montes Claros, ca.1330 m, 05 March 1997, *F. Rivadavia 599* (SPF); 14 July 1999, *F. Rivadavia & F. Pinheiro 1138* (SPF); 13 October 2001, *F. Rivadavia 1287* (SPF); 22 April 2010, *P.M. Gonella et al. 292* (SPF); à direita da estrada para Montes Claros, ca. 1300 m, 29 July 2002, *F. Rivadavia & R. Gibson 1365* (SPF). Município de Botumirim, alto da Serra da Canastra, ca. 1375 m, 13 October 2001, *F. Rivadavia 1269* (SPF); Várzea da Estiva, seguindo em direção ao paredão rochoso no fundo da várzea (vereda), 12 February 2011, *P.M. Gonella et al. 389* (SPF); margem direita do Rio do Peixe, 794 m, 6 September 2011, *F. Rivadavia 2705* (SPF). Município de Grão Mogol, Vale do Ribeirão das Mortes, próximo à nascente, 1050–1100 m, 23 May 1987, *J.R. Pirani & R. Mello-Silva CFCR 10843* (SPF); trilha da Tropa, 02 June 1994, *F. Rivadavia 277* (SPF); trilha saindo de Grão Mogol, 09 September 1994, *F. Rivadavia 304* (SPF).

5. *Genlisea exhibitionista* Rivadavia & A. Fleischm., *sp. nov.* (Figs. 3, 8, 12J, K, 13N, O)

Genliseae violaceae similis sed corolla 6–8 mm lata, palato subnullo, corollae labio superiore 2.0–2.5 mm longo, calcari breviore saccato vel breviter conico.

Type:—BRAZIL. Bahia: Município de Mucugê, no alto da Serra ao norte da cidade, ca. 1400 m, 24 July 1995, *F. Rivadavia 472* (holotype SPF!)

Rosetted annual or facultative short lived perennial; delicate herb, to ca. 12 cm tall; rosette lax, comprising few leaves, usually 3–8. *Leaves* spathulate, up to 30 mm long, dark green or wine red; petiole up to 15 mm long and 1–2 mm wide, gradually widening into the lamina; lamina obovate to oblong-spathulate, up to 5 mm wide and 15 mm long, glabrous. *Inflorescence* a simple raceme, bearing 1–6 flowers. *Scapes* 1–3, 30–120 mm long, terete, diam. up to 0.5 mm, with base ascending, covered with both glandular capitate and simple eglandular hairs up to 0.5 mm long. *Bracts* narrowly lanceolate, 1.0–1.3 mm long, up to 0.3 mm wide, with glandular capitate hairs. *Bracteoles* subulate to narrowly lanceolate, up to 1 mm long and 0.2 mm wide, with glandular capitate hairs. *Pedicel* 3–10 mm, growing during anthesis, adaxial side covered with both glandular and shorter eglandular hairs, abaxial side only sparsely hairy; pedicels erect at anthesis, recurved in fruit. *Sepals* 5, lanceolate, 1.5–1.8 mm long and 0.4–0.7 mm wide, adaxial side with capitate glandular hairs and few eglandular hairs. *Corolla* up to 11 mm long, bilabiate, tubular, lacking a gibbous palate (or only very shallowly pronounced), pale lilac, base of lower lip white and with a pale greenish-yellow marking at the margin of the mouth; outer surface of corolla lobes sparsely covered with capitate glands on ca. 0.5 mm long translucent stalks, gland head dark, and longer eglandular hairs; upper lip 2.0–2.5 mm long, 3.5–4.0 mm wide, deeply bilobate, lobes spreading, each lobe ca. 1.5–2.0 mm wide; lower lip rhombic to transversely oblong in outline, 5.0–6.5 mm long, 6–8 mm wide, trilobate, with emarginate crests between the lobes, median lobe larger than the lateral ones, up to 4 mm wide and 3 mm long, lateral lobes ca. 2.5 mm wide and 2 mm long; palate on the upper part of lower corolla lip ca 1.5 mm long, ca. 1 mm wide, pale yellow, densely covered with translucent short-stalked glands; spur short, terete, saccate or shortly conical, obtuse, 2–3(–4) mm long, 2 mm wide; back of the corolla and spur sparsely covered with glandular and longer eglandular hairs, few long eglandular hairs on the palate. *Capsule* globose to broadly ovoid, ca. 2 mm in diameter, covered with glandular capitate and eglandular hairs. *Seeds* prismatic, 4–6-angled, ca. 300 µm long, 200–250 µm wide and 100 µm high (two to three times wider than high), narrowly and irregularly winged on the angles, testa reticulate, the testa cells polygonal to isodiametric, with the anticlinal walls much raised, slightly sinuate, and the periclinal walls tabular, on the distal surface with verrucose broad longitudinally striate bands that are continuing on the anticlines.

Distribution:—Brazil, endemic to Bahia, so far only known from three locations in the Chapada Diamantina highlands: two in the Serra do Sincorá, and one at the Cachoeira da Fumaça. It is the northernmost occurring member of the subgenus. Although apparently rare and occurring in very small localized populations (as already mentioned in Rivadavia 2000, referring to this plant as "*Genlisea* sp."), *G. exhibitionista* may in fact be more common, occurring in scattered populations on the rarely explored mountain tops of the Chapada Diamantina.

Habitat:—Growing in cracks of moss-covered vertical wet sandstone rocks in semi-shaded places with high humidity, or on saturated peat in shaded crevices of dripping rocks in (upper) montane regions (1000–1400 m). The *locus classicus* is high montane habitat receiving regular fog, and the site at the Cachoeira da Fumaça lies within the reach of the spray of the waterfall and the upriver Riacho da Fumaça. At both places, *G. exhibitionista* is often growing sympatric with the carnivorous bladderwort *Utricularia rostrata* Fleischmann & Rivadavia (2009: 155).

Etymology:—The specific epithet refers to the conspicuous opening between lower and upper corolla lobe, which is unique among *Genlisea*, exposing and exhibiting the styles and stamens.

Conservation Status:—Vulnerable (VU). Although it is currently known from only three small populations consisting of very few individuals, we assume that it is more widespread in poorly-explored areas of the Chapada Diamantina, especially at high elevations, on regularly fog-covered summits.

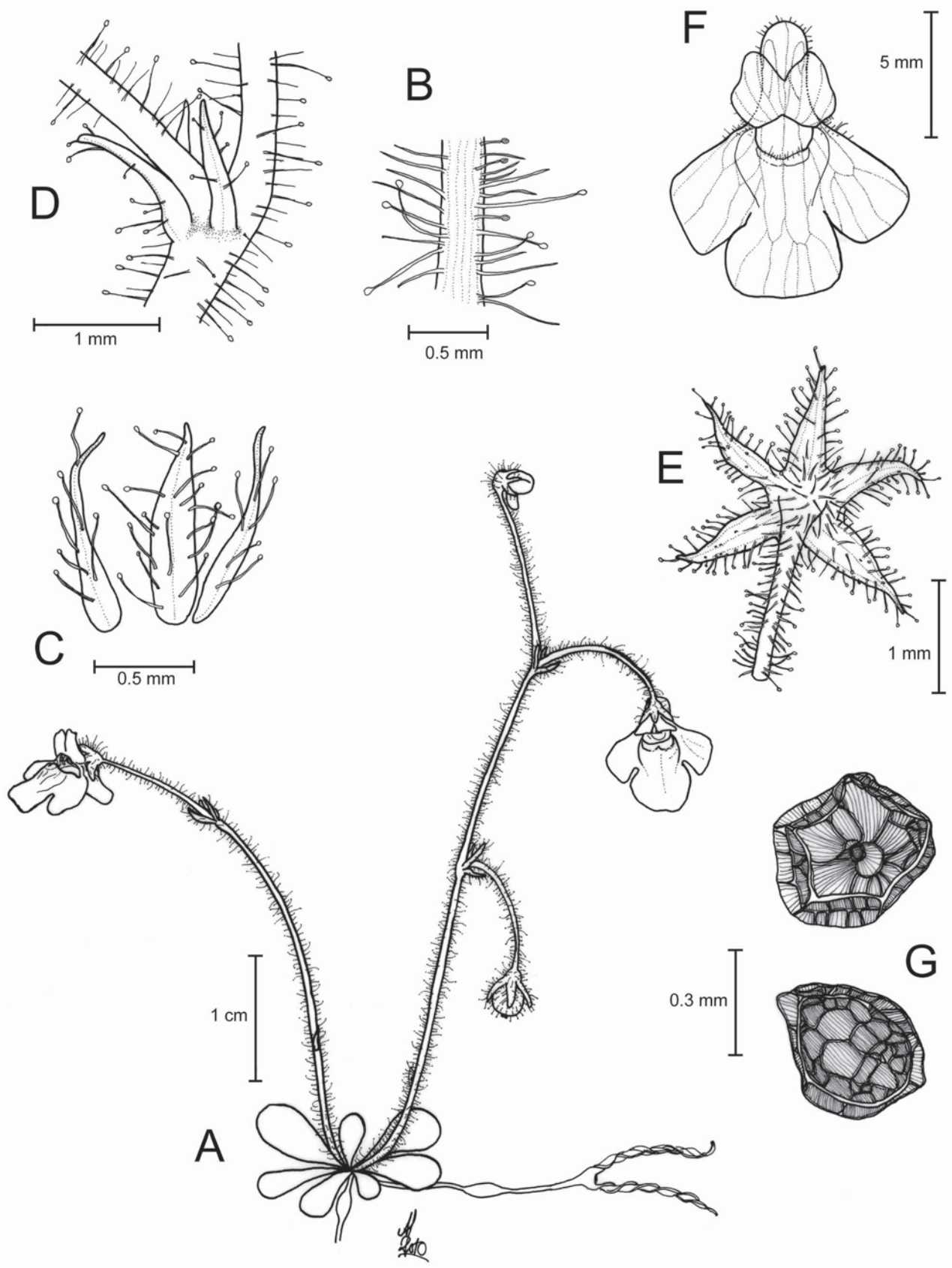


FIGURE 8. *Genlisea exhibitionista* (all from *F. Rivadavia 472*). **A,** habit. **B,** section of the scape. **C,** bract (centre) and bracteoles. **D,** insertion of bract and bracteoles to the peduncle. **E,** calyx. **F,** corolla. **G,** seeds. Drawing by A. Fleischmann.

Notes:—All members of *G.* subgenus *Tayloria* have hypocrateriform zygomorphic flowers, which possess a more or less well-developed small notch on the palate between upper and lower corolla lip, through which the corolla tube can be entered by pollinating insects. The bilobate stigma and the two anthers are firmly enclosed within the tubular part of the corolla. In some species (*G. flexuosa*, *G. lobata*, *G. nebulicola*, some local forms of *G. violacea*) the concave bulge of the upper lip base is well expressed, so that the ventral surface of the stigmatic lobe is visible when the flower is viewed from the front. In all these species, however, the entrance to the tubular part of the corolla remains a small slit, as it is narrowed by a prominent small, blotch-like protrusion of the lower lip (consisting of two yellow gibbous rims in all species except *G. uncinata*, *G. oligophylla* and *G. metallica*, which additionally bear a larger gibbous swelling). In *G. exhibitionista*, however, this protrusion is lacking. Furthermore, the palate is slightly concave, forming a relatively large, open, conical corolla tube (which is reminiscent of the open labiate flowers of the Lentibulariaceae genus *Pinguicula* [Linnaeus 1753: 17]), in which stigma and anthers are freely exposed. This unique corolla design among *Genlisea* might indicate that *G. exhibitionista* relies on a different pollinator. The very short, wide spur of this species (which is the shortest in relation to overall corolla size observed in any *Genlisea* species), together with the open conical corolla tube, suggest a short-tongued insect is acting as the main pollinator, which probably inserts its head deeply into the corolla tube to reach the nectar at the bottom of the spur. In contrast, the flowers of all remaining species of *G.* subgenus *Tayloria* (perhaps except those of *G. uncinata*) which have a slender, long corolla tube with a narrowed entrance and disc-like spreading corolla lobes (creating a landing platform), are typical salverform blossoms, which are pollinated by insects with a long proboscis, like butterflies, moths, or dipterans like bombyliid flies (bee flies). In salverform flowers, the pollinator does not enter the corolla tube with its head, and pollen is likely to be deposited on the proboscis of the pollinator (Leins & Erbar 2010).

Genlisea exhibitionista differs from the somewhat similar *G. violacea* in its much shorter, saccate to shortly conical spur, a smaller corolla (especially a much shorter and narrower upper lip) lacking a gibbous marking of the palate, and a slightly different indumentum of scape and calyx, consisting of more simple hairs than glandular hairs. Previous mention of *G. violacea* from the Chapada Diamantina (Harley & Mayo 1980) correspond to *G. exhibitionista*.

Additional specimens examined (paratypes):—BRAZIL. Bahia: Município de Palmeiras, cachoeira da Fumaça, ca.1320 m, 20 June 2005, *F. Rivadavia et al. 2086* (SPF). Município de Andaraí, approx. 15 km North of Mucugê on road to Andaraí, ca. 1100 m, 18 February 1977, *R.M. Harley et al. 18889* (K (alcohol material), RB).

6. *Genlisea nebulicola* Rivadavia, Gonella & A. Fleischm., *sp. nov.* (Figs. 3, 9, 12L,M, 13P,Q)

Genliseae lobatae similis sed corollae lobis integris obtusis, scapis tantum pilis glanduligeris sparsis obsitis, sepalis perangustis et subglabris, pedicellis fructigeris recurvatis (non reflexis) differt.

Type:—BRAZIL. Minas Gerais: Município de São Roque de Minas, Parque Nacional da Serra da Canastra, base da Cachoeira Casca d'Anta, 27 March 2011, *P.M. Gonella 408* (holotype SPF!).

Small, perhaps perennial, lithophyte, to 6 cm tall. *Leaves* rosulate, broadly spathulate, 4.5–15.0 mm long and up to 5 mm wide. *Inflorescence* a lax raceme, often more or less one-sided, 2–8-flowered; scapes and pedicels very sparsely covered with short-stalked glandular capitate hairs 0.08–0.20 mm long. *Scapes* 1–4, up to 60 mm long, erect, terete, filiform, ca. 0.5 mm wide near the base, 0.25 mm in diam. towards the apex. *Bracts* narrowly oblong, to 1.5 mm long and 0.2 mm wide, glabrous or with occasional capitate glandular hairs. *Bracteoles* subulate to narrowly oblong, 0.5–0.8 mm long and up to 0.15 mm wide, glabrous or with occasional capitate glandular hairs. *Pedicels* terete, 0.1–0.3 mm in diam., to 5 mm long and erect or slightly curved downwards at anthesis, recurved from the base and ca. 10 mm long in fruit; pedicels inserted from distinctive swollen nodes on the scape. *Sepals* 5, subulate to narrowly oblong, 0.8–1.2 mm long and 0.15–0.40

mm wide, adaxial side glabrous or with few single capitate glandular hairs; calyx densely covered with glandular capitate hairs and eglandular hairs. *Corolla* bilabiate, 6.0–6.5 mm long, lower lip pale lilac-lavender or whitish and with a mark of two yellow rims at the base of the palate, upper lip with dark purple streaks along the nerves; lower lip trilobate, ovate to broadly ovate in outline, to 4.5 mm long and 4 mm wide, the median lobe circular to transversely elliptic, 2.0–2.5 mm long and to 2.5 mm wide, the smaller lateral lobes ovate to elliptic, ca. 1 × 1.0–1.5 mm, lobes with apex obtuse to slightly undulate; upper lip bilobate, 1.5–2.0 mm long and 2.5–3.0 mm wide, lobes spreading; spur narrowly cylindrical to oblong, straight or slightly curved downwards, shorter than lower corolla lip, up to 3 mm long and 0.5 mm in diameter, apex obtuse; corolla margins and lower surface glabrous, spur with few scattered glandular capitate and eglandular hairs. *Capsule* broadly obovoid to ellipsoid, up to 2 mm long and 1.5 mm wide, glabrous at the base, with capitate glandular hairs towards the tip. *Seeds* prismatic, 5–6-angled, ca. 400 µm long, 300 µm wide, and 100 µm high (three to four times wider than high), the testa cells more or less isodiametric (to elongate on the distal surface), with the anticlinal walls raised, slightly sinuate, the periclinal walls tabular, microscopically rugose.

Distribution:—Brazil. Minas Gerais, endemic. Thus far only found at the Serra da Canastra and the Serra das Sete Voltas (Alex Melo, pers. obs.). Rare and very localized populations.

Habitat:—Lithophyte. At the Casca d'Anta waterfall *G. nebulicola* is growing on algae- or moss-covered sandstone rocks dripping constantly with water from the spray of the waterfall, always on the side facing south, away from the falls. At least during the wet season, plants are continuously fogged by the spray from the waterfall, often causing flowers and fruits to be enveloped by water droplets. It occurs sympatrically with the carnivorous bladderwort *Utricularia laciniata* Saint-Hilaire & Girard (1838: 870). At the Serra das Sete Voltas, it has been observed growing on a dripping moss-covered vertical sandstone wall with *Drosera communis* (Saint-Hilaire 1824: 267) and *Utricularia subulata* (Linnaeus 1753: 18) (Alex Melo, pers. obs.).

Etymology:—The epithet *nebulicola* meaning 'adherent to clouds', refers to the unique habitat at the Casca d'Anta, where the plant grows in the fog of the spray from the waterfall.

Conservation Status:—Critically Endangered (CR). The type location, although within a National Park, is easy accessible and frequently visited by tourists, and the very small population could face potential risk of destruction or overcollection. At the second known location, this plant is known from a few individuals only.

Notes:—The minute *G. nebulicola* is apparently most closely allied to *G. lobata*, with which it shares the cylindrical oblong spur of the corolla, the pale corolla colour, and the very slender fragile scapes. It differs from *G. lobata* in the indumentum of capitate glandular hairs (consisting of both glandular and numerous eglandular hairs in *G. lobata*), in its more delicate flowers, which have entire corolla lobes, by shorter scapes less than 10 cm high, and by its very short pedicels at anthesis, which are about as long as (or even shorter than) the entire corolla length, and which are recurved (not reflexed) in fruit. It resembles *G. violacea* in the vegetative parts, but differs from that species in the more delicate inflorescence and flowers, the oblong cylindrical spur, and the very sparse indumentum of glandular hairs, which make the inflorescences of *G. nebulicola* appear almost glabrous. *Genlisea nebulicola* has a very diagnostic calyx indumentum and it is the only species within *G.* subgenus *Tayloria* that has (semi)glabrous bracts and sepals.

Genlisea violacea is also found in the Serra da Canastra at the Casca d'Anta (e.g., *F. Rivadavia & M. Peixoto 880*), and it occurs in close proximity to the known populations of *G. nebulicola*. However the two species apparently do not grow sympatrically, with *G. violacea* only being known from seasonally humid habitats around pools at the top of the waterfall and in moist sandy soils halfway down from the top (F. Rivadavia, pers. obs.).

Additional specimens examined (paratype):—BRAZIL. Minas Gerais: Município de São Roque de Minas, Parque Nacional da Serra da Canastra, base da Casca d'Anta, ca. 1000 m, 01 April 1999, *F. Rivadavia & M. Peixoto 864* (SPF, M).

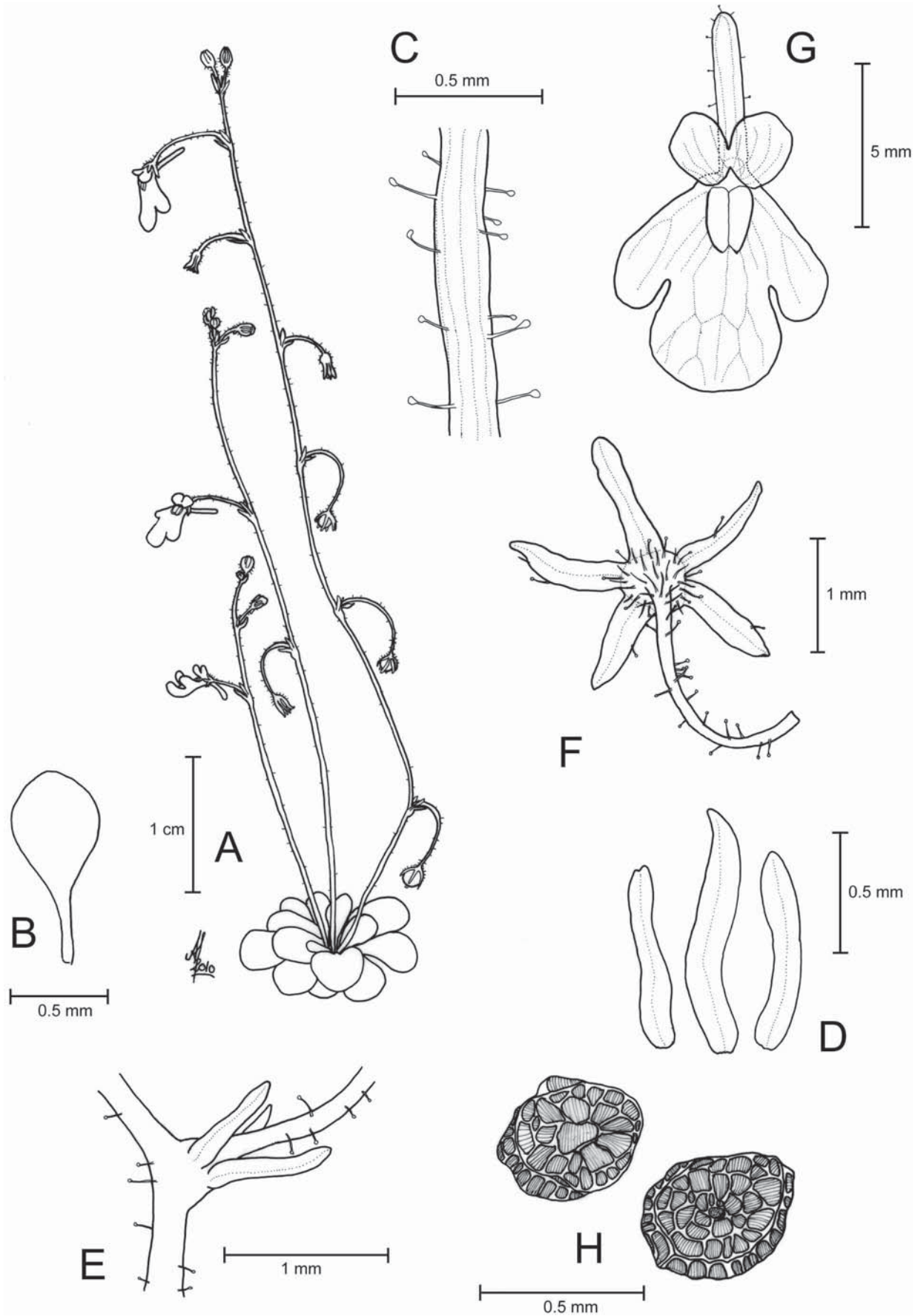


FIGURE 9. *Genlisea nebulicola* (all from *F. Rivadavia & M. Peixoto 864*). **A,** habit. **B,** leaf. **C,** section of the pedicel. **D,** bract (centre) and bracteoles. **E,** insertion of bract and bracteoles to the peduncle. **F,** calyx and entire pedicel. **G,** corolla. **H,** seeds. Drawing by A. Fleischmann.

7. *Genlisea lobata* Fromm (Fig. 3, 10, 12N,O, 13L,M)

Type:—BRAZIL. Minas Gerais: Serra do Caparaó, na primeira formação rochosa em direção à Tronqueira, sobre rochas em local úmido, com água escorrendo, 19 March 1988, *R.F.N. Camargo et al. FPNC 0002* (holotype CESJ!, isotypes SPF!, R photo!, MO image!).

Small to medium sized rosetted annual herb, up to 22 cm tall. *Leaves* few, rosulate, obovate to obovate-spathulate, 7.0–12.5 mm long and 1.5–5.0 mm wide. *Inflorescence* a lax raceme, 1–14-flowered; scapes, bracts, bracteoles, pedicels and ovary covered with glandular capitate hairs of 0.1–0.4 mm length and numerous eglandular simple hairs to 0.5 mm length. *Scapes* 1–4, 60–220 mm long, erect, filiform, to 0.35 mm in diameter. *Bracts* narrowly lanceolate, ovate or oblong, 0.8–1.5 mm long, 0.2–0.5 mm wide, with apex emarginate (bidentate), acute or retuse. *Bracteoles* lineate, linear-lanceolate, lanceolate or narrowly oblong, 0.5–1.0 mm long, ca. 0.2 mm wide. *Pedicels* terete, 0.05–0.20 mm in diam., to 15 mm long, erect at anthesis, in fruit sharply reflexed from a slightly swollen base. *Sepals* 5, unequal, ovate, ovate-lanceolate, slightly elliptical to oblong or narrowly cuneate, with apex obtuse, acute, retuse or emarginate, 3-nervate, 1.0–1.3 mm long and 0.4–0.5 mm wide. *Corolla* bilabiate, 8.5–9.0 mm long, lower lip white or pale lilac with a blotch consisting of two yellow rims at the base of the palate, upper lip pale lilac or white, with dark purple reticulation, spur dark purple; upper lip bilobate, lobes rectangular to cuneate, 2.0–3.5 long and wide, with apex emarginate to bilobate; lower lip trilobate, lobes subequal, median one larger than the lateral ones, 5.0–6.5 mm long and 4.5–8.0 mm wide, with apex emarginate or crenulate, lateral lobes with apex emarginate; corolla margins glabrous; spur cylindrical (or slightly narrowed towards the tip), oblong, 3–5 mm long, to 1 mm in diameter near the base, shorter than the lower corolla lip, with apex obtuse; spur and corolla lower surface sparsely covered with few glandular capitate hairs and eglandular simple hairs, yellow marking on the palate densely covered with eglandular hairs. *Capsule* ellipsoid to obovoid, 2.0–2.5(–4.0) mm in diam., glabrous at the base, with glandular and eglandular hairs towards the tip. *Seeds* prismatic, yellowish brown, 4–6-angled, 300–400 µm long, 200–250 µm wide and ca. 100 µm high (two to three times wider than high), very narrowly winged on the angles, testa reticulate, the testa cells large, polygonal to isodiametric, with the anticlinal walls raised, and the periclinal walls tabular, on the distal surface with verrucose broad longitudinally striate bands that are continuing on the anticlines.

Distribution:—Brazil, endemic to South-Eastern Brazil, where it is known from the Serra do Caparaó highlands at the border between the states Minas Gerais and Espírito Santo, the Pico do Forno Grande inselberg (Serra do Castelo), Muniz Freire, Santa Maria de Jetibá, and from Pedra Azul in Espírito Santo, and from the Serra da Araponga and the Serra das Cabeças in Minas Gerais.

Habitat:—Montane (1000–1722 m), growing on marshy depressions on granitic hills (inselbergs), on seepage sites over granitic rock, among mosses (often *Sphagnum* spp.) in partly shaded seasonally wet areas, or in humid sandy soil among grasses (Rivadavia 2002). In contrast to all other members of *G.* subgenus *Tayloria*, *G. lobata* does not grow in the *campo rupestre* vegetation of the quartzite highlands (constituting the Espinhaço Range), but is instead confined to the granitic outcrops of the easterly mountain chains and surrounding inselbergs.

Conservation Status:—Vulnerable (VU). *Genlisea lobata* is known from a few locations, but population sizes of this annual plant are generally small. It grows in more developed areas that have suffered extensive granite mining activities, deforestation and habitat change in the lowlands over recent decades, which also can affect the local climate and hydrology of the montane regions.

Notes:—*Genlisea lobata* is readily distinguished from all other species of *G.* subgenus *Tayloria* by the distinctly emarginate petal tips, and by the colour pattern of the corolla. Although white flowered specimens of *G. violacea* are known to rarely occur in some populations, they never have a purple spur and bicoloured upper corolla lip. The sharply reflexed pedicels of fruiting specimens are unique in the subgenus, too, as all other species have either arcuate-recurved or circinate pedicles in fruit.

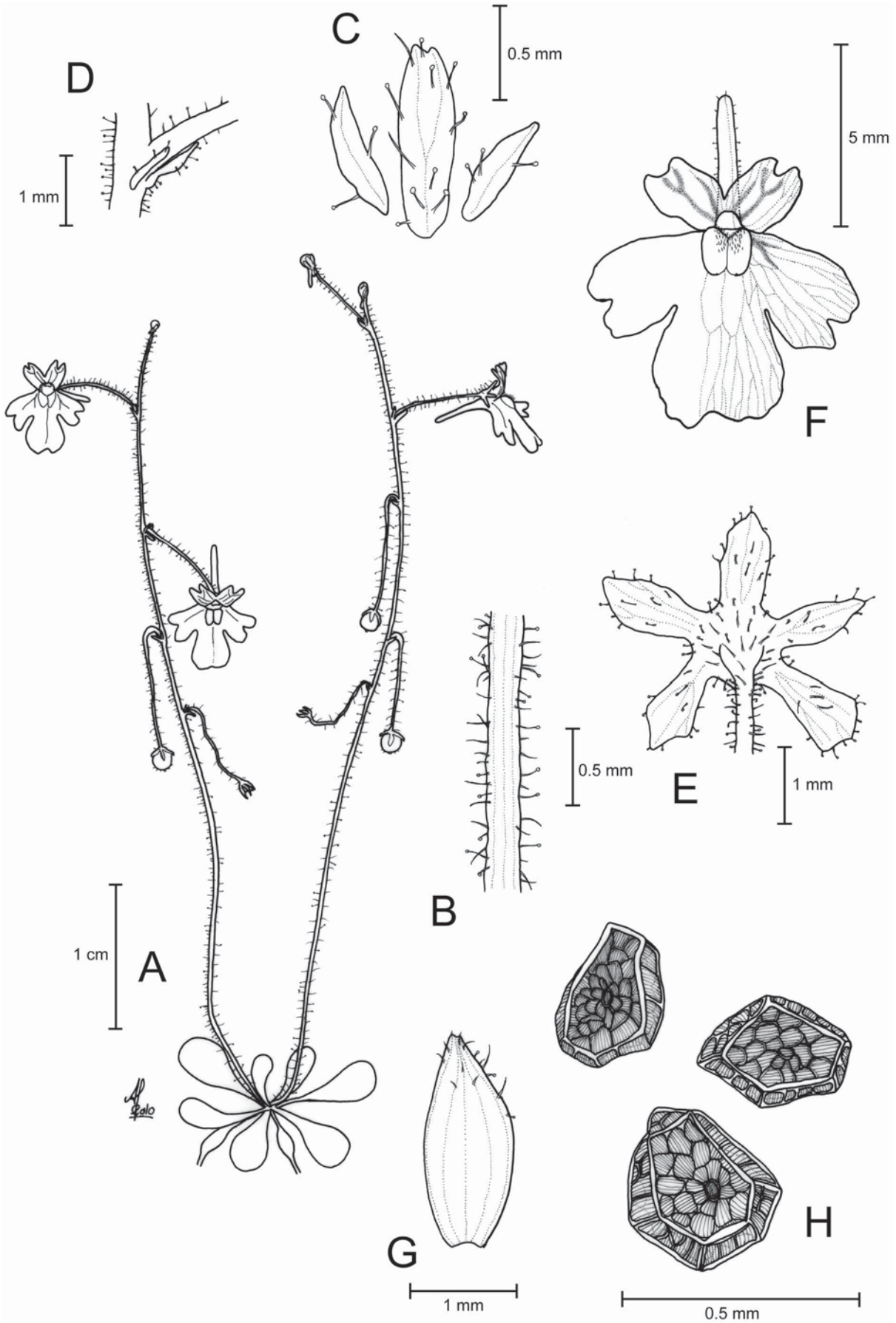


FIGURE 10. *Genlisea lobata* (all from *F. Rivadavia et al. 517*). **A,** habit. **B,** section of the scape. **C,** bract (centre) and bracteoles. **D,** insertion of bract and bracteoles to the peduncle. **E,** calyx. **F,** corolla. **G,** valve of the seed capsule. **H,** seeds. Drawing by A. Fleischmann.

An artificial hybrid has been created in cultivation between *G. lobata* and *G. flexuosa* by carnivorous plant breeder Jan Flisek (named as "*G. lobata* x *violacea*"; K. Pasek, pers. com.), and between *G. lobata* and *G. violacea* by P.M. Gonella. This indicates that there are no reproductive barriers between these closely related taxa (see Fleischmann *et al.*, 2010), and that rather geographical isolation and edaphic factors have driven speciation.

Genlisea lobata is the only member of *G.* subgenus *Tayloria* that occurs in Espírito Santo state, and previous records of *G. violacea* from this state (Fromm-Trinta 1979, Taylor 1991) result from misidentified specimens of *G. lobata*.

Specimens examined:—BRAZIL. Espírito Santo: Município de Castelo, Parque Estadual do Forno Grande, Trilha para o Forninho, 1100–1400 m, 17 July 2008, *R. Goldenberg 1166* (RB); Pedra abaixo do Mirante, 1100–1400 m, 09 April 2009, *R. Goldenberg 1428* (RB). Município de Ibitirama, Serra do Caparaó, 24 March 1970, *A.P. Duarte 12691* (BHCB). Município de Domingos Martins, Rodovia Vitória–Belo Horizonte, Pedra Azul, 16 June 1980, *O.J. Pereira 451* (UEC). Município de Muniz Freire , estrada entre Manhuaçú e Vitória, 1000 m, 07 September 1977, *G.J. Shepherd et al. 5835A* (UEC). Município de Santa Maria de Jetibá, Alto São Sebastião, 1000–1170 m, 16 May 2006, *A.P. Fontana & R.R. dos Santos 2118* (RB). Minas Gerais: Município de Araponga, Parque Estadual Serra do Brigadeiro, Serra das Cabeças, 1722 m, 30 January 2002, *A.N. Caiafa & F.M. Martins 214* (UEC); Serra da Araponga, Fazenda Neblina, ca. 1400 m, 17 February 1996, *F. Rivadavia et al. 517* (SPF). Município de Alto Caparaó: Parque Nacional do Caparaó, Vale Verde, 02 April 1989, *L. Krieger & al. 23586* (R); Vale Verde, ca. 1300 m, 10 June 1993, *F. Rivadavia 216* (SPF); Vale Verde, 28 May 1999, *W. Forster & L.S. Leoni 74* (ESA); Vale Verde, ao longo do Rio Caparaó, 16 February 2000, *V.C. Souza et al. 23187* (ESA); próximo à entrada do parque, ca. 1300 m, 27 February 2005, *F. Rivadavia 1964* (SPF).

8. *Genlisea violacea* A.St.-Hil. (Figs. 3, 11, 12P–U, 13J,K)

Type:—BRAZIL. Minas Gerais: Minas Geraes Montagnes élevées, bord de ruisseaux dans la Serra da Lapa [Serra do Cipó], without date, *A. de Saint-Hilaire B2 2162* (holotype P!; isotypes P!, B+ (photo M!)).

Genlisea biloba Benjamin (1847: 254). Type: BRAZIL. São Paulo: Guaratinguetá, without date, *C.F.P. Martius s.n.* (holotype M!).

Genlisea cylindrica Sylvén (1909: 4). Type: BRAZIL. Minas Gerais: Serra de Caldas (infra Pedra Branca), 01 June 1874, *C.W.H. Mosén 1996* (holotype S, K *fragmentum typi*! photo K! photo M!).

Possible synonym: *Genlisea reflexa* Benjamin (1847: 254). Probable type: BRAZIL. without locality, without date, *F. Sellow s.n.* (B+, photo K! photo M!)

Small to medium sized annual herb, up to 25(–35) cm tall. *Leaves* spathulate, (1.5–)3.0–12.0 mm long and (0.5–)1.5–7.0 mm wide, petiole short, 1–5 mm long, lamina obovate-spathulate to obovate. *Inflorescence* a lax raceme, often more or less one-sided, 1–6(–12)-flowered; scapes, bracts, pedicels, sepals and ovary covered by glandular capitate and fewer eglandular hairs, or by glandular capitate hairs only. *Scapes* 1–2(–4), erect, terete, (25–)30–250(–350) mm tall, (0.1–)0.2–1.0 mm in diam., occasionally bifurcate or multiple branched. *Bracts* ovate, ovate-lanceolate, rarely elliptical, oblong or linear-lanceolate, with apex acute or pointed, rarely emarginate or retuse, dorsal surface and margins glandular, 1.0–2.0(–3.2) mm long and 0.2–0.8 mm wide.*Bracteoles* subulate, linear-lanceolate, linear or lanceolate, oblong or ovate-lanceolate, with apex acute, 0.5–2.0 mm long and 0.1–0.3 mm wide. *Pedicel* terete, 0.2–0.3 mm in diam., erect and 7–20 mm long at anthesis, reflexed and enlarged to 32 mm in fruit (sometimes arcuated more than 360° and then pointing upwards). *Sepals* 5, oblong, ovate-lanceolate or elliptical, rarely linear-lanceolate, with apex acute, pointed, rarely obtuse or emarginate, 1.0–3.0 mm long and 0.2–1.2 mm wide. *Corolla* bilabiate, (5–)7–12(–16) mm long, violet or lilac, with gibbous yellow mark on the palate of the lower lip; upper lip deeply bilobed, obcordate, 3.0–5.0(–7.0) mm long and (3.0–)5.0–8.5 mm wide, lobes oblong or circular, overlapping or

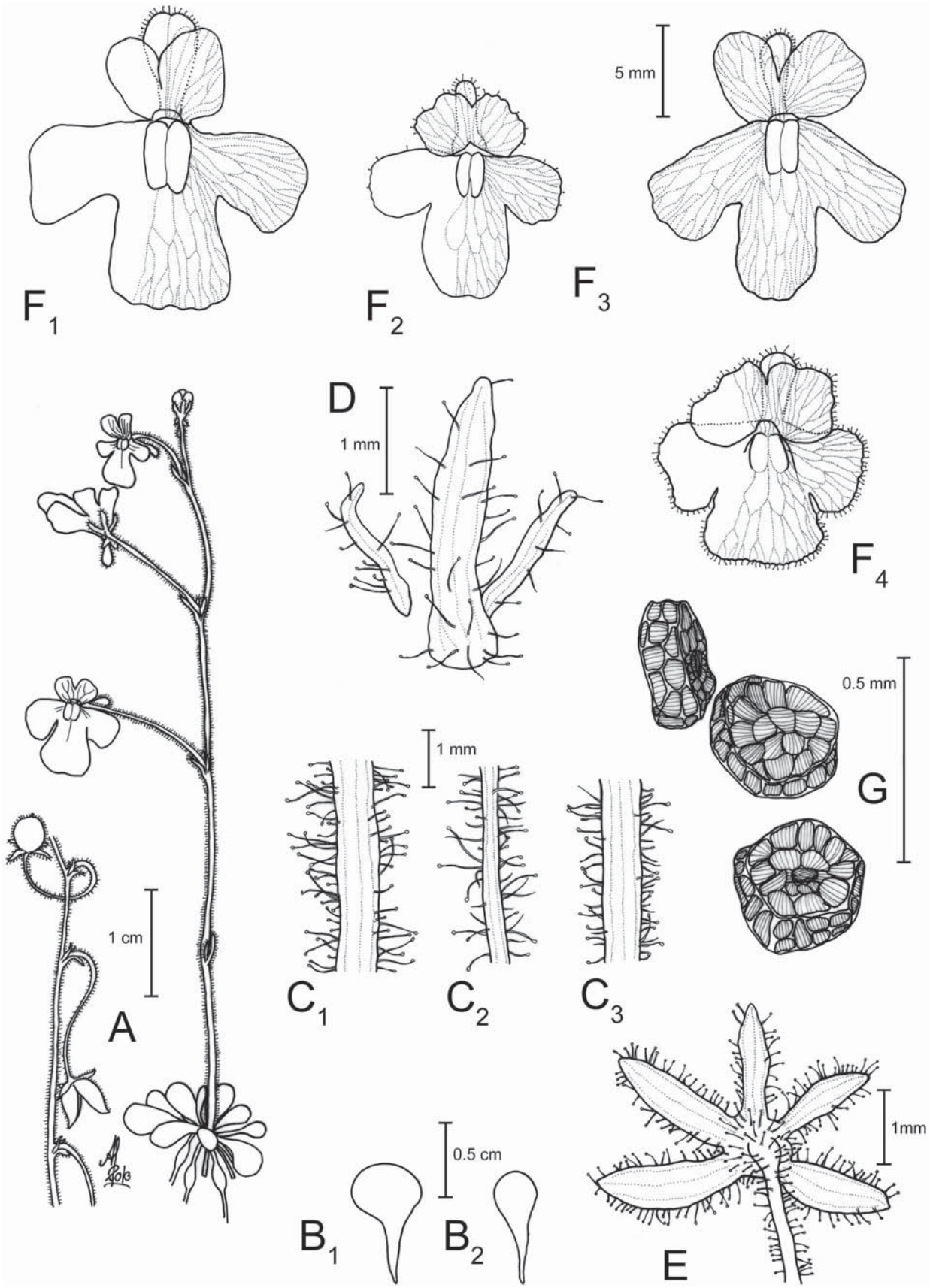


FIGURE 11. *Genlisea violacea*. **A,** habit and infructescence. (*St.-Hilaire B2 2162*, type; corolla details adopted from photographs of plants *in situ*). **B,** leaf (1 from *F. Rivadavia & J. Mullins 536*; 2 from *F. Rivadavia 2532*). **C,** section of the pedicel (1 from *Glaziou 8218a*; 2 from *Regnell III848*; 3 from *Martius s.n.*). **D,** bract and bracteoles (*F. Rivadavia 2560*). **E,** calyx (*F. Rivadavia 2560*). **F,** corolla (1 from *F. Rivadavia 1952*; 2 from *F. Rivadavia 2532*; 3 from *F. Rivadavia 2560*; 4 from *F. Rivadavia & J. Mullins 536*). **G,** seeds (from *F. Rivadavia 2532*). Drawing by A. Fleischmann.

slightly spreading; lower lip (5.5–)8.0–10.0 mm long and (6.5–)10.0–15.0 mm wide, distinctly 3-lobate, lobes oblong, spreading, with apex rounded, obtuse, retuse, emarginate or truncate, margin often slightly undulate, lobes subequal, median lobe bigger; spur cylindrical, straight (or sometimes slightly curved downwards near the apex), widened towards the apex, with apex rounded, obtuse or retuse, shorter than (rarely almost equalling) the lower corolla lip, (2.3–)3.0–5.0(–6.0) mm long and 0.5–2.0 mm wide. *Capsule* globose, 2.0–3.0(–5.0) mm in diam., with glandular capitate hairs. *Seeds* prismatic, 5–6-angled, 250–350 µm long, 200–300 µm wide, and 100–150 µm high (about twice as wide as high), the testa cells isodiametric, with the anticlinal walls raised, slightly sinuate, the periclinal walls tabular, microscopically rugose, on the distal surface with verrucose longitudinally striate bands that are continuing on the anticlines.

Distribution:—Brazil, endemic to the states of São Paulo and Minas Gerais. Previous erroneous reports of *G. violacea* from Espírito Santo (Fromm-Trinta 1979, Taylor 1991) and Bahia (Harley & Mayo 1980) correspond to *G. lobata* and *G. exhibitionista* respectively. In São Paulo state, only two historic collections of *G. violacea* have been made in the late 19th century in the Serra do Mar. It was found growing "in moist plains near Guaratinguetá" (Benjamin 1847) and in the Serra da Bocaina in "Campos da Bocaina, au Bom Jardim, dans les bourbiers" (Glaziou 1911).

In Minas Gerais, *G. violacea* is common along the Serra do Espinhaço, especially in the central (Serra do Cipó, Diamantina Plateau and Serra do Cabral) and southern parts (Serra de Ouro Branco and Serra do Caraça), whereas it is more rarely found in the northern part (Serra de Grão Mogol). Furthermore, *G. violacea* is encountered in the northeastern part of the Serra da Mantiqueira highlands, and reaches its westernmost limit at the Serra da Canastra Range.

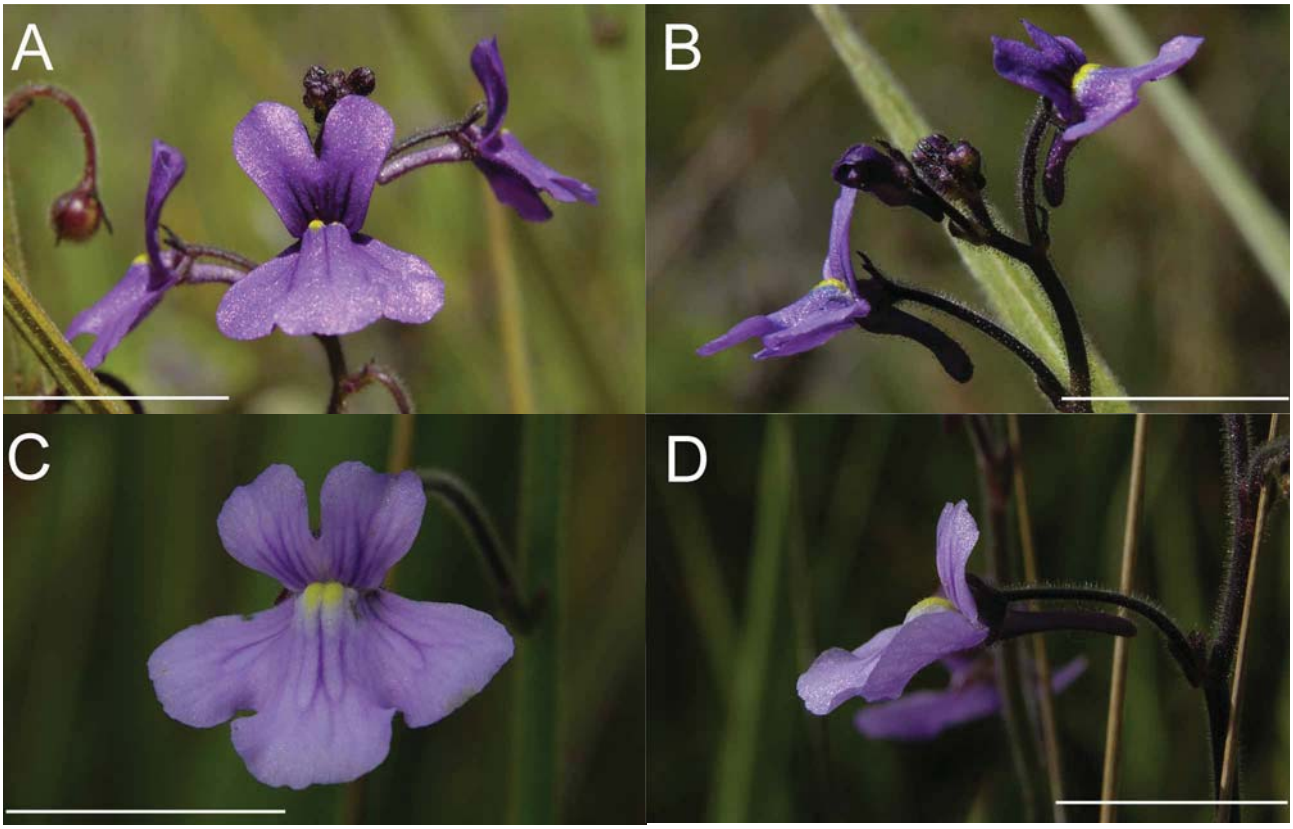
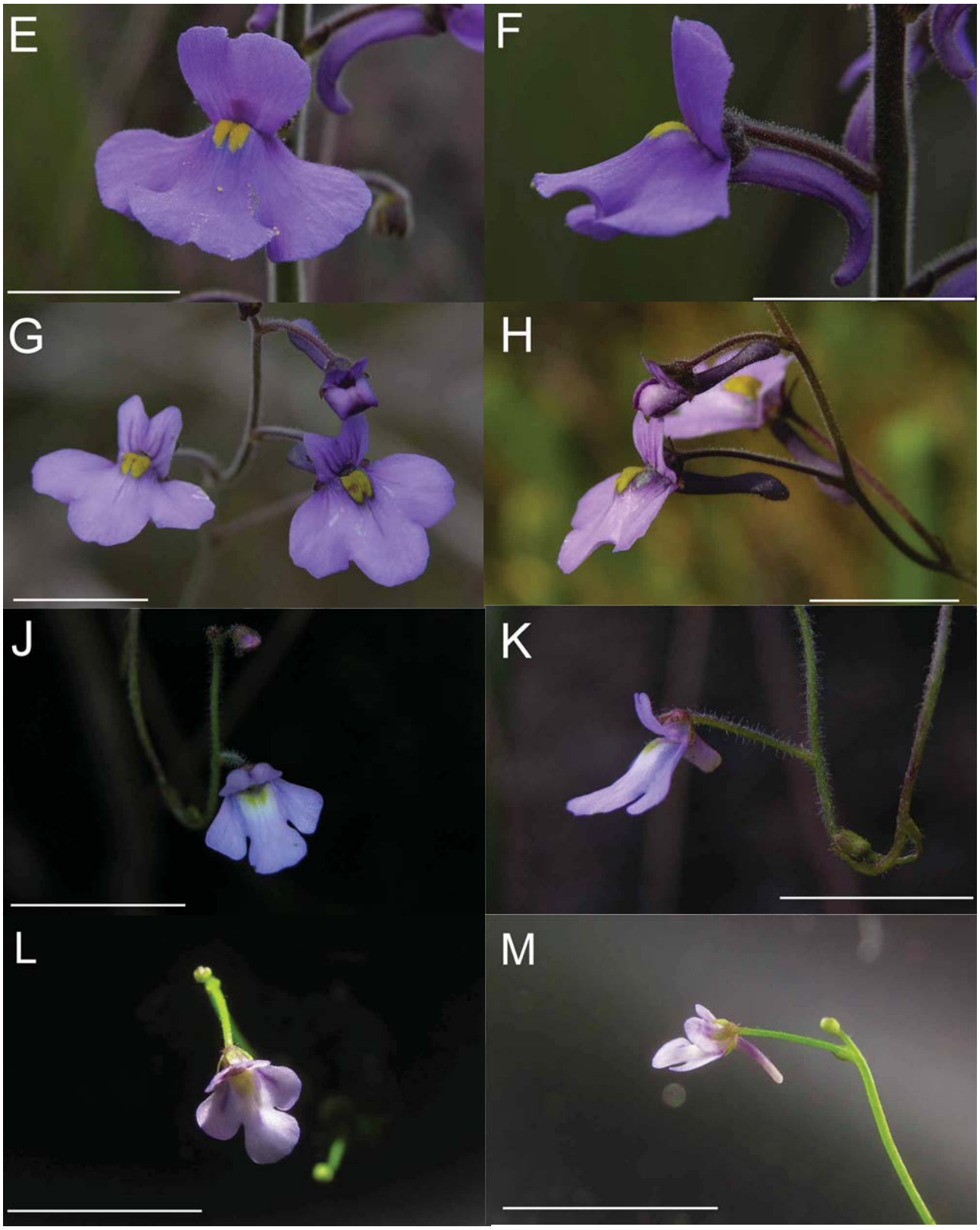
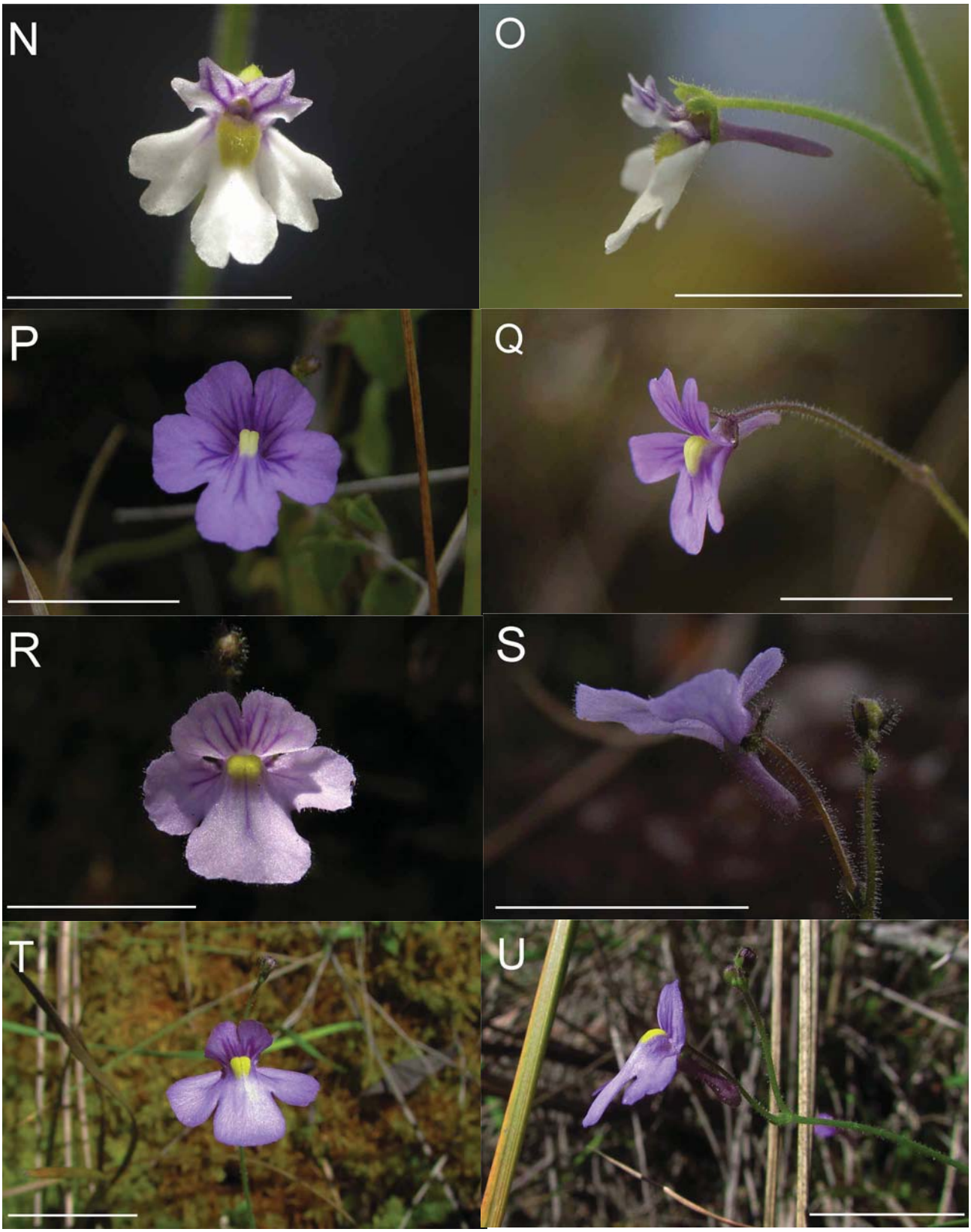


FIGURE 12. Flower photographs of *Genlisea* subgenus *Tayloria* in situ, corolla shown in ventral and lateral view. **A, B,** *G. metallica*, Itacambira. **C, D,** *G. oligophylla*, Serra do Cipó. **E, F,** *G. uncinata*, Serra do Sincorá. **G, H,** *G. flexuosa*, Itacambira. **J, K,** *G. exhibitionista*, Cachoeira da Fumaça. **L, M,** *G. nebulicola*, Casca D'Anta. **N, O,** *G. lobata*, cultivated plant. **P, Q,** *G. violacea*, Diamantina. **R, S,** *G. violacea*, Serra do Cipó. **T, U,** *G. violacea*, Ibitipoca. Scale bars 1 cm.

..... *continued on the next page*



..... continued on the next page


N
O
P
Q
R
S
T
U

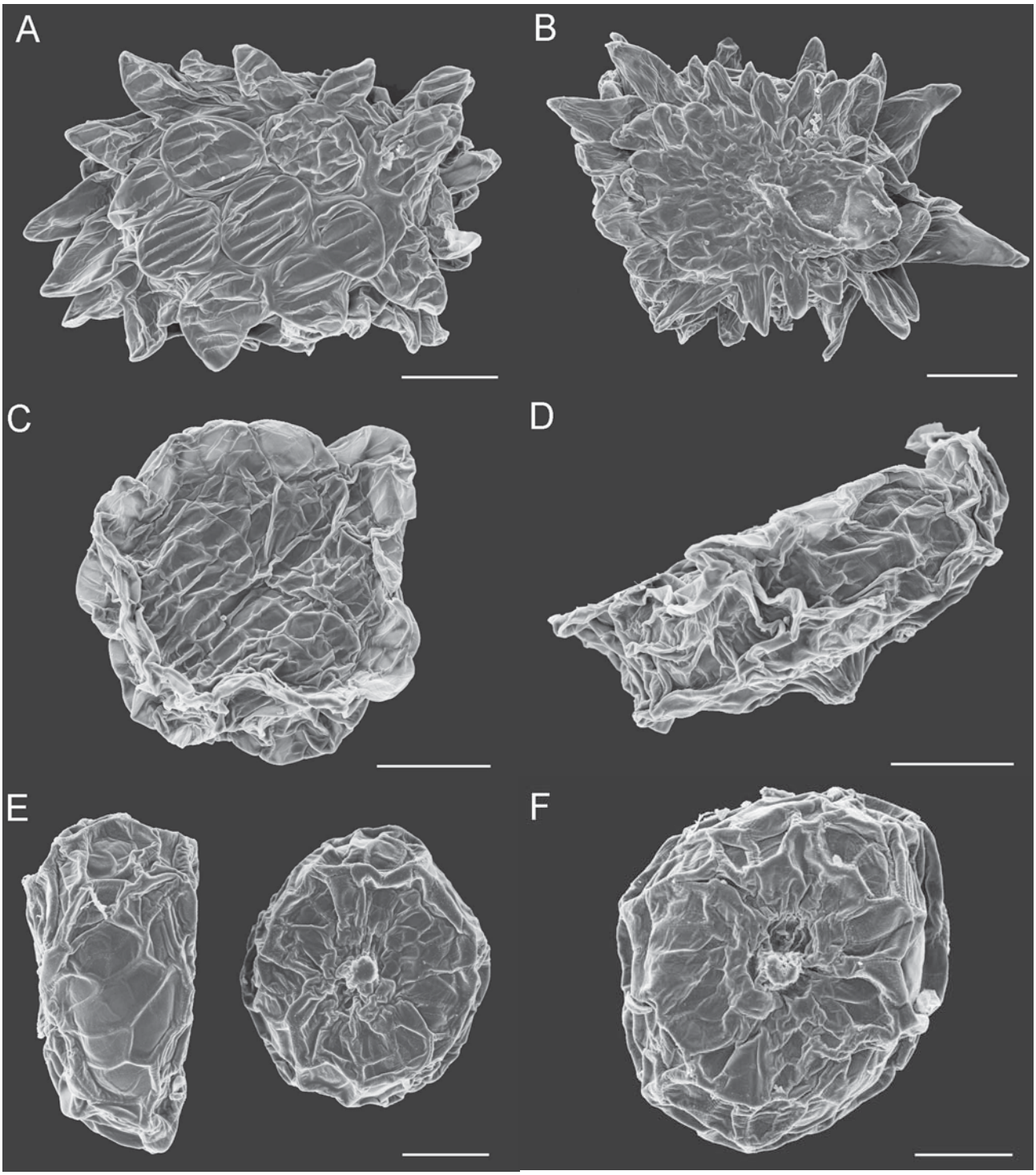
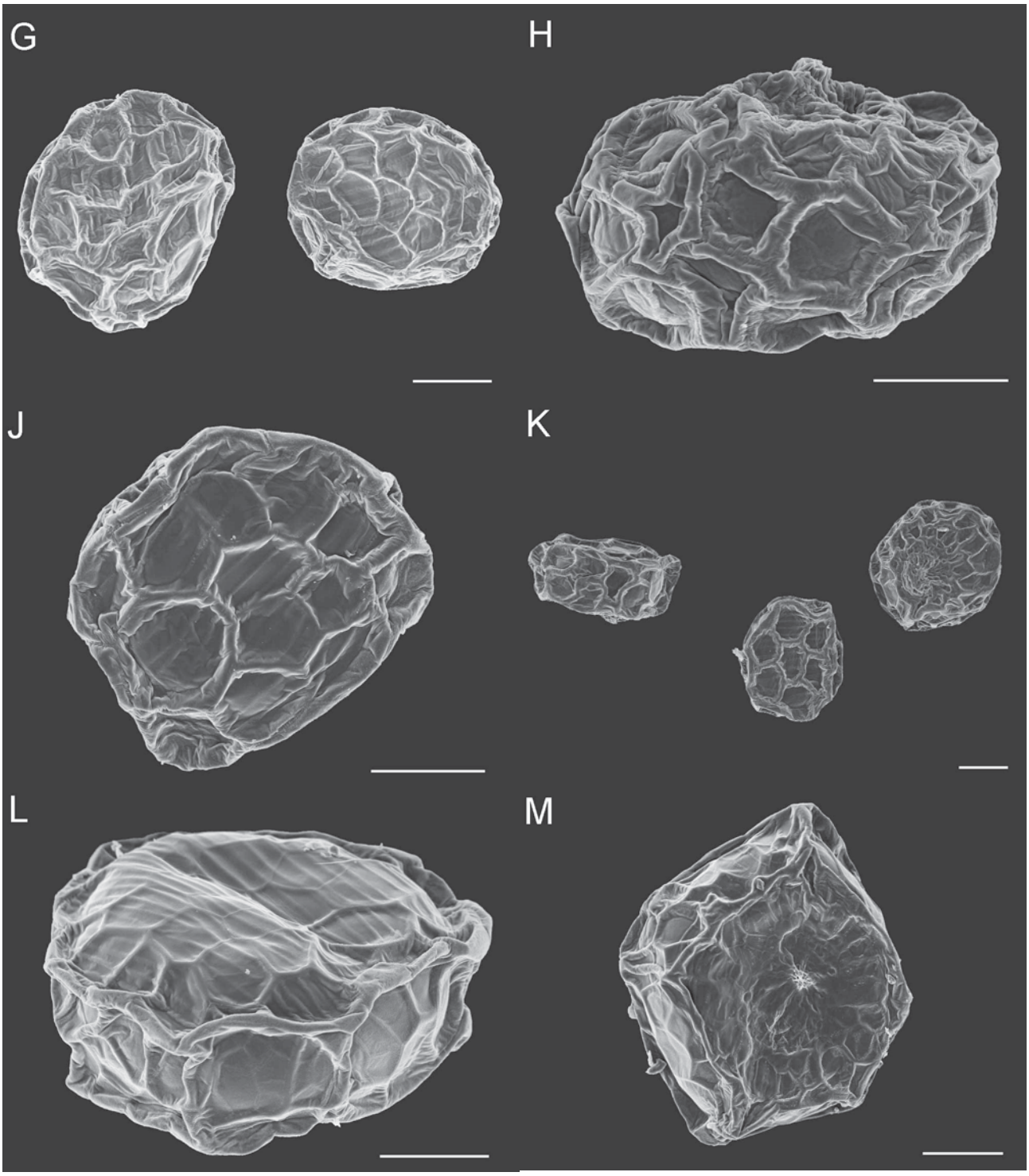


FIGURE 13. SEM microphotographs of seeds of *Genlisea* subgenus *Tayloria*. *Genlisea uncinata* (*F. Rivadavia 471*): **A,** distal view. **B,** proximal view. *Genlisea oligophylla* (*P.M. Gonella et al. 227*): **C,** distal. **D,** lateral. *Genlisea metallica* (*P.M. Gonella et al. 293*): **E,** lateral and proximal views. **F,** proximal. *Genlisea flexuosa* (*P.M. Gonella et al. 292*): **G,** distal. **H,** lateral. *Genlisea violacea* (*F. Rivadavia 2532*): **J,** distal. **K,** lateral, distal, and proximal views. *Genlisea lobata* (*F. Rivadavia et al. 517*): **L,** lateral-distal view. **M,** proximal. *Genlisea exhibitionista* (*F. Rivadavia et al. 2086*): **N,** distal. **O,** proximal. *Genlisea nebulicola* (*F. Rivadavia & M. Peixoto 864*): **P,** distal. **Q,** proximal. Scale bars 100 µm.

..... continued on the next page



..... *continued on the next page*

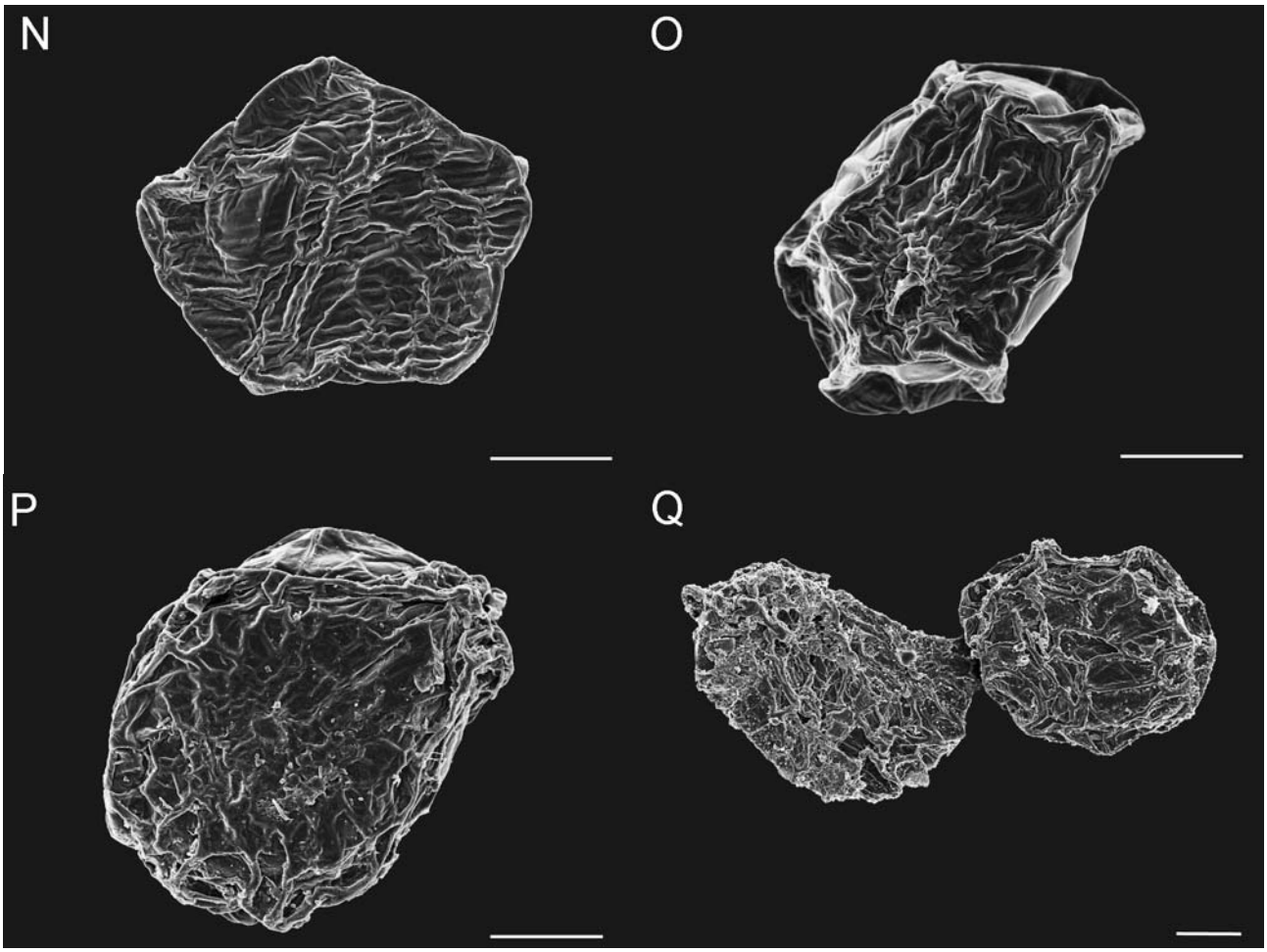


FIGURE 14. Thickened underground stem of *Genlisea metallica*.

Habitat:—Lower montane to subalpine, (680–)900–1950 m, at higher elevations especially in the Serra do Caraça. Seasonally wet places and dripping walls, on sandy quartzitic ground or in thin organic layer over rocks, rarely growing in *Sphagnum*. Usually in moist and open places among sparse grasses in *cerrado* and *campo rupestre* vegetation, but also in open gallery forest.

Conservation Status:—Least Concern (LC) for Minas Gerais, where it is widespread, including several well-established populations in National Parks and State Parks. However, several local populations of this variable species that lie outside protected areas may be threatened to different degrees by human activities. Probably Extinct (EX) in the state of São Paulo, from where only historic collections exist, from areas which have been highly developed in the past century.

Notes:—Saint-Hilaire's type of *G. violacea* is based on a collection made in the central part of the species' range, in the Serra do Cipó ("Serra da Lapa"), however no collection date is given. Nevertheless, the collection number of the type, *Saint-Hilaire B2 2162* (representing Catalogue B^2 = Volume 2 of his collection Series B, Section 1) indicates that it must have been collected on a voyage between December 1816 and March 1818 (Dwyer 1955). But apparently Auguste de Saint-Hilaire also encountered *G. violacea* at the southern part of its range, in the eastern Serra da Mantiqueira, because in his protologue he states the Serra do Ibitipoca as a second locality: "ad rivulos montis altissimi Serra da Lapa [Serra do Cipó] et in arenosis humidis montis Serra da Ibitipoca" (Saint Hilaire 1833). However no herbarium specimens made by Saint-Hilaire have been found for the latter record.

Due to the wide distribution, *G. violacea* is highly diverse in overall size, indumentum, corolla size, shape and venation, especially in the northern part of its range, and in the Serra da Canastra and Furnas region. The notable variability resulted in the description of three further species, *G. reflexa*, *G. biloba* and *G. cylindrica*, which are all considered to be conspecific with *G. violacea* (Fromm-Trinta 1979, Taylor 1991). This opinion is also shared by the authors of the present study. The examination of the available type material (*Martius s.n.*!, *Mosén 1996*!), left no doubt that *G. biloba* and *G. cylindrica* both represent *G. violacea*. The case of *G. reflexa*, however, is more difficult (as no physical type can be found anymore). In his protologue of *G. reflexa*, Benjamin (1847) did not cite a type specimen, neither did he mention the collector. The *locus classicus* is also denoted rather broadly as "*Habitat in Brasilia*". However in 1976, Peter Taylor (on herbarium annotations) recognized a photo of a collection made by Sellow (*s.n.*, without collection date) as the type of Benjamin's *G. reflexa*, as this was the only material of a *G. violacea*-like plant belonging to the given time frame and which did not have an earlier identification or name, and thus could without doubt be associated with Benjamin's description. This opinion was also followed by Fromm-Trinta (1979), and is also agreed with by the authors of the present study. Unfortunately, no further locality other than the country is given with the Sellow collection, which could help to determine the *locus classicus* of *G. reflexa*.

German plant collector Friedrich Sellow travelled across Brazil on six different expeditions, and collected near putative areas of distribution of all members of *Genlisea* subgenus *Tayloria,* especially in the state of Minas Gerais, which he travelled through on three expeditions (Urban 1906). The original material of the Sellow specimens in B very probably got destroyed in World War II, and thus black and white photos thereof deposited in K(!) and M(!) seem to represent the single remaining phototypes. Both the M and K photos (labelled "Key negative 16807, Nov. 1976") are obviously a photo print of a much older photo, which was taken from the original specimen. It has become evident from the first author's enquiry that there is no more original left of the photo taken from the holotype directly, neither in B nor in K. Sadly, the photo in M does not show the whole type sheet, but represents only a detail of the original herbarium specimen, as it only depicts two partial scapes, most likely belonging to two different individuals. According to the description of the plant by Benjamin (1847), the single specimen he studied had four visible scapes ("*scapi in unico specimine mihi viso 4,* [...]").

Unfortunately, the photo at M does not allow the identification of crucial floral characters of *G. reflexa*, but the upper corolla lip is somewhat larger as generally found in *G. violacea*. Most notably, the scape appears to be relatively robust (a fact also noted in the protologue: "*scapi* [...] *stricti, crassiusculi*"). Therefore, the

only taxon this specimen could possibly match, other than *G. violacea*, is *G. metallica* (as *G. lobata*, *G. exhibitionista* and *G. nebulicola* have much more slender scapes and very different and smaller corollae, *G. uncinata* and *G. oligophylla* have a thicker scape and a shorter pedicel at anthesis, which is distinctly circinate in fruit, and in *G. flexuosa* the spur would be much longer, and the upper corolla lip would be distinctly smaller). However we can further exclude that *G. reflexa* might represent the large-flowered *G. metallica*, as Benjamin's protologue states that the corolla of *G. reflexa* is "*2.5–3 lin. longi*" (ca. 5.6–6.7 mm), which is distinctly smaller than the corolla of *G. metallica* - but also only half the size of the corolla length given for *G. biloba* in the same text (from upper to lower lobe "*circiter 6 lin. longa*", which equals ca. 13.5 mm). Fortunately, the type material of *G. biloba* is available for comparison, and it is without doubt conspecific with Saint-Hilaire's *G. violacea*. Therefore Benjamin's *G. reflexa* very likely represents a stunted specimen of *G. violacea*, as this is the only known taxon that matches both corolla shape and size.

Earlier reports of large, many flowered specimens of *G. violacea* with scapes up to 64 cm and bearing up to 17 flowers (Fromm-Trinta 1979, Fromm-Trinta 1981) are referring to *G. flexuosa*, *G. oligophylla*, and *G. uncinata*. *Genlisea violacea* is a small to medium sized plant across most of its range, and usually has scapes not exceeding 25 cm, with up to six flowers. Only rarely the scapes reach up to 35 cm tall (*F. Rivadavia et al. 588*), with a maximum of 12 flowers.

Specimens examined:—BRAZIL. without locality, without date, *J. Kuhlmann 11199* (RB); without date, without locality, *F. Sellow s.n.* (B+, photo K! photo M!, probably type of *G. reflexa*).

São Paulo: Guaratinguetá, without date, *C.F.P. Martius s.n.* (M 173411, holotype of *G. biloba*); Campos da Bocaina, 16 February 1876, *A.F.M. Glaziou 8218a* (K *fragmentum*!).

Minas Gerais: without locality, 1892, *A.F.M. Glaziou 19871* (K); Serra de Caldas (infra Pedra Branca), 01 June 1874, *C.W.H. Mosén 1996* (S [not seen], K *fragmentum typi*! photo K! photo M!, holotype of *G. cylindrica*); Cidade de Caldas, Pedra Branca, March 1875, *A.F. Regnell III.848* (R, K *fragmentum*!, photo M!); Caldas, Pedra Branca, ca. 1950 m, 24 January 1980, *A. Krapovickas & C.L. Cristóbal 35475* (K). Município de Lima Duarte, Distrito de Conceição de Ibitipoca, Parque Estadual Florestal de Ibitipoca, 21 January 1987, *H.C. de Souza s.n.* (BHCB); ca. 1350 m, 23 November 2001, *F. Rivadavia 1322* (SPF); ca. 1380 m, 26 February 2005, *F. Rivadavia 1952* (SPF); Parque Estadual da Serra do Ibitipoca, 17 December 2010, *P.M. Gonella et al. 368* (SPF); Serra Negra, Fazenda Serra Negra, Cachoeira da Mamãe Oxum, 961 m, 02 March 2008, *F.R.G. Salimena et al. 2690* (CESJ). Município de Tiradentes, alto da Serra de São José, ca.1170 m, 08 June 2007, *F. Rivadavia et al. 2588* (SPF). Município de Capitólio, Furnas, entre Furnas e Piumhi, 22 March 1991, *F. Rivadavia 47* (SPF); Furnas, estrada Passos–Piumhi (MG050), ca. 820 m, 08 March 1997, *F. Rivadavia 636* (SPF); estrada Furnas–Capitólio, 13 February 1998, *R. Goldenberg et al. 512* (UEC) [mixed collection of *G. violacea* and *Utricularia amethystina*]; estrada Passos–Piumhi (MG-050), 26 March 2011, *P.M. Gonella 405* (SPF). Município de São Roque de Minas, Serra da Canastra, vale da nascente do rio São Francisco, 12 December 1996, *L.S. Kinoshita & A.O. Simões 96155* (UEC); Parque Nacional da Serra da Canastra, trilha entre a base e o alto da Casca D'Anta, ca. 1200 m, 02 April 1999, *F. Rivadavia & M. Peixoto 880* (SPF); Parque Nacional da Serra da Canastra, na parte alta da Cachoeira Casca D'Anta, 27 March 2011, *P.M. Gonella 409* (SPF). Município de Moeda, Serra da Moeda, estrada para Moeda, ca. 1140 m, 12 March 2002, *F. Rivadavia 1340* (SPF); 26 April 2002, *F. Rivadavia 1350* (SPF); estrada para Moeda, 1331 m, 26 April 2007, *J.A.N. Batista & C.A.N. Martins 2057* (BHCB). Município de Catas Altas, Caminho para Pico do Sol, ca. 1850 m, *I. San Martin Gaiardo & I. Freitas 162001* (UEC); Serra do Caraça, Pico do Inficionado, 1950 m, 16 February 2000, *M.F. Vasconcelos s.n.* (BHCB 52583); Serra do Caraça, 17 February 2001, *R.C. Mota 1204* (BHCB); RPPN Santuário do Caraça, Pico da Carapuça, 1919 m, 17 February 2009, *C.T. Oliveira & A.J. Arruda 319* (BHCB). Município de Santa Bárbara, sandstone summit of Serra do Caraça, ca. 1750–1950 m, 25 January 1971, *H. S. Irwin et al. 29060* (K); Parque Natural do Caraça, trilha para Gruta do Padre Caio, 1400 m, 02 March 1992, *F. Rivadavia 110* (SPF); subida do Pico do Sol, 1800 m, 04 March 1992, *F. Rivadavia 119* (SPF); subida do Pico da Carapuça, 1700–1850 m, 05 March 1992, *F. Rivadavia 121* (SPF). Município de Santa Luzia, Serra do Cipó, 15 April 1935, *H.L. Mello Barreto 1264* (RB); Serra do Cipó, km 138, Estrada Pilar, 15 April 1935, *H.L. Mello Barreto 1067 & A.C. Brade 14424* (BHCB, RB). Município de

Jaboticatubas, rodovia Lagoa Santa–Conceição do Mato Dentro–Diamantina, 04 March 1972, *A.B. Joly et al. 771* (UEC); rodovia Lagoa Santa–Conceição do Mato Dentro–Diamantina, 27 May 1972, *A.B. Joly et al. 2109* (UEC); Fazenda da Serra do Cipó, 26 February 1992, *F. Rivadavia 98* (SPF); Serra do Cipó, próximo a estátua do Juquinha, 10 April 1993, *F. Rivadavia 214* (SPF); Serra do Cipó, morro entre a sede do IBAMA e a estátua do Juquinha, ca. 1500 m, 04 July 1995, *F. Rivadavia 442* (SPF); Serra do Cipó, km 112–113 da estrada para Conceição do Mato Dentro, ca. 1360 m, 04 April 2003, *F. Rivadavia 1546* (SPF). Município de Santana do Riacho, rodovia Belo Horizonte–Conceição do Mato Dentro, 30 March 1980, *I. Cordeiro et al. CFSC 6074* (SPF); rodovia Belo Horizonte–Conceição do Mato Dentro, 23 July 1980, *N.M. Menezes et al. CFSC 6383* (SPF). Município de Santana do Pirapama, rodovia Belo Horizonte–Conceição do Mato Dentro, Fazenda Inhame (Serra Mineira), Serra do Cipó, 22 March 1982, *J.R. Pirani et al. CFSC 8086* (SPF) [mixed collection with *Utricularia amethystina*]; 23 March 1982, *I. Cordeiro et al. CFSC 8190* (SPF); rodovia Belo Horizonte–Conceição do Mato Dentro, Fazenda Palácio, 19 July 1985, *D.C. Zappi CFSC 9341* (SPF 40380); rodovia Belo Horizonte–Conceição do Mato Dentro, 26 February 1987, *D.C. Zappi et al. CFSC 10021* (SPF); estrada Belo Horizonte–Conceição do Mato Dentro, bifurcação para Morro do Pilar, 14 February 1988, *J.M. Piliackas et al. CFSC 10884* (SPF); bifurcação da estrada para Conceição do Mato Dentro e Morro do Pilar, ca. 1450 m, 23 February 1996, *F. Rivadavia & J. Mullins 536* (SPF); Distrito de Lapinha, trilha para o Pico do Breu, ca. 1400 m, 30 January 2005, *F. Rivadavia 1947* (SPF); Serra do Cipó, distrito de São José da Cachoeira, trilha da Captação da Fazenda Toucan Cipó, 680 m, 17 February 2007, *V.C. Souza et al. 32551* (ESA); estrada S. José da Cachoeira–Inhame, 1500 m, 19 February 2007, *D.C. Zappi et al. 817* (ESA, RB, SPF); Serra do Cipó (Serra da Lapa), distrito de São José da Cachoeira, trilha da Senhorinha, 1300 m, 19 February 2007, *V.C. Souza et al. 32835* (ESA, RB). Serra do Cipó, without attribution to administrative area: Environs de Rio Janeiro et D'Ouro Preto, 1883–1884, *A.F.M. Glaziou 15182* (K); Serra do Cipó, 20 July, without year, *L. Damazio s.n.* (RB 112382); Serra do Cipó, 06.1908, *L. Damazio 2068* (RB). Município de Congonhas do Norte, Serra da Mangabeira, ca.1240 m, 26 February 1997, *F. Rivadavia & F. Pinheiro 562* (SPF); Serra da Carapina (Serra Talhada segundo folha do IBGE), 1310 m, 02 March 1998, *R.C. Forzza et al. 687* (SPF). Município de Serro, Serro Frio [Serro], in paludibus supra montes adamantinus, propre Tejuco [Diamantina], without date, *C.F.P. Martius s.n.* (M 173410); de Serro em direção a Milho Verde, 1000 m, 11 March 1995, *V.C. Souza et al. 8327* (ESA); Serro para Diamantina, 25 February 1967, *A.P. Duarte 10385* (RB); BR 259, nascente do Rio Jequitinhonha, 04 March 1999, *F. Feres et al. 99/47* (UEC); estrada para Gouveia, 980 m, 27 February 2002, *V.C. Souza et al. 28517* (ESA); Distrito de Milho Verde, cachoeira do Lajeado, ca.1050 m, 05 April 2003, *F. Rivadavia & J.P.A. Neves 1566* (SPF); estrada de Milho Verde para Diamantina, ca.1220 m, 06 April 2003, *F. Rivadavia 1578* (SPF); estrada de Milho Verde para Capivari, ca.1210 m, 13 May 2007, *F. Rivadavia 2560* (SPF). Município de Santo Antonio do Itambé, ca. 18 km N of Serro on road (MG 2) to Diamantina, 1200 m, 23 February 1968, *H. S. Irwin et al. 20665* (K); Trinta Réis, estrada Serro–Diamantina, 27 January 1986, *H.L. Wagner et al. CFCR 9232* (BHCB, SPF). Município de Gouveia, estrada Diamantina km 98, 03 June 1985, *H.F. Leitõa f. et al. 17225* (UEC); estrada Curvelo–Diamantina, Serra do Barro Preto, 09 April 1982, *A. Furlan et al. CFCR 3209* (SPF); Contagem, Fazenda Galheiros, 06 February 2009, *R. Mello-Silva & M.G. Sajo 3159* (SPF); rodovia Gouveia–Curvelho (BR 259), ao sul da fazenda Contagem, norte da Serra do Indaial, base de inselberg próximo ao Ribeirão do Contagem, 07 February 2009, *R. Mello-Silva & M.G. Sajo 3164* (SPF). Município de Datas, vicinity of Datas, 1300 m, 24 January 1969, *H. S. Irwin et al. 22558* (NY photo!); estrada Datas–Serro, povoado de Trinta Réis, 19 April 1987, *D.C. Zappi et al. CFSC 10668* (SPF). Município de Diamantina, Riacho das Varas [Conselheiro Mata], 21 March 1892, *Schwacke 7945* (OUPR); Conselheiro Mata, June 1934, *A.C. Brade s.n.* (RB 28479); Diamantina, January 1947, *W.A. Egler s.n.* (RB 59862); ca. 18 km E of Diamantina, 900 m, 18 March 1970, *H. S. Irwin et al. 27877* (K); estrada Turmalina/Diamantina, MG-2, 14 km de Diamantina, 1200 m, 14 May 1979, *G. Martinelli 5963* (RB); estrada para Biri-Biri, ca. 944 m [3098 feet; elevation given as "3068 m" certainly not correct], 08 April 1982, *N. Hensold et al. CFCR 3119* (K, SPF); Diamantina, ao leste da cidade, 22 February 1992, *F. Rivadavia 61* (SPF); Serro dos Cristais, estrada vicinal da rodovia para Araçuaí (BR 367), 30 January 2000, *R. Mello-Silva & R.C. Forzza 1758 (*SPF); Area de Proteção Ambiental Pau de Fruta, 13 February 2001, *J.A. Lombardi 4217*

(BHCB); estrada entre Diamantina e Conselheiro Mata, 1180 m, 08 July 2001, *V.C. Souza et al. 25437* (BHCB, ESA, SPF); rodovia para Mendanha (BR-367), 1300 m, 23 January 2007, *J.R. Pirani et al. 5680* (SPF); estrada para a Gruta do Salitre, ca. 1150 m, 13 May 2007, *F. Rivadavia 2546* (SPF); estrada para Milho Verde, ca. 1120 m, 13 May 2007, *F. Rivadavia 2551* (SPF); ao sul da cidade de Diamantina, 1140 m, 24 July 2008, *P.M. Gonella et al. 169* (SPF); ao norte do distrito de Inhaí, 05 September 2011, *P.M. Gonella et al. 461* (SPF). Município de Rio Vermelho, Serra da Torre, ca. 1300 m, 11 July 1999, *F. Rivadavia et al. 1112* (SPF). Município de Couto de Magalhães de Minas, ca. 30 km ao leste de Diamantina ca. 905 m, 12 May 2007, *F. Rivadavia 2523* (SPF); ca. 40 km ao leste de Diamantina, ca.1330 m, 12 May 2007, *F. Rivadavia 2532* (SPF, M). Município de São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, junto ao córrego da Lapa, 07 April 2000, *J.A. Lombardi et al. 3764* (BHCB); Parque Estadual do Rio Preto, 08 April 2000, *J.A. Lombardi et al. 3900* (BHCB); cerca de 1 km depois da portaria, ca. 828 m, 05 February 2009, *P.M. Gonella & R.V.R. Viana 181* (SPF); trilha para o alto da chapada, ca. 1107 m, 05 February 2009, *P.M. Gonella & R.V.R. Viana 187* (SPF). Município de Augusto de Lima, Serra do Cabral, ca. 12 km da cidade em direção à Fazenda Serra do Cabral, 1000 m, 20 March 1994, *C.M. Sakuragui et al. CFCR 15259* (SPF) [mixed collection with *Utricularia* spp.]. Município de Buenópolis, Parque Nacional das Sempre Vivas, atrás do alojamento de guarda-parques, 04 September 2011, *P.M. Gonella et al. 446* (SPF). Município de Itacambira, estrada Juramento–Itacambira (MG-308), 13 February 2011, *P.M. Gonella et al. 400* (SPF).

The following specimens are somewhat atypical for *G. violacea*, and differ in having very long scapes, numerous flowers, and/or a prolonged spur, resembling *G. flexuosa* in these characters. All of these were collected in populations that occur in the northernmost part of the range of *G. violacea*, close to the range of *G. flexuosa*, therefore introgression with the latter species cannot be excluded. The specimens, however, are here assigned to *G. violacea*, as they agree with this species in the majority of combinations of characters.

BRAZIL. Minas Gerais: Município de Gouveia, estrada Diamantina–Curvelo, ca.1260 m, 02 August 2002, *F. Rivadavia & R. Gibson 1403* (SPF); 06 April 2003, *F. Rivadavia 1589* (SPF); 03 September 2011, *P.M. Gonella et al. 423* (SPF). Município de Diamantina, Cachoeira dos Cristais, ca.1000 m, 01 March 1997, *F. Rivadavia et al. 588* (SPF). Município de São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, descendo o Ribeirão das Éguas, ca. 890 m, 28 June 2003, *F. Rivadavia & Deco 1611* (SPF). Município de Buenópolis, Serra do Cabral, estrada para Lapa Pintada, 1100-1200 m, 13 October 1988, *R.M. Harley & al. 24937* (SPF, K photo!). Município de Joaquim Felício, Serra do Cabral, Fazenda da Onça, ca. 1100 m, 01 September 1985, *J.R. Pirani et al. CFCR 8179* (SPF); Serra do Cabral, descida da Serra, 1000 m, 12 February 1988, *J.R. Pirani et al. 2222* (SPF); Serra do Cabral, estrada que sobe a serra a partir da rua do Cruzeiro em J. Felício, ca.1080 m, 06 March 1997, *F. Rivadavia 617* (SPF); Serra do Cabral, km34 da estrada que sobe a serra ao norte de Joaquim Felício, ca.1200 m, 07 March 1997, *F. Rivadavia 630* (SPF); Serra do Cabral, 16 May 1999, *V.C. Souza et al. 22422* (ESA, SPF); estrada da 'Serra do Cabral Agro Indústria S.A.' entre J.Felício e Augusto de Lima sobre a Serra do Cabral, a 10km da câmara municipal de J.Felício, ca. 990 m, 29 June 2003, *F. Rivadavia 1635* (SPF); estrada da 'Serra do Cabral Agro Indústria S.A.' entre J.Felício e Francisco Dumont, ca. 1200 m, 03 July 2003, *F. Rivadavia 1667* (SPF); Serra do Cabral, 1016 m, 21 April 2010, *P.M. Gonella et al. 285* (SPF). Município de Grão Mogol, próximo ao Morro do Jambeiro, 03 June 1994, *F. Rivadavia 281* (SPF); estrada Grão Mogol–Montes Claros, 715 m, 12 August 2010, *P.M. Gonella 356* (SPF); 07 Sepember 2011, *P.M. Gonella et al. 480* (SPF); trilha para Cachoeira Véu das Noivas, 11 February 2011, *P.M. Gonella et al. 383* (SPF).

Discussion

The more robust, perennial species (*G. uncinata*, *G. oligophylla*, *G. flexuosa*, and *G. metallica*) represent the early branching taxa in *G.* subgenus *Tayloria*, which are consecutive sisters to the annual species (Fleischmann *et al.* 2010; unpublished data for *G. oligophylla*). The origin of *G.* subgenus *Tayloria* can be

assumed in the northern part of its range, as the extant most basally branching species, *G. uncinata*, is confined to the high mountain tops of the Chapada Diamantina, Bahia. From there, a phylogeographic southward migration is observed in the subsequently branching species: *G. flexuosa* occurs further south, but still represents the northernmost outpost of *G.* subgenus *Tayloria* in the Espinhaço Range of Minas Gerais, followed by the slightly more southerly occurring *G. metallica*, and finally *G. violacea* and *G. lobata*, which extend the range far to the south and south-west, and east respectively (for phylogenetic trees, see Fleischmann *et al.*, 2010; a comprehensive phylogeny of the subgenus including the newly described taxa is in preparation).

In *Genlisea* subgenus *Tayloria*, the different pattern of the fruiting pedicel reflexion is a diagnostic character, and it can be somewhat helpful for species delimitation. *Genlisea uncinata* has pedicels that are strongly circinate in fruit (pedicels strongly curved inwardly and evenly along their whole length), often forming arcs of about 360°, this means the capsule is almost tangent to the pedicel base. *Genlisea oligophylla* has much more laxly circinate pedicels in fruit (pedicels evenly curved inwardly along their whole length, but less strong than in *G. uncinata*; sometimes stronger curved in the basal part than in the apical part). In the majority of species, namely *G. metallica*, *G. exhibitionista*, *G. flexuosa*, *G. nebulicola*, and *G. violacea*, the fruiting pedicels are recurved in fruit (pedicels arcuate-curved downwards only near the base, the apical part often more or less paralleling the peduncle; in *G. flexuosa* usually subsequently curved or twisted upwards at half length when the capsules mature). In *G. violacea*, some fruiting pedicels are occasionally curved as full arc of a circle, thus the capsule is facing upwards. *Genlisea lobata* is the only species in which the fruiting pedicels are not recurved, but sharply reflexed from the base (pedicels bent downwards abruptly from the base, not curved, but more or less paralleling the peduncle for their whole length).

Although the overall rhizophyll morphology is very similar in all members of *G.* subgenus *Tayloria*, and all species produce only one type of rhizophyll traps (no trap dimorphism has been observed, such as that found by Studnicka (1996) in some species of *G.* subgenus *Genlisea*), at least some differences in the length ratio of trap stalk to the helically twisted trap arms are found: in the phylogenetically early branching species *G. uncinata*, *G. oligophylla* and *G. metallica*, the trap stalk is much prolonged, reaching deep into the substrate (Rivadavia 2000), and the trap arms are relatively short. In contrast, in the other members of the subgenus, the long helically twisted trap arms comprise the major part of the entire trap length. The orientation of the traps in the soil is also different, with the former three species having rhizophylls reaching radially deep into the soil, whereas the remaining species *G. violacea*, *G. lobata*, *G. nebulicola*, *G. exhibitionista* and *G. flexuosa* form trap leaves that are usually spreading horizontally just below the soil surface. This most likely is an adaptation to different substrates, as these species (with the exception of *G. flexuosa*) grow in shallow soils, often lithophytically, whereas *G. uncinata*, *G. oligophylla*, *G. metallica* can be found growing in deeper soils. However, as the underground parts of *Genlisea* are usually not well-preserved in herbarium specimens, and difficult to observe as they require carefully excavated plants, trap characters are not considered for this taxonomic treatment.

Acknowledgements

The authors would like to thank the curators of the respective herbaria for access to the specimens for study, or for sending specimens on loan to the first author; Renato Goldenberg for contributing information and photographs of *Genlisea* from Espírito Santo; Hajo Esser for providing further information on early Brazilian plant collectors and comments on the manuscript. Nílber Gonçalves da Silva for helpful contributions and for sending photos of specimens from R; Adilson Peres, Nílber Gonçalves da Silva, Vitor Albuquerque, Joe Mullins, Ed Read, Fabio Pinheiro, Vitor Miranda, Wilson Ganev, Joás Brandão, Aline Amália do Vale, Vinicius Dittrich, Mauro Peixoto, Robert Gibson, and Stewart McPherson for help with fieldwork; Linilson Padovese for discovering *G. nebulicola* at the Serra da Canastra, Alex Melo for reporting his finding of *G. nebulicola* at the Serra do Sete Voltas; Fernanda Antunes Carvalho for help with DIVA-GIS analysis; Eva

Facher for SEM analysis; Jan Schlauer and Kamil Pasek for helpful comments; Xander van der Burgt, Melissa Barington, and Martin Cheek for assistance at Kew; Maarten Christenhusz, Miloslav Studnicka and an anonymous reviewer for helpful comments on the manuscript. Permits for fieldwork were issued by ICMBIO for PMG. AF received a grant of the graduate funding of the Universität Bayern e.V., PMG was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Alves, R.J.V., Cardin, L. & Kropf, M.S. (2007) Angiosperm disjunction "*Campos rupestres* - *restinga*": a re-evaluation. *Acta Botanica Brasilica* 21: 675–685.

Antonelli, A., Verola, C.F., Parisod, C. & Gustafsson, A.L.S. (2010) Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society* 100: 597–607.

Angiosperm Phylogeny Group [Bremer, B., Bremer, K., Chase, M.W., Fay, M.F., Reveal, J.L., Soltis, D.E., Soltis, P.S. & Stevens, P.F. (comp.)] (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society of London* 161: 105–121.

Benites, V.M., Schaefer, C.E.G.R., Simas, F.N.B. & Santos, H.G. (2007) Soils associated with rock outcrops in the Brazilian mountain ranges Mantiqueira and Espinhaço. *Revista Brasileira de Botânica* 30: 569–577.

Benjamin, L. (1847) Utricularieae. *In*: Martius, C.F.P. von (ed.), *Flora Brasiliensis 10*. R. Oldenbourg, Munich and Leipzig, pp. 233–256.

Dwyer, J.D. (1955) The botanical catalogues of Auguste de St. Hilaire. *Annals of the Missouri Botanical Garden* 42: 153–170.

Echternacht, L., Trovó, M., Oliveira, C.T. & Pirani, J.R. (2011) Areas of endemism in the Espinhaço Range in Minas Gerais, Brazil. *Flora* 206: 782–791.

Fischer, E., Porembski, S. & Barthlott, W. (2000) Revision of the genus *Genlisea* (Lentibulariaceae) in Africa and Madagascar with notes on ecology and phytogeography. *Nordic Journal of Botany* 20: 291–318.

Fleischmann, A. & Rivadavia, F. (2009) *Utricularia rostrata* (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil. *Kew Bulletin* 64: 155–159.

Fleischmann, A., Schäferhoff, B., Heubl, G., Rivadavia, F., Barthlott, W. & Müller, K.F. (2010) Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae). *Molecular Phylogenetics and Evolution* 56: 768–783.

Fromm-Trinta, E. (1977) *Tayloria* Fromm-Trinta - Nova Seção do gênero *Genlisea* St.-Hil. (Lentibulariaceae). *Boletim do Museu Nacional Rio de Janeiro, Botanica* 44: 1–4.

Fromm-Trinta, E. (1979) Revisão das espécies do gênero *Genlisea* St.-Hil (Lentibulariaceae) das regiões sudeste e sul do Brasil. *Rodriguésia* 31(49): 17–139.

Fromm-Trinta, E. (1981) Revisão do gênero *Genlisea* St.-Hil. (Lentibulariaceae) no Brasil. *Boletim do Museu Nacional Rio de Janeiro, Botanica* 61: 1–21.

Fromm-Trinta, E. (1989) *Genlisea lobata* Fromm-Trinta – Uma nova espécie para o gênero *Genlisea* St.-Hil. Sect. *Tayloria* (Lentibulariaceae). *Bradea* 4: 152–155.

Fromm-Trinta, E. (1996) Flora da Serra do Cipó, Minas Gerais: Lentibulariaceae. *Boletim de Botânica da Universidade de São Paulo* 15: 105–118.

Fromm-Trinta, E. (2004) Flora de Grão-Mogol, Minas Gerais: Lentibulariaceae. *Boletim de Botânica da Universidade de São Paulo* 22: 267–271.

Funch, R.R., Harley, R.M. & Funch, L.S. (2009) Mapping and evaluation of the state of conservation of the vegetation in and surrounding the Chapada Diamantina National Park, NE Brazil. *Biota Neotropica* 9: 21–30.

Giulietti, A.M., Menezes, N.L., Pirani, J.R., Meguro, M. & Wanderley, M.G.L. (1987) Flora da Serro do Cipó, Minas Gerais: caracterização e lista das espécies. *Boletim de Botânica da Universidade de São Paulo* 9: 1–151.

Giulietti, A.M. & Pirani, J.R. (1988) Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. *In*: Vanzolini, P.E. & Heyer, W.R. (Eds), *Proceedings of a workshop on neotropical distribution patterns*. Academia Brasileira de Ciências, Rio de Janeiro, pp. 39–69.

Giulietti, A.M., Pirani, J.R. & Harley, R.M. (1997) Espinhaço Range region, Eastern Brazil. *In*: Davis, S.D., Heywood, V.H., Herrera-MacBryde, O., Villa-Lobos, J. & Hamilton, A.C. (Eds), *Centres of plant diversity: a guide and strategy for their conservation*. *Vol. 3: The Americas*. WWF/IUCN Publications Unit, Cambridge, pp. 397–404.

Glaziou, A.F.M. (1911) Utricularicacées. *In*: Plantae Brasiliae centralis a Glaziou lectae. Liste des plantes du Brésil Central recueillies en 1861-1895. *Bulletin de la Société Botanique de France, Mémoires* 3f: 511–512.

Harley, R.M. & Mayo, S.M. (1980) *Towards a checklist of the flora of Bahia*. Royal Botanic Gardens, Kew.

Harley, R.M. & Simmons, N.A. (1986) *Florula of Mucugê, Chapada Diamantina, Bahia, Brazil*. Royal Botanic Gardens, Kew.
Harley, R.M. (1988) Evolution and distribution of *Eriope* (Labiatae), and its relatives, in Brazil. *In*: Vanzolini, P.E. & Heyer, W.R. (Eds), *Proceedings of a workshop on neotropical distribution patterns*. Academia Brasileira de Ciências, Rio de Janeiro, pp. 71–120.
Hijmans, R.J., Guarino, L., Jarvis, A., O'Brien, R., Mathur, P., Bussink, C., Cruz, M., Barrantes, I. & Rojas, E. (2005) DIVA-GIS 5.2. A geographic information system for the analysis of species distribution data. Program and manual available at http://www.diva-gis.org (accessed 01.04.2011).
IUCN (2001) *IUCN red list categories and criteria, version 3.1.* IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.
Leins, P. & Erbar, C. (2010) *Flower and fruit. Morphology, ontogeny, phylogeny, function and ecology.* Schweizerbarth Science Publishers, Stuttgart.
Linnaeus, C. (1753) *Species Plantarum*. Impensis Laurentii Salvii, Stockholm.
Płachno, B.J., Faber, J. & Jankun, A. (2005) Cuticular discontinuities in glandular hairs of *Genlisea* A.St.-Hil. in relation to their functions. *Acta Botanica Gallica* 152: 125–130.
Rapini, A., Mello-Silva, R.D. & Kawasaki, M.L. (2002) Richness and endemism in Asclepiadoideae (Apocynaceae) from the Espinhaço Range of Minas Gerais, Brazil—a conservationist view. *Biodiversity and Conservation* 11: 1733–1746.
Reut, M.S. (1993) Trap structure of the carnivorous plant *Genlisea* (Lentibulariaceae).*Botanica Helvetica* 103: 101–111.
Rivadavia, F. (2000) The giant *Genlisea uncinata* P.Taylor & Fromm-Trinta. *Carnivorous Plant Newsletter* 29: 83–86.
Rivadavia, F. (2002) *Genlisea lobata* Fromm-Trinta. *Carnivorous Plant Newsletter* 31: 116–118.
Rivadavia, F. (2003) Four new species of sundews, *Drosera* (Droseraceae), from Brazil. *Carnivorous Plant Newsletter* 32: 79–92.
Rivadavia, F. (2009) *Drosera x fontinalis* (Droseraceae), the first natural sundew hybrid from South America. *Carnivorous Plant Newsletter* 38: 121–125.
Rivadavia, F. & Gonella, P.M. (2011) *Drosera quartzicola* (Droseraceae), a new and threatened species from the Serra do Cipó, Brazil. *Phytotaxa* 29: 33–40.
Saint-Hilaire, A. (1824) *Histoire des Plantes les plus Remarquables du Brésil et du Paraguay: tome premier*. A. Belin, Paris.
Saint-Hilaire, A. (1833) *Voyage dans le district des Diamans du Brésil*. Librairie-Gide, Paris.
Saint-Hilarie, A. & Girard, F. (1838) Monographie des Primulacées et des Lentibulariées du Brésil méridional. *Comptes Rendus Hebdomadaires des Séances de l'Academie des Sciences, Series. D: Sciences Naturelles* 7: 868–870.
Studnicka, M. (1996) Several ecophysiological observations in *Genlisea*. *Carnivorous Plant Newsletter* 25: 14–16.
Sylvén, N. (1909) Die Genliseen und Utricularien des Regnell'schen Herbariums. *Arkiv för Botanik* 8(6): 1–48.
Taylor, P. (1989) *The genus Utricularia – a taxonomic monograph*. Royal Botanic Gardens, Kew, London.
Taylor, P. (1991) The genus *Genlisea*. *Carnivorous Plant Newsletter* 20: 20–26.
Taylor, P. & Fromm-Trinta, E. (1983) Uma nova espécie para o gênero *Genlisea* St.-Hil. sect. *Tayloria* (Lentibulariaceae): *Genlisea uncinata* P.Taylor & Fromm-Trinta. *Bradea* 3: 365–368.
Urban, I. (1906) Vitae itineraque collectorum botanicorum, notae collaboratorum biographicae. *In*: Martius, C.F.P. von (ed.), *Flora Brasiliensis 1, 1*. R. Oldenbourg, Munich and Leipzig, pp. 2–211.
Zappi, D.C., Lucas, E., Stannard, B.L., Lughadha, E.N., Pirani, J.R., de Queiroz, L.P., Atkins, S., Hind, D.J.N., Giulietti, A.M., Harley, R.M. & de Carvalho, A.M. (2003) Lista das plantas vasculares de Catolés, Chapada Diamantina, Bahia, Brasil. *Boletim de Botânica da Universidade de São Paulo* 21: 345–398.

Pinguicula chuquisacensis (Lentibulariaceae), a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula*

Beck, S.G., Fleischmann, A., Huaylla, H., Müller, K.F., Borsch, T., 2008.
Willdenowia 38: 201-212.

Article IV

STEPHAN G. BECK, ANDREAS FLEISCHMANN, HIBERT HUAYLLA, KAI F. MÜLLER & THOMAS BORSCH

Pinguicula chuquisacensis (Lentibulariaceae), a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula*

Abstract

Beck, S. G., Fleischmann, A., Huaylla, H., Müller, K. F. & Borsch, T.: *Pinguicula chuquisacensis (Lentibulariaceae),* a new species from the Bolivian Andes, and first insights on phylogenetic relationships among South American *Pinguicula.* – Willdenowia 38: 201-212. – ISSN 0511-9618; © 2008 BGBM Berlin-Dahlem.
doi:10.3372/wi.38.38115 (available via http://dx.doi.org/)

Pinguicula chuquisacensis is described and illustrated as a new species from the department of Chuquisaca of Bolivia. Data on distribution, ecology and a key for identification among other central Andean species are given. DNA sequence data are presented for this new taxon and related Andean species of *Pinguicula* for the first time. The plastid tree reveals a lineage of central Andean species (within the clade of tropical growth type species), whereas the northern Andean (Colombia, Venezuela) *P. elongata* appears distantly related and sister to all remaining taxa of the Mexican-Central American-Caribbean clade of *Pinguicula.*

Resumen

Se describe y se ilustra una nueva especie de *Pinguicula* de Chuquisaca, Bolivia, *P. chuquisacensis.* Se discuten la distribución, el hábitat y la ecologías y se presenta una clave de identificación entre las especies de las Andes Centrales. Se presentan una filogenia obtenida sobre la base de secuencias de ADN incluyendo este nuevo taxón y de las especies Andinas relacionadas por la primera vez. *P. chuquisacensis* pertenece a un grupo monofilético de las especies de las Andes Centrales pero *P. elongata* de las Andes del Norte (Colombia, Venezuela) es una línea hermana de todos los otras especies de *Pinguicula* que representan un clado distribuido en Mexico, Centro America y en el Caribe.

Additional key words: carnivorous plants, Andean biogeography, taxonomy, phylogenetics, *matK/trnK*

Introduction

The genus *Pinguicula* L. belongs to the carnivorous angiosperm family *Lentibulariaceae,* and is the sister group of the *Genlisea-Utricularia* clade (Jobson & al. 2003; Müller & al. 2004). *Pin-*

guicula is well supported as monophyletic by both molecular and morphological characters (Müller & al. 2004). Characteristic are flypaper traps with a special type of mucilage glands that loose turgor upon stimulation. To the contrary, the other two genera possess morphologically complex eel or bladder traps. Casper monographed *Pinguicula* in 1966, thereby recognizing 48 species. In a more recent synopsis Legendre (2000) already lists 85 species, and this number is still growing. Zamudio (2001, 2003, 2005) described several new taxa from Mexico; Casper & Urquiola Cruz (2003) and Casper (2003, 2004, 2007) from Cuba; and even in the European Alps a well isolated and easily distinguishable new species was discovered (Steiger & Casper 2001). Therefore, *Pinguicula* can be estimated to contain an approximated 100 species.

The first comprehensive phylogenetic analysis of *Pinguicula* used plastid *trnK/matK* sequence data (Cieslack & al. 2005). Rather than supporting a previous subgeneric and sectional classification system that had largely been based on floral morphology (Casper 1966), several geographically distinct radiations were unravelled. The most speciose of these radiations is a Mexican-Central American-Caribbean clade (*P. filifolia* Griseb., *P. moranensis* Kunth and relatives) with *P. alpina* L. as sister group. A Eurasian radiation with shallow branches probably resulting from rapid speciation comprises north temperate and sub-boreal taxa (*P. leptoceras* Rchb., *P. vulgaris* L. and relatives), whereas East Asian (*P. villosa* L. and relatives) and Mediterranean (*P. lusitanica* L. and relatives) appear as distinct lineages (Cieslack & al. 2005). From the South American butterworts *P. antarctica* Vahl (Patagonia, Tierra del Fuego) was resolved as the sister group to a clade of species from the southeastern United States (*P. lutea* Walter and relatives). So far, *P. antarctica* was the only South American *Pinguicula* included in any phylogenetic analysis. Degtarajeva & al. (2006) generated nrITS trees for *Pinguicula,* focussing on the Eurasian clade with particularly dense taxon sampling. Several major clades were congruently discovered with high bootstrap support such as the Eurasian clade *(P.* sect. *Pinguicula)* or the clade of species with tropical growth type from the southeastern United States (*P. lutea* and relatives), whereas deeper nodes only received confidence in Bayesian analysis of the data. Incongruent placements of *P. lusitanica* and *P. vulgaris* in the chloroplast and nuclear gene trees may point to the presence of ancient hybridisation in the evolution of *Pinguicula* but with the currently available data no conclusive assessment of reticulate evolution or other modes of speciation is possible. Using ITS sequences Shimai & al. (2007) recently analysed relationships among Cuban species of *Pinguicula*. These authors were also able to show that most of these narrow endemic species are distinguishable by their ITS ribotypes.

South American species of *Pinguicula* are known to grow in high Andean Paramo, Puna and Tierra Fria habitats (*P. calyptrata* Kunth, *P. elongata* Benj., *P. involuta* Ruiz & Pav.) or in Antarctic rain forests (*P. antarctica*, *P. chilensis* Clos; Casper 1966). All five South American species of *Pinguicula* were considered as closely related by Casper (1966) and classified as *P.* sect. *Ampullipalatum* Casper. In Bolivia the only known *Pinguicula* species was *P. involuta,* occurring in the Yungas of the La Paz departamento.

During preparation of the treatment of *Lentibulariaceae* for the Catalogo de las Plantas Vasculares de Bolivia morphologically and ecologically deviating plants of a new species of *Pinguicula* were encountered. Aims of this study were to evaluate their relationships among the South American taxa of *Pinguicula* using molecular and morphological data, to describe them as a new species, *P. chuquisacensis,* and to provide a key for identification of the currently accepted Andean species.

Material and methods

Plants were studied in their natural habitat by Hibert Huaylla at the type locality in Chuquisaca, Bolivia, and as herbarium specimens (see specimens citations). The validating description was provided by the authors of the new species. Measurements were taken from herbarium specimens and corollas were dissected using a binocular for detailed morphological analysis. Since herbar-

Table 1. List of the *Pinguicula* species and sources of the plant material from which *matK/trnK* sequences were generated and added to the *Pinguicula* alignment of Cieslack & al. (2005).

Species	Source	GenBank number	Geographic distribution
P. calyptrata	Peru, Depto. Amazonas, Prov. Chachapoyas, Balsas road to Leymebabamba, just below Abra Callacalla, 3559 m, *M. Weigend, E. Rodriguez, H. Förther & N. Dosert 2000/867* (M)	FM200225	central Andes (Colombia, Ecuador, Peru)
P. chuquisacensis	Bolivia, *C. E. Hinchliff, W. B. Warrington & A. Lliully 595* (without voucher but of same population as *Lliully & al. 587*, see specimens, below)	FM200223	central Andes (Bolivia)
P. elongata	Colombia, from tissue culture (no voucher)	FM200224	northern Andes (Colombia, Venezuela)
P. involuta	Peru, Dept. Huanuco, Weg von Laguna Copra zur Negro Coche, c. 3800 m, *T. Hofreiter & T. Franke 1/38* (M)	FM200226	central Andes (Bolivia, Peru)

ium material did not yield genomic DNAs in a quality that could be used for amplification of *matK/trnK* or ITS (T. Borsch, unpublished data), an additional specimen had to be recollected *(A. Lliully 895)* with some leaves preserved on silica gel.

For molecular phylogenetic analysis, *matK/trnK* sequences of *P. elongata, P. involuta, P. calyptrata* and of the new species were generated and added to the already existing *Pinguicula* alignment of Cieslack & al. (2005). Laboratory and alignment methods basically followed those described in Cieslack & al. (2005). The *trnK* intron including the *matK* gene was amplified and sequenced in two halves using primers trnK3914Fdi (GGGGTTGCTAACTCAACGG) and Le1 (ATAGAAATAGATTCGTTC) as well as ACmatK500F (TTCTTCTTTGCATTTATTACG) and trnK2R (AACTAGTCGGATGGAGTAG). The four new sequences generated for this study can be downloaded from EMBL/GenBank (Table 1). The alignment is available from the senior author upon request.

PAUP* (Swofford 2002) was used to execute parsimony ratchet command files generated by PRAP (Müller 2004). 10 random addition cycles with 200 ratchet iterations each were employed. During bootstrapping, searches for the optimal trees were far less intense, with only one tree swapped upon via TBR branch swapping in each replicate (Müller 2005). To minimize standard errors of bootstrap proportions, 10 000 replicates were run.

MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) was used for Bayesian analyses under the GTR+Γ model. Default priors were used, i.e. flat dirichlets (1.0,…,1.0) for state frequencies and instantaneous substitution rates, a uniform prior (0.0, 50.0) for the shape parameter of the gamma distribution, a uniform prior (0.0, 1.0) for the proportion of invariable sites, a uniform topological prior, an exponential prior Exp(10.0) for branch lengths. Four categories were used to approximate the gamma distribution. Two runs with 2 million generations each were run, and four chains were run in parallel for each run, with the temperature set to 0.2. The chains were sampled every 100th generation. To check for convergence of the independent runs under a given model, we ensured that plots of both runs indicate that stationary phase was reached in both runs, the potential scale reduction factor approached 1 for all parameters, and no significantly conflicting nodes were found among the consensus trees from the individual runs.

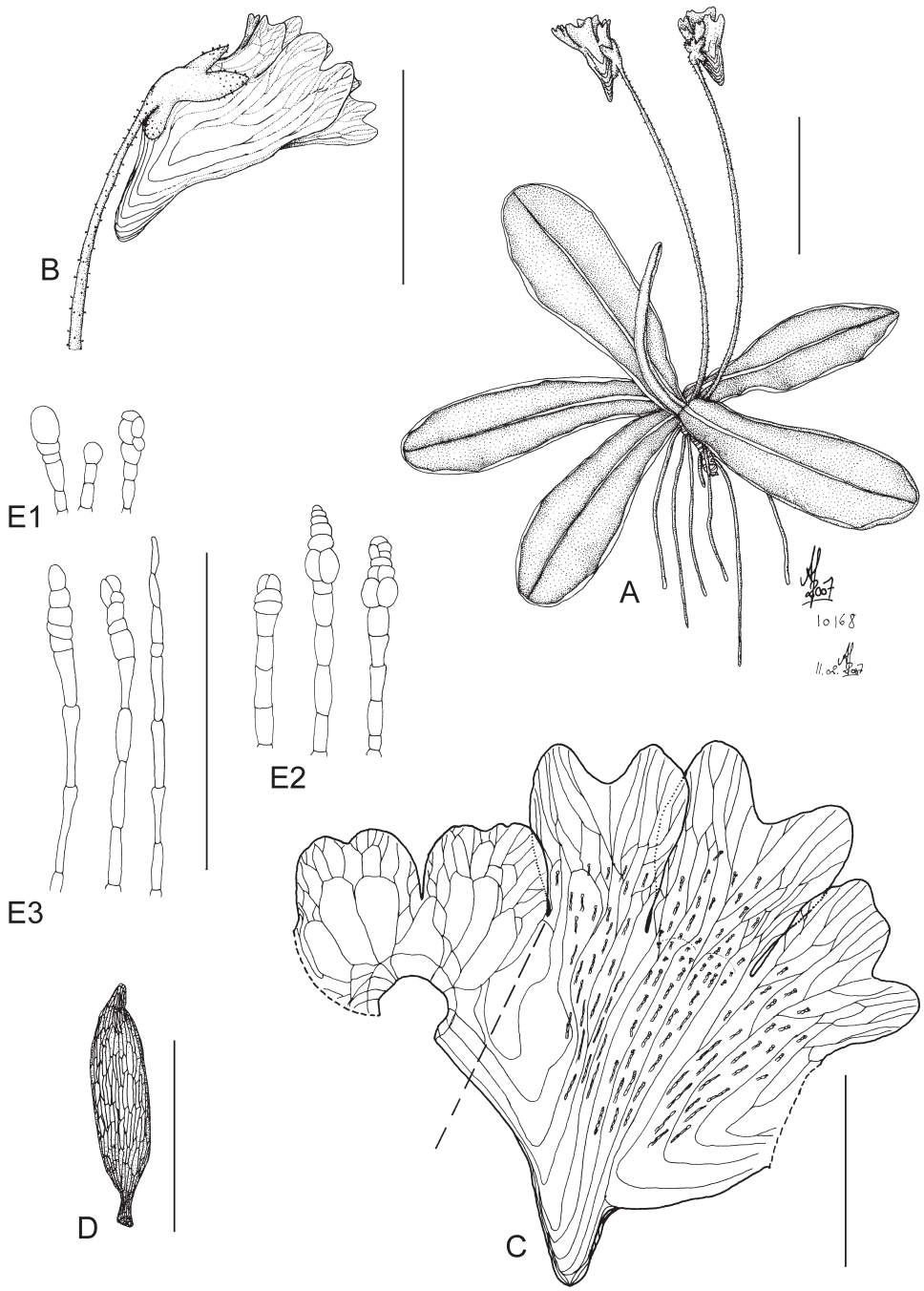


Fig. 1. *Pinguicula chuquisacensis* – A: habit of flowering plant; B: lateral view of the flower; C: corolla, opened; D: seed; E: hairs of the corolla tube, E1: hairs of the palate region, E2: hairs of the base of the lobes of the lower lip, E3: hairs of the three rows in the corolla tube interior. – Scale bars: A = 20 mm, B = 10 mm, C = 5 mm, D = 50 µm, E = 100 µm; drawn from the type collection by Andreas Fleischmann.

Results

Pinguicula chuquisacensis S. Beck, A. Fleischm. & Borsch, **sp. nov.**
Holotype: Bolivia, Depto. Chuquisaca, Prov. Belisario Boeto, 1 km de Nuevo Mundo a Padilla, 2250 m, 5.7.1995, *M. Kessler 5149 & J. Gonzales & K. Bach* (LPB; isotype: B).

Paratypes. – Bolivia: Chuquisaca: Boeto, Nuevo Mundo, NE of Villa Serrano, steep base hills with wooded valleys and rough grazing, 2400 m, 23.11.1995, *J. R. I. Wood 10168* (HSB, K, LPB); Boeto, 1 km de la comunidad de Nuevo Mundo, camino a Serrano, 2518 m, 14.5.2004, *H. Huaylla 1121* (HSB, MO); Boeto, Nuevo Mundo, Quebrada Nuevo Mundo, cañón húmedo al sudoeste del pueblo, 2145 m, 30.11.2005*, J. Villalobos con M. Paredes & D. Villalobos 501* (B, HSB, LPB); same locality, 2.7.2007, *C. E. Hinchliff* with *W. B. Warrington & A. Lliully 587* (HSB, LPB, M, WS).

Pinguicula calyptrata Kunth affinis sed foliis 4-6 (non 5-10), valde majoribus (4-11 cm non 1-4.5 cm), oblongis ad ovato-oblongis (non obovatis ad rotundato-obtusis), corolla albida (non purpurea ad pallide lilacina), lobis labii superioris lobis labii inferioris distincte brevioribus (non similibus), subrectangulis et subemarginatis vel integerrimis (non profunde emarginatis) differt.

Herba perennis. *Rhizoma* simplex, ca. 15 mm longum, radicibus adventitiosis filiformibus numerosis. *Folia* 4-6 radicalia rosulata, solum adpressa vel suberecta, succulenta (sicco membranacea), (3-)4-6(-11) cm longa et (1-)2-3 cm lata, oblonga vel ovato-oblonga, apice rotundata, basin versus abrupte angustata, margine leviter involuta, laete viridia, superne glandulis obsita. *Hibernacula* nulla. *Scapi* 1-4(-5), erecti, teretes, glandulis stipitatis disperse obsiti, 5-7(-11) cm alti. *Flores* parvi, 13-15 mm longi (calcari incluso). *Calyx* bilabiatus, extus glandulis stipitatis modice dense obsitus; labium superum usque ad 1/3 longitudinis trilobum, lobis subtriangulis vel ovatis; labium inferum usque ad 1/3 longitudinis, bilobum, lobis ovato. *Corolla* bilabiata, albida, venis obscuris, extus glandulis stipitatis disperse obsita; labium superiorum bilobum, lobis subrectangulis angulis rotundatis, ca. 5 mm longis 2-3.5 mm latis, subemarginatis vel integerrimis; labium inferior paulo longius, trilobum, lobis obovato-oblongis vel subrectangulis angulis rotundatis, longioribus quam latis, 4.5-5 mm longis, 4-5 mm latis, perspicue emarginatis, basi pilis multicellularibus cylindricis vel subcapitatis obsitis. *Tubus* subcylindricus, venis obscuris, intus pilosus, parte inferiore pilis multicellularibus longis (ad 100 µm) subulatis, subclavatis vel subcapitatis in triabus lineis ordinatis cum palato; palatum bivesiculatum sparse pilosum pilis multicellularibus cylindricis vel capitatis, brevibus; tubus ad faucem ca. 3 mm diametro, in calcar conicum obtusum vel acutiusculum 2-3 mm longum gradatim transiens. *Stamina* 2, ca. 2 mm longa, basi ovarii adnata, filamenta brevia, incurvata. *Ovarium* subglobosum, glabrescens, in stylum brevissimum productum, stigma bilobum. *Capsula* subglobosa, ca. 3 mm longa, glabrescens. *Semina* numerosa, 0.5-0.65 mm longa, 0.15-0.25 mm lata, ellipsoidea, superficie reticulata.

Perennial herb, rosette forming, scapose. *Rhizome* c. 15 mm long, with numerous adventitious fibrous roots. *Leaves* 4-6, rosetted, flat on the ground or somewhat erect, succulent (drying membranous), (3-)4-6(-11) cm long, (1-)2-3 cm wide, oblong to ovate-oblong in outline, rounded at the tip and attenuated to the base into a short petiole c. 5 mm wide, with margins more or less uprolled, yellowish green, upper surface of lamina glandular, covered with carnivorous glands. *Hibernacula* (dormant buds) absent. *Scapes* 1-4(-5), erect, 5-7(-11) cm long, terete, 0.5-1 mm tick, 1-flowered, sparsely glandular. *Flowers* small, 13-15 mm long, including spur. *Calyx* two-lobed, upper surface of sepal tips sparsely covered with stipitate glands; upper lip divided to 1/3 of its length into 3 lobes, the lobes ovate to subtriangular, each 2-3 mm long, outer lobes 1 mm wide at the base, central lobe 2 mm wide at the base; lower lip divided to 1/3 of its length into 2 ovate lobes c. 2 mm long and 1 mm wide. *Corolla* two-lipped, whitish, lobes and tube striated by veins; upper lip two-lobed, lobes obovate c. 5 mm long and 2-3.5 mm wide, subemarginate at the apex; lower lip little larger than the upper lip, with 3 oblong to obovate-oblong lobes of nearly equal size, 4.5-5 mm long and 4-5 mm wide, each with emarginate apex, at the base near the throat with long multicellular uniseriate hairs. *Tube* funnel-shaped, at the entrance to the throat c. 3 mm wide,

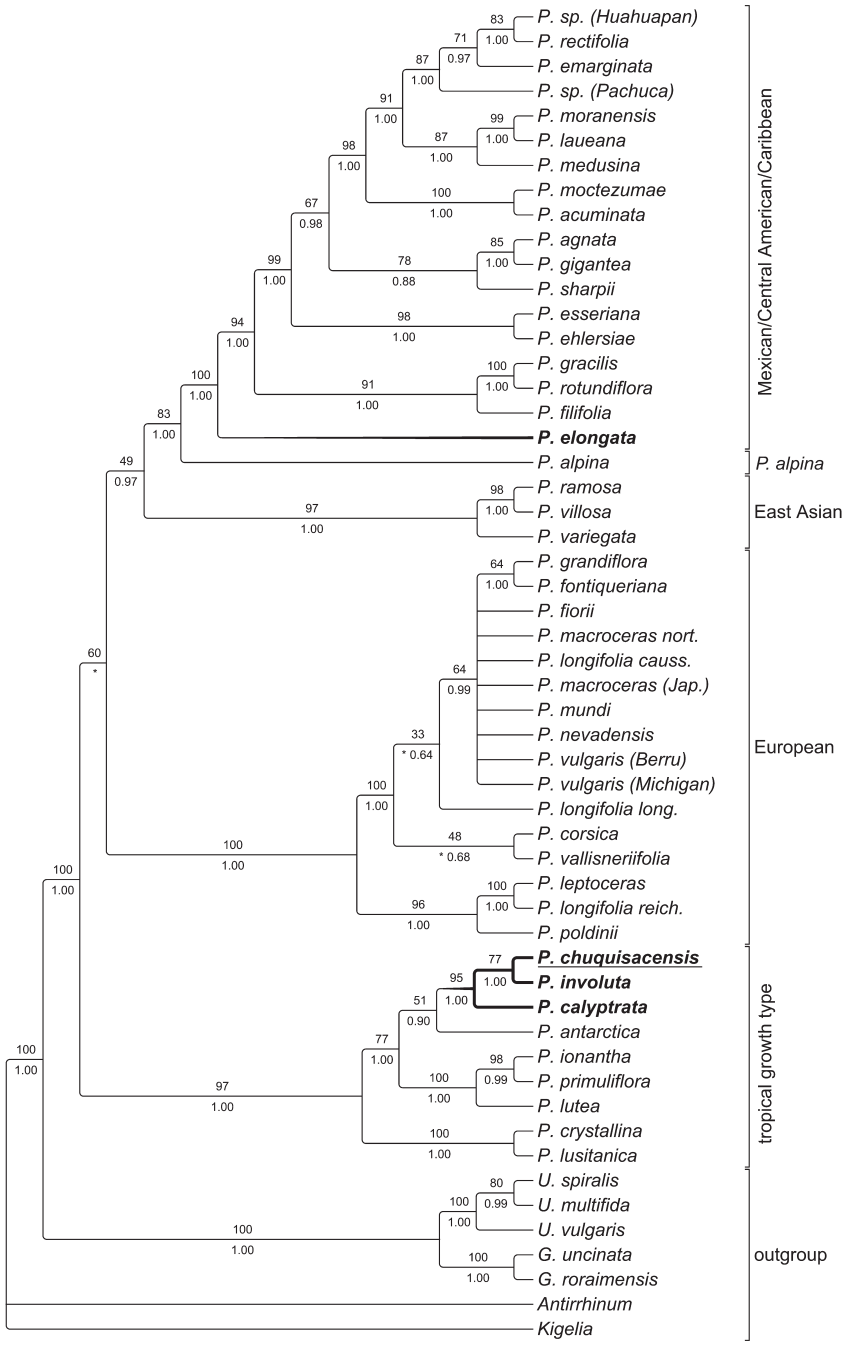


Fig. 2. Phylogenetic relationships in *Pinguicula* based on *trnK* sequences. Above nodes are bootstrap proportions from maximum parsimony analysis; below branches are posterior probabilities from the Bayesian MCMC analysis. An asterisk (*) marks nodes that collapse in the strict consensus tree from the nine most parsimonious trees found. Species not already analysed by Cieslack & al. 2005 (newly added in this study) are in bold; *P. chuquisacensis* described here as a new species is underlined.

Fig. 3. Bayesian phylogram based on the *matK/trnK* dataset. Note the longer branch of *Pinguicula chuquisacensis* relative to its sister group *P. involuta,* caused by autapomorphies. The branches of the species of *Genlisea* and *Utricularia* are distinctly longer, caused by their distinctly higher mutation rates.

without palate. Spur short, 2-3 mm long, straight, grading into the tube, tapering into the blunt apex. *Stamens* with filaments incurved, c. 2 mm long, anthers c. 1.2 mm long. *Ovary* subglobose, subsessile, glabrous. *Capsule* subglobose, c. 3 mm in diameter. *Seed* fusiform, 0.5-0.65 mm long and 0.15-0.25 mm wide, with appendages on micropylar and chalazal end, brownish, seed coat surface with rectangular ornamentation.

Ic. – Fig. 1; see also colour photograph (leaf rosettes at the type locality) in the electronic supplement at www.bgbm.org/willdenowia/willd38/beck+al.htm.

Distribution. – *Pinguicula chuquisacensis* is endemic to the department Chuquisaca of Bolivia and so far only confirmed from the valley of Nuevo Mundo. It is the only species of *Pinguicula* known from the Bolivian-Tucuman biogeographic province (Arbo 1999; Navarro 2002; Wood 2005). Its altitudinal distribution ranges from about 2100 to 2500 m. According to field observation by Hibert Huaylla, the species can only be found in the area of one small cliff in the valley. The different altitudes possibly have to be explained by errors of GPS data.

Habitat and ecology. – *Pinguicula chuquisacensis* grows on steep, seasonally wet, dripping sandstone rock faces in the valleys of the seasonal forests of the Bolivian-Tucuman formation, in areas with frequent cloud covering. It is confined to the open upper parts of the acidic, wet sandstone cliffs, where it grows in small populations besides the road. It grows together with *Lycopodiella cernua* (L.) Pic. Serm. The evergreen seasonal *Myrtaceae* forest extends around with *Blepharocalyx salicifolia* O. Berg and *Myrcianthes pseudo-mato* (D. Legrand) McVaugh *(Myrtaceae)* and some *Lauraceae*. No hibernacula (dormant resting buds) have been observed in *P. chuquisacensis*, therefore this species can be assigned to the tropical homophyllous growth type, although it grows in seasonally wet habitats. All other known Andean species of *Pinguicula* are restricted to permanently wet soils. The plants have been collected in flower in July (*Kessler & al. 5149; Hinchliff & al. 587)* and in flower and fruit in November *(J. R. I. Wood 10168; Villalobos & al. 501*).

Conservation status. – *Pinguicula chuquisacensis* is a very rare local endemic species, known to exist only at a single location in the valley of Nuevo Mundo. Hibert Huaylla tried to find the species in other areas of the valley, but without success. Its area of occupancy is estimated to be less than 10 km² and with a significant decline projected in the area of occupancy by road construction. We therefore recommend to classify the new species in the Red List category "Critically Endangered (CR)" according to criteria B2a+b (IUCN 2001).

Molecular data. – Maximum parsimony analyses recovered 9 shortest trees with 1771 steps (CI = 0.761, RI = 0.803). The topology is shown in Fig. 2, with bootstrap proportions indicated above the branches, and posterior probabilities from the Bayesian analysis below the branches. Bayesian inference yielded a congruent tree that was slightly better resolved within the European clade. A Bayesian phylogram is shown in Fig. 3 indicating several autapomorphies in the *trnK/matK* sequence of the sample of *Pinguicula chuquisacensis* studied. Our new species belongs to a clade with the other two central Andean species *P. involuta* and *P. calyptrata* and the southern South American *P. antarctica,* whereas the northern South American *P. elongata* is resolved as sister to the remaining species of the Mexican/Central American/Caribbean Clade (Fig. 2-3). The second southern South American species, *P. chilensis* was not included in our analysis.

Delimitation. – *Pinguicula chuquisacensis* differs from the related *P. calyptrata* in having very long (up to 11 cm) oblong to ovate-oblong leaves, white flowers, less truncate corolla margins and short lobes of the upper lip, whereas in *P. calyptrata* the leaves are broad-ovate to rotundate-obtuse, the flowers are pale lilac to purple, have strongly truncate corolla lobes and relatively long lobes of the upper lip.

Key to the central Andean species of *Pinguicula*

1. Small plants; leaves obovate, leaf margins strongly incurved; upper lip of corolla much shorter than lower lip . . . . . *P. involuta*

– Plants of medium size; leaves ovate-oblong, leaf margins poorly incurved; upper lip of corolla only slightly shorter than, or as long as lower lip 2
2. Leaves 5-10, obovate to rotundate-obtuse, 10-35(-45) mm long and 7-20 mm wide; all corolla lobes deeply emarginate, lobes of upper lip equal in size to lobes of lower lip; flowers purple to pale lilac; plant of high altitudes (Paramos and cloud forest vegetation at > 3000 m altitude) . . . . . *P. calyptrata*
– Leaves 4-6, oblong to ovate-oblong, 40-60(-110) mm long and 20-30 mm wide; lobes of upper corolla lip shorter than lobes of lower lip, lobes of upper corolla lip subemarginate; flowers white; plant of submontane to montane altitudes (Bolivian Tucumano Forest in 2000-2500 m altitude) . . . . . *P. chuquisacensis*

Discussion

Species classification of Andean *Pinguicula*

Morphologically speaking the new species described here first appeared most closely allied to *Pinguicula calyptrata* but is clearly different (see above). Molecular data were thus elusive in further underscoring the difference to that species. As the *matK/trnK* trees indicates, the next relative may be *P. involuta* rather than *P. calyptrata.* Moreover, *P. chuquisacensis* has several autapomorphies in its *matK/trnK* sequence (see phylogram in Fig. 3) that seem rather many for within species variability. Nevertheless, the inclusion of further populations of *P. involuta, P. calyptrata* and *P. chuquisacensis* is needed, as is the use of nuclear markers to infer speciation patterns and better understand the origin of these species. At the species level, and sampling several geographically distinct populations per species, so far only the Eurasian *Pinguicula* clade (*P.* sect. *Pinguicula*) was analysed with molecular markers (Degtarajeva & al. 2006). These authors resolved different ITS ribotypes from the different populations of several respective taxa in as clades, and most likely, species-non monophyly (e.g. in *P. longifolia*) is caused by improper taxonomy rather than biological phenomena like reticulate evolution or incomplete lineage sorting. DNA sequences therefore appear to help recognizing groups of individual specimens, and are useful for species recognition.

The best known species in the central Andes of the high eastern Yungas slopes of Peru and Bolivia (as far south as Cochabamba) is *Pinguicula involuta* (Casper 1966; Brako & Zarucchi 1993). This species is a small plant growing on acid black soils in high elevations (up to 3500 m) that are rich in organic matter and permanently humid. Another species, *P. calyptrata*, grows in a similar habitat in wet Paramo soil (up to 4000 m) but has a more northern range from Peru through Ecuador to Colombia (Taylor 1975; Brako & Zarucchi 1993). *P. elongata* is very distinct and restricted to the Andes of Colombia and Venezuela (Luteyn 1999).

Relationships in South American *Pinguicula*

Pinguicula chuquisacensis belongs to a well supported clade of central Andean species within *P.* sect. *Ampullipalatum,* to which possibly also the southern Andean taxa represented here by *P. antarctica* belong. Although not sampled here, *P. chilensis* appears morphologically very closely allied to *P. antarctica* (Casper 1966) and may therefore be hypothesised as its closest relative. However, further sequence data are needed to corroborate the sister group relationship of these south Andean species to the central Andean *Pinguicula* clade, since there is only weak statistical support for this.

Our result that *Pinguicula elongata* is not related to the Andean species but to the Mexican-Carribean clade means that section *Ampullipalatum* of subgenus *Temnoceras* is polyphyletic in the circumscription of Casper (1966). All members of section *Ampullipalatum* are of the tropical growth type, and share a two-lobed corolla with conical tube, and a short, saccate spur which gradually widens into the corolla tube. However, *P. elongata* is of a tropical-heterophyllous growth type, while all other members of the section are homophyllous, i.e. they do not produce hi-

bernacula or non-glandular "winter rosettes" but form rosettes of glandular carnivorous leaves instead all year round (Casper 1966). This difference from the other South American *Pinguicula* led Casper (1966) to differentiate a monotypic subsection *Heterophylliformis* for *P. elongata*. Although the position of *P. elongata* as sister lineage to all remaining species of the *Pinguicula* radiation in the Mexican-Central American-Caribbean region is a surprising result, it may mark a biogeographical pattern that can be explained by the proximity of continental South America. Further studies are needed to examine a possible convergent evolution of the tropical growth type in *P. elongata*.

Casper (1966) already hypothesized an origin of South American *Pinguicula* species in relation to the raise of the Andes based on the assumption that any of the high Andean elements were migrated from the North via Central America to South America in Pliocene ("Nordic Invasion") including *Pinguicula* (Diels 1937). Ancestors of Andean *Pinguicula* were considered to have lived in the southern Caribbean area (south of the Isthmus of Tehuantepec) even considering *P. elongata,* which is the northernmost species of the extant South American *Pinguicula,* as a member of such an ancestral lineage (Casper 1966). The hypothesis that many of the high Andean species belong to north temperate genera and have migrated southwards via Mesoamerica with cooling climates after about the Mio-Pliocene have been confirmed by palaeobotanical evidence (Burnham & Graham 1999). A recent analysis using methods of molecular phylogeography and speciation in the genus *Lupinus* by Hughes & Eastwood (2006) indicated that there may have been several southward migration phases from North America with subsequent radiation in habitats offered by the uprising Andes as recently as late Pliocene or Pleistocene. According to our study, the origin of the Andean species of *Pinguicula* appears different from Casper's original assumption, because the clade of *P. lutea* and relatives from the southeastern United States is much more closely related than the Caribbean taxa. A further understanding of the origin of the central Andean *Pinguicula* will thus require a better understanding of speciation patterns in the tropical growth clade first recognized by Cieslack & al. (2005).

Acknowledgements

The authors wish to thank Michael Kessler, John Wood, Jeanneth Villalobos, Ariel Lliully and Cody Hinchliff for providing the herbarium specimens for this rare endemic. Special thanks are due to Ariel Lliully who made available silica gel dried material for the molecular analysis. This study was supported in part by the Deutsche Forschungsgemeinschaft (DFG grant MU2875/2 to Kai Müller, project "Carnivory in Lamiales"). A grant by the graduate funding of the Universität Bayern e.V to Andreas Fleischmann is acknowledged. Bastian Schäferhoff helped in the lab of the Nees Institute in Bonn with generating the *trnK* sequences. Norbert Kilian, Hans Walter Lack and Ludwig Martins (Berlin) provided helpful comments to an earlier version of this manuscript.

References

Arbo, M. M. 1999: *Lentibulariaceae.* – Pp. 786-788 in: Zuloaga, F. O. & Morrone, O. (ed.), Catálogo de las plantas vasculares de la República Argentina II. – Monogr. Syst. Bot. Missouri Bot. Gard. **74.**

Brako, L. & Zarucchi, J. L. 1993: Catalogue of the flowering plants and gymnosperms of Peru. – Monogr. Syst. Bot. Missouri Bot. Gard. **45.**

Burnham, R. J. & Graham, A. 1999: The history of neotropical vegetation: new developments and status. – Ann. Missouri Bot. Gard. **86:** 546-589. [CrossRef]

Casper, S. J. 1966: Monographie der Gattung *Pinguicula* L. – Biblioth. Bot. **127/128.**

— 2003: Two new *Pinguicula* species *(Lentibulariaceae)* from east Cuba (Cuba oriental). – Haussknechtia **9:** 141-155.

— 2004: Two new *Pinguicula* species (*Lentibulariaceae; P. benedicta*-group) from the eastern mountain range of Cuba (Greater Antilles) with reddish flowers. – Wulfenia **11:** 1-13.

— 2007: *Pinguicula lippoldii* nova spec. and *Pinguicula toldensis* nova spec. – two endemic *Pinguicula* species *(Lentibulariaceae)* from East Cuba new to science. – Wulfenia **14:**75-96.
— & Urquiola Cruz, A. J. 2003: *Pinguicula cubensis (Lentibulariaceae),* a new insectivorous species from western Cuba (Cuba occidental). – Willdenowia **33:** 167-172.
Cieslack, T., Polepalli, J. S., White, A., Müller, K., Borsch, T., Barthlott, W., Steiger, J., Marchant, A. & Legendre, L. 2005: Phylogenetic analysis of *Pinguicula (Lentibulariaceae):* chloroplast DNA sequences and morphology support several geographically distinct radiations. – Amer. J. Bot. **92:** 1723-1736. [CrossRef]
Degtjareva, G. V., Casper, S. J., Hellwig, F. H., Schmidt, A. R., Steiger, J. & Sokoloff, D. D. 2006: Morphology and nrITS phylogeny of the genus *Pinguicula* L. *(Lentibulariaceae),* with special attention to embryo evolution. – Pl. Biol. **8:** 778-790. [CrossRef]
Diels, L. 1937: Beiträge zur Kenntnis der Vegetation und Flora von Ecuador. – Biblioth. Bot. **116.**
Hughes, C. & Eastwood, R. 2006: Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. – Proc. Natl. Acad. Sci. USA **103:** 10334-10339. [CrossRef]
IUCN 2001: IUCN Red List categories and criteria. Version 3.1. – Gland & Cambridge.
Jobson, R. W., Playford, J., Cameron, K. M. & Albert, V. A. 2003: Molecular phylogenetics of *Lentibulariaceae* inferred from plastid rps16 intron and *trnL-F* DNA sequences: implications for character evolution and biogeography. – Syst. Bot. **28:** 157-171.
Legendre, L. 2000: The genus *Pinguicula* L. *(Lentibulariaceae):* an overview. – Acta Bot. Gallica **147:** 77-95.
Luteyn, J. L. 1999: Páramos – a checklist of plant diversity, geographical distribution, and botanical literature. – Mem. New York Bot. Gard. **84**: 112.
Müller, K. 2004: PRAP – computation of Bremer support for large data sets. – Molec. Phylogen. Evol. **31:** 780-782. [CrossRef]
— 2005: The efficiency of different search strategies in estimating parsimony jackknife, bootstrap, and Bremer support. – BMC Evol. Biol. **5:** 58. [CrossRef]
— , Borsch, T., Legendre, L., Porembski, S., Theisen, I. & Barthlott, W. 2004: Evolution of carnivory in *Lentibulariaceae* and the *Lamiales*. – Pl. Biol. **6:** 477-490. [CrossRef]
Navarro, G. 2002: Provincia biogeográfica Boliviano-Tucumana. – Pp. 349-451 in: Navarro, G. & Maldonado, M. (ed.), Geografia ecológica de Bolivia. Vegetación y ambientes acuáticos. – Cochabamba.
Ronquist, F. & Huelsenbeck, J. P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics **19:** 1572-1574. [CrossRef]
Shimai, H., Masuda, Y., Panfet Valdés, C. M. & Kondo, K. 2007: Phylogenetic analysis of Cuban *Pinguicula (Lentibulariaceae)* based on internal transcribed spacer (ITS) region. – Chromosome Bot. **2:** 151-158.
Steiger, J. & Casper, S. J. 2001: A new *Pinguicula (Lentibulariaceae)* from the pre-alpine region of northern Italy (Friul-Venezia Giulia) : *Pinguicula poldinii* Steiger & Casper spec. nov. – Wulfenia **8:** 27-37.
Swofford, D. L. 2002: PAUP*4b10. Phylogenetic Analysis Using Parsimony (*and other methods). – Sunderland.
Taylor, P. 1975: *Lentibulariaceae*. – Pp. 9-21 in: Harling, G. & Sparre, B. (ed.), Flora of Ecuador. – Opera Bot., Ser. B, **4.**
Wood, J. R. I. (ed.) 2005: La guía "Darwin" de las flores de los valles bolivianos. Darwin Initiative. – La Paz.
Zamudio Ruiz, S. 2001: Una especie nueva notable de *Pinguicula (Lentibulariaceae)* de los estados de Querétaro y San Luis Potosí, Mexico. – Bol. Soc. Bot. México **68:** 85-88.
— 2003: *Pinguicula conazattii (Lentibulariaceae),* una especie nueva del estado de Oaxaca, México. – Acta Bot. Mex. **62:** 15-20.
— 2005: Dos especies nuevas de *Pinguicula* (*Lentibulariaceae*) de la sierra madre oriental Mexico. – Acta Bot. Mex. **70:** 69-83.

Addresses of the authors:
Stephan G. Beck, Herbario Nacional de Bolivia, Cota Cota Calle 27, Campus Universitario, Casilla 10077 Correo Central, La Paz, Bolivia; e-mail: lpb.dir@acelerate.com

Andreas Fleischmann, Institut für Systematische Botanik, Ludwig-Maximilians-Universität München, Menzinger Straße 67, D-80638 Munich, Germany; e-mail: fleischmann@lrz.uni- muenchen.de

Hibert Huaylla, Herbario Chuquisaca, Universidad Mayor Real y Pontifícia de San Francisco Xavier de Chuquisaca, Casilla 1046, Sucre, Bolivia; e-mail: pterid2000@yahoo.es

Kai F. Müller, Nees Institut für Biodiversität der Pflanzen, Friedrich-Wilhelms Universität Bonn, Meckenheimer Allee 170, D-53115 Bonn, Germany; e-mail: kaimueller@uni-bonn.de

Thomas Borsch, Botanischer Garten und Botanisches Museum Berlin-Dahlem und Institut für Biologie, Freie Universität Berlin, Königin-Luise-Straße 6-8, D-14195 Berlin, Germany; e-mail: t.borsch@bgbm.org

Utricularia rostrata (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil

Fleischmann, A., Rivadavia, F., 2009. *Kew Bulletin* 64: 155-159.

Article V

KEW BULLETIN VOL. 64: 155–159 (2009)

Utricularia rostrata (Lentibulariaceae), a new species from the Chapada Diamantina, Brazil

Andreas Fleischmann[1] & Fernando Rivadavia[2]

Summary. *Utricularia rostrata* A. Fleischm. & Rivadavia (Lentibulariaceae) is described from the Chapada Diamantina highlands in Bahia state, north-eastern Brazil. The morphological characteristics which distinguish this new species are discussed, together with its distribution and ecology.

Key Words. Brazil, Chapada Diamantina, Lentibulariaceae, new species, *Utricularia*.

Introduction

The sandstone formation known as the Chapada Diamantina highlands in the central part of Bahia state, north-eastern Brazil, is well-known for its floral diversity and high rate of endemism (Stannard 1995). Nearly 20 species of the carnivorous plant genus *Utricularia* L. are known to occur on these highlands, of which three are endemic: *U. blanchetii* A. DC., *U. flaccida* A. DC. and *U. parthenopipes* P. Taylor (Taylor 1989).

In late 1992, a minute unidentified species of *Utricularia* was observed by one of us (F. Rivadavia) growing at different places on the Chapada Diamantina. After several trips to this region over the years, it became clear that this was an undescribed species. Surprisingly, this new species has proved to be very common and widespread on the Chapada Diamantina and therefore its absence from herbarium collections can only be explained by its diminutive size.

Based on field studies, herbarium specimens and plants in cultivation, this new species of *Utricularia* is described below.

Utricularia rostrata *A. Fleischm. & Rivadavia* **sp. nov.** *Utricularia costatae* P. Taylor affinis sed calcar incurvatum corolla brevius vel maxime subaequans et lobus superus calicis obovatus apice acuto capsulam circumcludens differt. Typus: Brazil, Bahia, Município de Mucugê, cachoeira do Tiburtino, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 1983 (holotypus SPF!; isotypi K, M).

Small delicate terrestrial annual (occasionally perennial). Stolons few, filiform, up to 7 cm long, c. 0.1 mm thick. *Leaves* from the stolons, petiolate, lamina narrowly linear with apex subacute, c. 0.5 mm wide, 1-nerved; up to 20 mm long. *Traps* numerous on the leaves and stolons, ovoid, on stalk of c. 0.2 mm length, 0.40 – 0.45 mm long by 0.35 – 0.40 mm wide, mouth lateral with a single, conical, dorsal appendage of 0.1 mm and a longer, deeply bifid, multicellular, ventral appendage; outer surface of traps papillose; trap interior with both quadrifid glands and bifid threshold glands, both on stalk c. 10 μm long; quadrifid glands X-shaped, apical cells 70 – 80 μm long. *Inflorescence* a raceme, erect, simple, up to 13 cm long; peduncle filiform, terete, glabrous, c. 0.2 mm thick. Scales few, similar to the bracts, basifixed, ovate-deltoid, with apex acute, 0.6 – 0.8 mm long. *Bracts* basifixed, ovate-deltoid, with apex acute, c. 0.6 mm long and 0.25 mm wide, 3-nerved. Bracteoles narrowly ovate, with apex acute, c. 0.6 × 0.15 mm. Flowers 1 – 3 (4); pedicel filifom, terete, 0.4 – 1.2 mm long, c. 0.1 mm in diam. *Calyx* lobes unequal, upper lobe longer than the lower lobe at anthesis, membranous, glabrous, with few simple parallel nerves; upper lobe obovate with apex acute, strongly convex, c. 2 mm long; lower lobe shorter at anthesis (c. 1.3 mm, but elongated to c. 2.5 mm in fruit), convex, margins curved inwards, narrowly elliptic to obovate, with apex rounded or minutely bifid, 1.3 – 1.5 mm long, spreading in fruit. *Corolla* white, mauve or violet, lower lip with a yellow blotch at the palate and white or

Accepted for publication October 2008.

[1] LMU Munich, Systematic Botany, Menzinger Str. 67, 80638, Munich, Germany. e-mail: fleischmann@lrz.uni-muenchen.de

[2] 1 Daniel Burnham Ct, San Francisco, CA, 94109, USA.

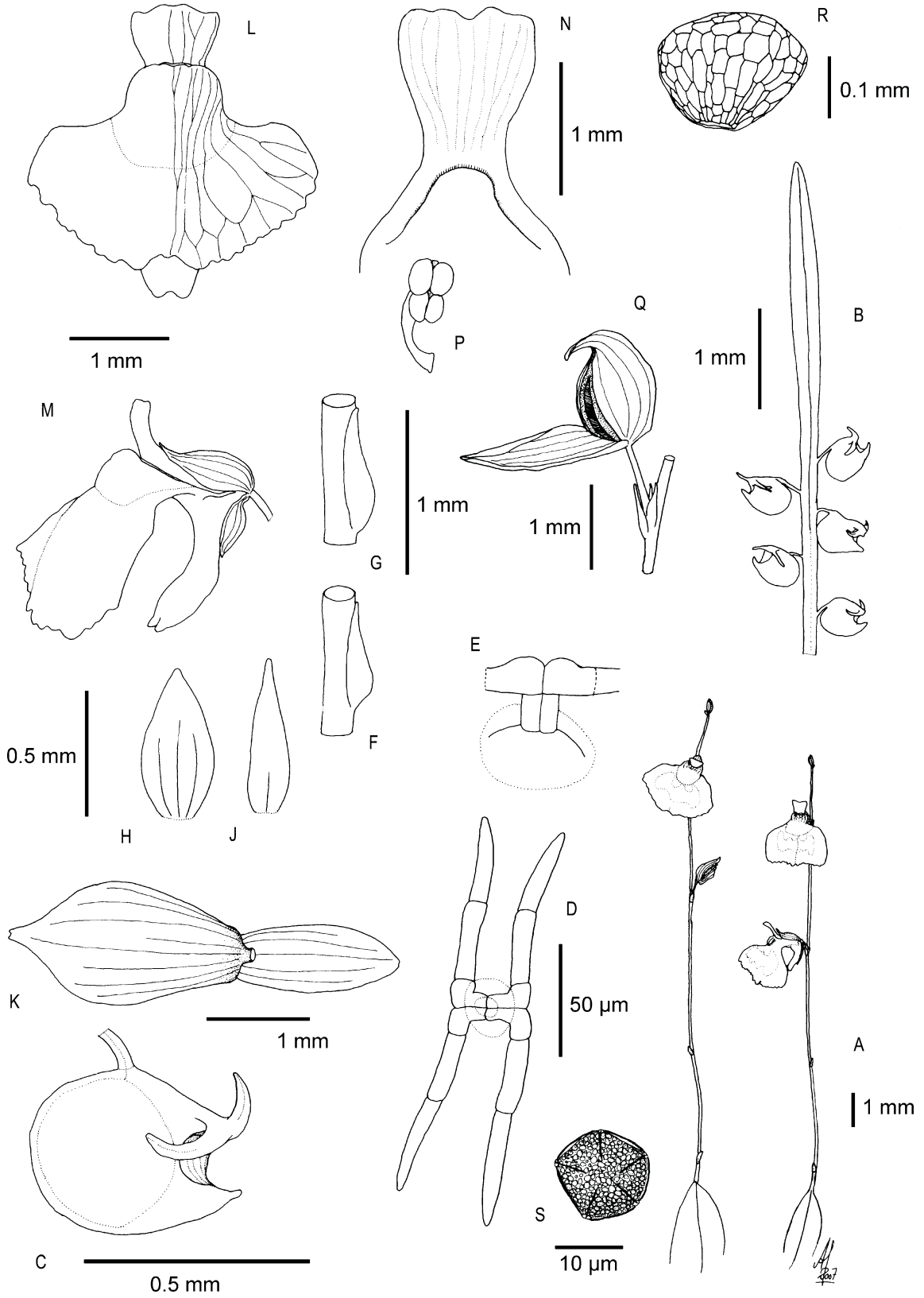


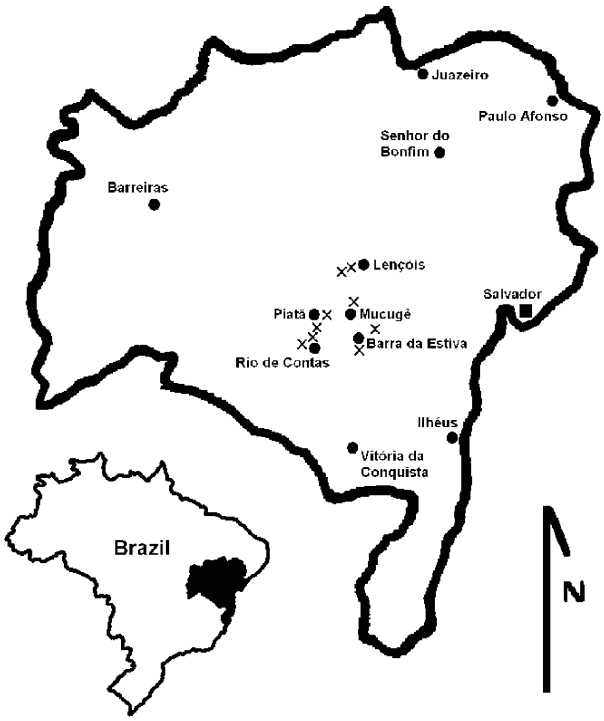
Fig. 1. *Utricularia rostrata*; **A** habit; **B** leaf with traps; **C** trap, lateral view; **D** quadrifid internal trap gland; **E** base of internal trap gland; **F** scale from base of peduncle; **G** scale from upper part of peduncle; **H** bract; **J** bracteole; **K** calyx; **L** corolla; **M** flower, lateral view; **N** corolla upper lip; **P** stamen; **Q** capsule and calyx in fruit; **R** seed; **S** pollen; Same scale bars used for **D** & **E**; for **F** & **G**; for **H** & **J**; for **K** & **L**; for **N** & **P**. All from *Rivadavia et al.* 1983 (SPF). DRAWN BY A. FLEISCHMANN.

cream markings on the limb, 3 – 4 mm long; upper lip constricted below the middle, the superior part quadrate to elliptic with apex crenate, the inferior part transversely elliptic with prominent basal sac with raised rim, the whole upper lip only slightly longer and not wider than the calyx, 2.5 – 3.0 × 1.0 mm; lower lip limb transversely elliptic to trapezoid in outline with a prominent rounded palate at the base, the apex rounded, crenate, 1.7 – 4.0 × 3.5 – 5.0 mm; palate margin with short ciliae; spur narrowly conical, with apex shortly bidentate, curved upwards, slightly shorter than or equalling the lower lip, 0.7 – 1.0 mm in diam., c. 3 mm long. Filaments curved, c. 0.6 mm long, the anther thecae subdistinct, c. 0.5 mm by 0.3 mm. Ovary globose; style short; stigma bilabiate. *Capsule* globose, to 1.2 mm in diam., shorter than the calyx lobes, totally covered by enrolled upper calyx lobe, wall membranous, dehiscence by a single, longitudinal, ventral, marginally thickened slit. Seed truncate-obovoid, compressed, c. 250 × 200 µm; testa surface rugose, testa cells ± rectangular, elongate, with conspicuously raised anticlines. Pollen 5-colporate, circular in outline, 22 × 27 µm. Fig. 1.

DISTRIBUTION. Brazil: Bahia, endemic to the Chapada Diamantina highlands. *Utricularia rostrata* is known to occur from the northern part of the Chapada Diamantina, around the town of Lençóis and at the Fumaça waterfall, to the southern part of these highlands, c. 100 km to the south near the town of Barra da Estiva and c. 125 km to the South West near the town of Rio de Contas. Map 1.

BRAZIL. Bahia: Município de Mucugê, cachoeira do Tiburtino, 11 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 1983 (holotype SPF!; isotypes K; M); Abaíra, Serra do Barbado, 14 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2011 (SPF!); Abaíra, Serra da Mesa, 15 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2029 (SPF!); Barra da Estiva, ao sul da cidade, 22 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2093 (SPF!); Piatã, Serra do Santana, 13 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 1998 (SPF!); Ibicoara, cach. do Buracão, 12 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 1990 (SPF!); Lençóis, Vale do Ribeirão, 29 Dec. 1992, *F. Rivadavia* 160 (SPF!); Lençóis, cach. do Sossego, 21 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2091 (SPF!); Mucugê, estr. p/Andaraí, 22 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2092 (SPF!); Palmeira, cach. da Fumaça, 20 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2084 (SPF!); Rio de Contas, Pico do Itobira, 18 July 2005, *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 2066 (SPF!).

HABITAT. Commonly found growing in moist sandy soils in semi-shaded habitats along streams and waterfalls; also common with mosses on semi-shaded vertical rocks dripping with water; occasionally found growing in open sandy seepages over rocks; c. 550 m – 1570 m.



Map 1. Distribution of *Utricularia rostrata* in Bahia state, Brazil (X).

CONSERVATION STATUS. Least Concern (LC) (IUCN 2001). *Utricularia rostrata* is widespread and common, and present in protected areas of a national park. Thus it is currently not under threat.

ETYMOLOGY. The specific epithet *rostrata* refers to the unique beak-like rostrate apex of the upper calyx lobe, which is especially obvious in fruiting material of this species.

NOTES. *Utricularia rostrata* is placed in sect. *Aranella* (Barnhart) P. Taylor because of its trap morphology (i.e. trap mouth with a single dorsal appendage and a pair of multicellular ventral appendages). However, the raised rim surrounding the basal sac of the upper corolla lip is not as prominent in *U. rostrata* as is typical with other members of that section. This new species is apparently most closely related to *U. costata* P. Taylor, because of the shape and sculpture of its seed (Fig. 2), and because calyx lobes, scales, bracts and bracteoles all have entire margins (at least lowermost scales fimbriate to dentate in all other members of *Aranella* except *U. costata* and *U. purpureocaerulea* A. St. Hil. & Girard).

Utricularia rostrata differs from all other members of sect. *Aranella* in its rostrate upper calyx lobe, which totally encloses the seed capsule in fruit. Furthermore, *U. rostrata* differs from the related *U. costata* in its short and curved spur (straight and 3 times as long as the

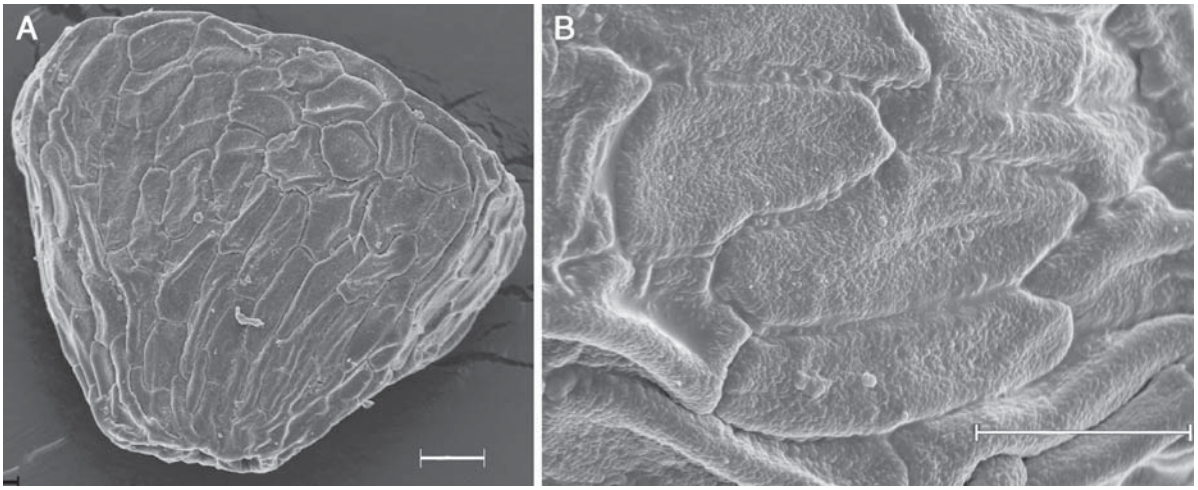


Fig. 2. Scanning electron micrographs of seed of *Utricularia rostrata*. A overview; B seed surface detail. All from *F. Rivadavia, V. Miranda, E. Read & J. Mullins* 1983 (SPF). Scale bars=30 μm.

lower corolla lip in *U. costata*), in its calyx with apex acute (upper lobe with obtuse, denticulate apex, lower lobe truncate to shortly bidentate), in the corolla upper lip being rectangular (upper corolla lip ovate) and in the lower corolla lip 3 – 4 times as long as the lower calyx lobe (about as long as the lower calyx lobe). *Utricularia rostrata* can be distinguished from *U. purpureocaerulea* (with which it shares bracts and bracteoles that all have entire margins) by its quadrate to elliptic upper corolla lip, that is constricted below the middle, that has a crenate apex, and that is narrower than the calyx and by its spur that is curved upwards (upper corolla lip transversely elliptic, not constricted, with apex rounded, much wider than the calyx and spur straight in *U. purpureocaerulea*). Seed of *U. rostrata* is truncate-obovoid, not ovoid like the seed of *U. purpureocaerulea*.

When growing in shadier habitats the inflorescences tend to be green in colour and the corolla mauve to violet. In sunnier habitats the whole inflorescence and especially the calyx may be reddish, while the corolla becomes nearly white. The flowers of *Utricularia rostrata* are sweetly perfumed.

The upper calyx lobe encloses the whole capsule in fruit, the lower calyx lobe is horizontally spreading in fruit. Seed which is shed from the longitudinal vertical dehiscence line of the capsule will fall on the lower calyx, from which it is shed by wind, or — more effectively — splashed by rain drops. The lower calyx lobe will act like a catapult, when hit by a rain droplet. Other species of *Utricularia* also have a lower calyx lobe that is elongated in fruit, becoming conspicuously larger than both the upper lip and the capsule (like *U. pusilla* Vahl of sect. *Setiscapella* (Barnhart) P. Taylor or *U. nana* A. St. Hil. & Girard of sect. *Benjaminia* P. Taylor). In these species, the lower lip may act like a seed catapult for rain drop dispersal as well.

Key to *Utricularia* sect. *Aranella*

This key is adapted from Taylor (1989: 238) adding *Utricularia rostrata* as follows:

7. Lowermost scales deeply fimbriate . . . . . 64. *laciniata*
7. Lowermost scales entire
8. Spur of corolla with apex obtuse, 2 – 3 times as long as the lower lip; upper lip scarcely longer than wide; calyx very strongly nerved, the lower lobe longer . . . . . 66a. *costata*
8. Spur of corolla with apex acute or shortly bifid, shorter or scarcely longer than the lower lip; calyx not very strongly nerved, the lobes ± equal or the upper lobe longer at anthesis
9. Upper lip of corolla not wider than calyx, quadrate to elliptic, scarcely longer than wide, constricted below the middle; spur curved upwards; calyx upper lobe with apex acute, enclosing the capsule in fruit; seed truncate-obovoid . . . . . 66b. *rostrata*
9. Upper lip of corolla much wider than calyx, corolla transversely elliptic, much wider than long, not constricted; spur straight; calyx upper lobe with apex 3-dentate, not enclosing the capsule in fruit; seed ovoid . . . . . 65. *purpureocaerulea*

Acknowledgements

The authors would like to thank Ed Read, Vitor Miranda, and Joe Mullins for helping in the study of *Utricularia rostrata* in its natural habitat in 2005 and Paulo Gonella for helping with measurements from herbarium specimens.

References

IUCN (2001). IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Stannard, B. L. (ed.) (1995). *Flora of the Pico das Almas*. Royal Botanic Gardens, Kew.

Taylor, P. (1989). *The genus* Utricularia — *a taxonomic monograph*. Kew Bull. Addit. Ser. XIV. HMSO, London.

Overcoming DNA extraction problems from carnivorous plants

Fleischmann, A., Heubl, G., 2009.
Anales del Jardín Botánico de Madrid 66: 209-215.

Article VI

Anales del Jardín Botánico de Madrid
Vol. 66(2): 209-215
julio-diciembre 2009
ISSN: 0211-1322
doi: 10.3989/ajbm.2198

Overcoming DNA extraction problems from carnivorous plants

by

Andreas Fleischmann & Günther Heubl

LMU Munich, Systematic Botany and Mycology, Menzinger Strasse 67, D-80638 Munich, Germany
fleischmann@lrz.uni-muenchen.de

Abstract

Fleischmann, A. & Heubl, G. 2009. Overcoming DNA extraction problems from carnivorous plants. *Anales Jard. Bot. Madrid* 66(2): 209-215.

We tested previously published protocols for DNA isolation from plants with high contents of polyphenols and polysaccharides for several taxa of carnivorous plants. However, we did not get satisfying results with fresh or silica dried leaf tissue obtained from field collected or greenhouse grown plants, nor from herbarium specimens. Therefore, we have developed a simple modified protocol of the commercially available Macherey-Nagel NucleoSpin® Plant kit for rapid, effective and reproducible isolation of high quality genomic DNA suitable for PCR reactions. DNA extraction can be conducted from both fresh and dried leaf tissue of various carnivorous plant taxa, irrespective of high contents of polysaccharides, phenolic compounds and other secondary plant metabolites that interfere with DNA isolation and amplification.

Keywords: carnivorous plants, DNA extraction, polyphenols, polysaccharides, secondary metabolites, viscous mucilage.

Resumen

Fleischmann, A. & Heubl, G. 2009. Superando problemas de extracción de ADN de plantas carnívoras. *Anales Jard. Bot. Madrid* 66(2): 209-215 (en inglés).

Probamos algunos protocolos publicados previamente para el aislamiento del ADN de plantas con alto contenido de polifenoles y polisacáridos para varios táxones de plantas carnívoras. Sin embargo, no conseguimos muy buenos resultados ni con tejidos de hojas frescas, ni con tejidos de hojas secadas en gel de sílice obtenidas de plantas colectadas en el campo o cultivadas en los invernaderos, ni de especímenes de herbario. Por lo tanto, hemos desarrollado un protocolo sencillo, modificado del Macherey-Nagel NucleoSpin® Plant kit disponible en el mercado para el aislamiento rápido, eficaz y reproducible de ADN genómico de alta calidad conveniente para la reacción en cadena de la polimerasa. La extracción del ADN se puede realizar en tejidos de hojas frescas o secas de varios táxones de plantas carnívoras, sin importar el grado de contenido de polisacáridos, compuestos fenólicos u otros metabolitos secundarios que interfieren con el aislamiento y la amplificación del ADN.

Palabras clave: plantas carnívoras, extracción del ADN, polifenoles, polisacáridos, metabolitos secundarios, mucílago viscoso.

Introduction

Many carnivorous plants show bright coloured leaves, which serve as visual attractants for insect prey (Juniper & al., 1989). Most pitcher plants *(Cephalotus, Darlingtonia, Heliamphora, Nepenthes and Sarracenia)* often have dark red spots, markings, veins or bright coloured leaves which serve as insect guides and probably mimic flowers (Juniper & al., 1989). Quite often these colour marks result from polyphenolic secondary metabolites (flavonoids or tannins). Polyphenolic compounds are known from several carnivorous plants, for example flavonoids occur in Sarraceniaceae (Sheridan & Griesbach, 2001) and *Roridula* (Wollenweber, 2007). The carnivorous plant members of the order Nepenthales *(Aldrovanda, Dionaea, Drosera, Drosophyllum, Nepenthes and Triphyophyllum)* are additionally characterised by quinones like plumbagin (Schlauer & al., 2005). The phenolic metabolite group of phenylethanoid glycosides is mainly restricted to the Lamiales, an observation supported by the presence of acetoside, which was found in both carnivorous families of this order, Byblidaceae and Lentibulariaceae (Schlauer & al., 2004; Schlauer & al., 2005).

In those carnivorous plants that trap insects by sticky flypaper traps, such as *Drosera*, *Drosophyllum*,

Triphyophyllum, *Byblis* and *Pinguicula*, the leaves are usually densely covered with specialized glands that secrete a water-based viscous mucilage that contains predominantly acidic polysaccharides (Juniper & al., 1989; Rost & Schauer, 1977). In the two species of the pre-carnivorous genus *Roridula* from South Africa, the glue of the sticky traps is resin-based (Lloyd, 1934) and rich in phenolic compounds, such as flavonoids (Wollenweber, 2007).

Both phenolic secondary metabolites and leaf secretions in the mentioned carnivorous plants impede the extraction of DNA from leaf tissue. During homogenization of the plant samples, phenolic components can become oxidized (Loomis, 1974). Oxidized polyphenols are known to bind covalently and therefore irreversibly to DNA molecules and thus interfere with subsequent reactions such as DNA amplification, restriction digest and cloning (Katterman & Shattuck, 1983; Stange & al., 1998; Porebski & al., 1997). Polysaccharides inhibit the enzymatic activity of several enzymes such as polymerases, ligases and restriction endonucleases (Shioda & Marakami-Muofushi, 1987; Richards, 1988), including *Taq* polymerase (Fang & al., 1992) and thus interfere with PCR (polymerase chain reaction). The result is either a very low DNA yield or none at all, or if DNA can be extracted, the remaining secondary metabolites may inhibit further steps in DNA amplification. For example, total genomic DNA isolated from bright coloured leaves of several species of *Drosera, Heliamphora* and *Sarracenia* could not be amplified by PCR in our studies. Therefore, genomic DNA extraction in carnivorous plants was acquired from flower material in some cases (A. Fleischmann, unpublished; Müller & al., 2004), which usually contains lower amounts of polyphenols and polysaccharides than the carnivorous leaves. Unfortunately, flower material is not always available in abundance and many crucial species only flower rarely in cultivation, or removal of flowers from herbarium specimens is not allowed.

Five different protocols for DNA isolation from plants with high concentrations of polyphenols and polysaccharides have been tested and compared when conducting this study (Tel-Zur & al., 1999; Stange & al., 1998; Porebski & al., 1997; Lodhi & al., 1994), including one protocol specifically designed for DNA extraction from in vitro material of the carnivorous plant *Drosera rotundifolia* (Bekesiova & al., 1999). None of these methods, however, enabled us to obtain total genomic DNA suitable for PCR from the carnivorous plant taxa used in this study. Consequently our broad and long-term research on carnivorous plants and problems with DNA extraction spurred the development of a more reliable method of obtaining high quality DNA from leaves of carnivorous plants, irrespective of the plant tissue used for extraction. Therefore, a standard protocol for quick and easy genomic DNA extraction from a NucleoSpin Plant kit (Macherey-Nagel, Germany) was modified to fulfil the specific needs when dealing with DNA isolation from carnivorous plant material rich in secondary metabolites, polyphenols and polysaccharides.

Material and methods

Plant material

Plant samples from herbarium specimens and living plants from tissue culture were obtained from various sources (see Table 1 for details).

Reagents and solutions

- Buffer C0 solution (cat. no. 740570.250, Macherey-Nagel (M-N)).
- 5% sodium N-lauroyl sarcosine (w/v) (Sigma).
- 10% PVP (polyvinyl-pyrrolidone) (w/v) (Sigma).
- 5 M NaCl.
- RNase A solution (cat. no. 740505, M-N).
- Buffer C4 solution (cat. no. 740935, M-N).
- Phenol:chloroform (1:1 v/v).
- Chloroform:isoamyl alcohol (24:1 v/v).
- Ethanol (100%).
- Buffer CW (cat. no. 740932, M-N).
- Buffer C5 solution (cat. no. 740931, M-N).
- Elution buffer CE (cat. no. 740570.250, M-N).

PVP, sodium N-lauroyl sarcosine and NaCl solutions are sterilized by autoclaving.

DNA extraction

This method for DNA extraction is a modified version of the NucleoSpin® Plant kit standard user manual "Genomic DNA from Plant" (Macherey-Nagel, 2007).

The plant samples are homogenized, for fresh leaf tissue preferably by grinding in a mortar under liquid nitrogen. Dried herbarium specimens or silica dried material is best homogenized using an automatic retch. To achieve lysis of the plant cells, the (frozen) powder is transferred into a capped microfuge tube. Following the protocol of the manufacturer of the M-N NucleoSpin® Plant kit (Macherey-Nagel, 2007) 400 µl buffer C0 (preheated to 45°C) are added. In addition to the original recipe, we add 94µl 5M NaCl, 120µl of 5% sodium N-lauroyl sarcosine and 60µl

10% PVP to the suspension. Following the recommendations of the M-N protocol, 10 µl RNase A solution are added to the lysis mixture, and the suspension is vortexed thoroughly. The lysis mixture is now incubated for 60 min. at 60°C in a water bath, and mixed 3-4 times during the incubation step by inverting the tubes once or twice.

We extract the lysate with an equal volume (c. 680 µl) of phenol: chloroform. After centrifugation at 7,100 *g* for 10 min. at 4°C, the supernatant (aqueous phase) is transferred into a new microfuge tube. An equal volume (c. 680 µl) of chloroform:isoamyl alcohol is added, and the samples are centrifuged at 7,100 *g* for 10 min. at 4°C again. Now 300 µl of the supernatant (aqueous phase) is transferred to a new microfuge tube, and 300 µl of buffer C4 and 200 µl ethanol are added. The solution is mixed by inverting the tube 2-4 times. From this step on, the modified protocol is processed following exactly the protocol "5.1 Genomic DNA purification with NucleoSpin® Plant (lysis buffer C1 and C0)" in the user manual. A NucleoSpin® Plant column (cat. no. 740570.250, M-N) is placed into a new NucleoSpin® collecting tube (2ml, cat. no. 740600, M-N), on which the sample is loaded. It is centrifuged for 1 min. at 11,000 *g*, and the flow-through is discarded afterwards. For the first washing step, 400 µl of buffer CW is added to the column, the sample is centrifuged for 1 min. at 11,000 *g*, and the flow-through is discarded. The second washing step is performed by adding 700 µl of buffer C5 to the column, centrifugation for 1 min. at 11,000 *g* and discard of the flow-through.

In the following washing step, 200 µl of buffer C5 are added to the column, which is afterwards centrifuged for 2 min. at 11,000 *g* to remove residual buffer C5 and dry the silica membrane. The column is now placed into a new 1.5 ml centrifuge tube. For elution, we pipette 100 µl of elution buffer CE (preheated to 70°C) onto the membrane of the column (using 50 µl will result in higher concentrated DNA eluate). After a 5 min. incubation at room temperature, the column is centrifuged for 1 min. at 11,000 *g* to elute the DNA.

DNA quantification

The concentration of total genomic DNA isolated with both the standard protocol "Genomic DNA from Plant" (Macherey-Nagel, 2007) and our modified protocol was determined fluorimetrically on the basis of absorbance at 260 nm by using a PicoGreen® dsDNA Quantitation Reagent fluorescent nucleic acid stain (Molecular Probes), following the manufacturer's protocol.

DNA amplification

The isolated genomic DNA was analyzed by standard methods for PCR amplification and agarose gel electrophoresis. The primer pairs used for amplification were ITS1 and ITS4 (White & al., 1990) for members of Ericales and Nepenthales and rbcLaF and rbcLcR (Hasebe & al., 1994) of the *rbc*L chloroplast region for Lamiales taxa sampled. Reactives and concentrations used for the PCR amplifications were as follows, for a final content of 100 µl in the reaction tube: 1 µl of DNA template, 81.5 µl of aqua bidest., 5 µl of dNTP mix (2.5 mM, ABGene, Germany), 10 µl of PCR reaction buffer (10x ThermoPol, New England BioLabs), 0.25 µl of each primer solution (MWG-Biotech, Germany) and 2 µl of *Taq* polymerase suspension (1U/µl, AGS, Germany; diluted to 1:10 before use). The amplification profile consisted of 94°C (3 min.), 40 cycles of 94°C (30 s)/ 54.5°C (30 s)/ 72°C (75 s) and a final extension of 10 min. at 72°C for ITS and of 94°C (3 min.), 34 cycles of 94°C (60 s)/54.7°C (60 s)/ 72°C (90 s) and 10 min. at 72°C for *rbc*L respectively.

Results and discussion

Total genomic DNA prepared with our protocol showed no degradation. It was tested for molecular use by performing PCR-reactions and restriction analysis, both of which were successful.

The concentration of total genomic DNA is given in Table 1. In most samples, the concentration of total genomic DNA eluted was higher when following the Macherey-Nagel standard protocol, compared to our modified protocol (see Table 1 and DNA bands in Fig. 1). The loss of DNA yield in our extraction protocol was 30% on average compared to the standard protocol (but ranging from 5% to 60%, depending on the plant species; see Table 1). However, in most cases, DNA obtained from the standard extraction method could not be used for PCR reactions, even if the template was more diluted (1:1, 1:10, 1:100 using aqua dest.) in order to reduce the concentration of secondary metabolites.

PVP and N-lauroyl sarcosine were used to eliminate polyphenols from the DNA extraction procedure (Maliyakal, 1992; Doyle & Doyle, 1987; Bekesiova & al., 1999) during cell lysis. PVP binds effectively to polyphenolic compounds which can then be separated from DNA by centrifugation (Maliyakal, 1992). In our protocol this is achieved in the phenol:chloroform extraction step. In addition, N-lauroyl-sarcosine is used as an antioxidant to avoid oxidation of polyphenolic plant components in the suspension during lysis.

Table 1. Plant material and vouchers of carnivorous plant species used (fresh = fresh plant material; herb. = herbarium specimen; silica = silica preserved material. Capital letters in column 3 are used for samples taken for DNA isolation with our modified protocol, lower case letters are used for samples for DNA isolation with the Macherey-Nagel standard protocol. Voucher specimens cited as "Heubl-" are deposited in the private collection G. Heubl, Munich).

Species	**Plant tissue used for DNA extraction**	**Modified protocol / Standard protocol**	**Dry wt** [mg]	**DNA conc.** [ng/µl]	**DNA/ mg dry wt** [ng/µl]	**Voucher**
Nepenthes mira	leaf (pitcher lid), herb.	N	5.8	12.0	2.2	*Herb. A. Robinson, s.n.*
		n	5.5	18.9	3.4	
Drosera alba	leaf, silica	D1	0.4	2.2	5.5	*Rivadavia, Gibson & Fleischmann, s.n. (SPF)*
		d1	0.3	1.9	6.3	
Drosera zeyheri	leaf, herb.	D2	1.9	3.5	1.8	*cult. Fleischmann, Heubl-34*
		d2	2.1	5.5	2.6	
Roridula dentata	leaf, herb.	R1	2.8	2.5	0.9	*cult. Fleischmann, Heubl-29*
		r1	2.5	4.1	1.6	
Roridula gorgonias	leaf, herb.	R2	2.2	5.0	2.3	*cult. Fleischmann, Heubl-30*
		r2	1.8	7.0	3.9	
Triphyophyllum peltatum	leaf (carnivorous leaf), silica	T	5.9	10.7	1.8	*Heubl-31*
		t	6.1	17.7	2.9	
Heliamphora sp. 1	leaf, fresh	H1	6.8	24.8	3.6	*in vitro collection Wistuba, Heubl-33*
		h1	6.3	35.8	5.7	
Heliamphora tatei	leaf, fresh	H2	4.5	8.8	2.0	*in vitro collection Wistuba, Heubl-32*
		h2	4.9	10.5	2.1	
Sarracenia alata	leaf, silica	S	6.8	13.7	2.0	*cult. Fleischmann, Heubl-35*
		s	6.9	24.8	3.6	
Byblis gigantea	leaf, silica	B	7.0	8.9	1.3	*cult. Fleischmann, Heubl-36*
		b	7.2	9.8	1.4	
Pinguicula moctezumae	leaf, silica	P	2.7	4.0	1.5	*cult. Fleischmann, Heubl-37*
		p	3.0	4.8	1.6	
Genlisea aurea	leaf, herb.	G	1.5	1.7	1.1	*Rivadavia 2270 (SPF)*
		g	1.4	2.5	1.8	
Utricularia benjaminiana	leaf, herb.	U	1.6	3.4	2.1	*Rivadavia & Fleischmann, s.n. (SPF)*
		u	1.0	5.2	5.2	

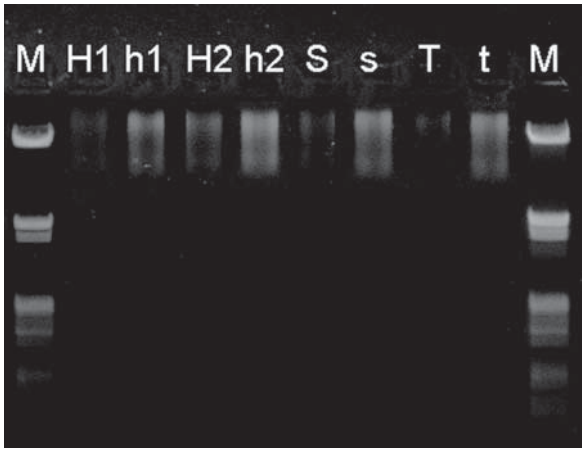


Fig. 1. DNA isolation from carnivorous plant taxa. Ethidium bromide-stained 1% agarose gel of undigested total genomic DNA extracted from leaves of various carnivorous plant taxa. Capital letters used for DNA obtained with the modified protocol, lower case letters used for DNA isolated using the standard Macherey-Nagel NucleoSpin Plant kit user manual "Genomic DNA from Plant". M: lambda DNA ladder (Thermo Fisher Scientific). Lanes H1/h1: *Heliamphora* sp. 1. H2/h2: *Heliamphora tatei*. S/s: *Sarracenia alata*. T/t: *Triphyophyllum peltatum*. Vouchers see Table 1.

The combination of both 10% PVP and N-lauroyl sarcosine during cell lysis of the plant tissue yields in increased amounts of pure total genomic DNA isolates compared to the separate usage of both agents. The presence of oxidized phenolic compounds can be reduced further by keeping plant material frozen during homogenization (Katterman & Shattuck, 1983), this is achieved by grinding fresh plant material in a mortar under liquid nitrogen.

The samples h1, h2, s, d2, n, t, r1, r2, b (for abbreviations, see Table 1) had a dark reddish brown colour after cell lysis (step 2) and this colour did not change until step 11. This colouration is due to the high presence of oxidized polyphenols in the suspension, which bind to the extracted nucleic acids and proteins (Loomis, 1974). During processing with M-N columns the brownish DNA-protein-polyphenol mix attaches to the column membrane and cannot be removed by the wash buffers used. However, the polyphenols are eluted together with the DNA in the elution step. This explains why the DNA obtained from these samples using the standard NucleoSpin® Plant protocol (Macherey-Nagel, 2007) could not be amplified by PCR (see Fig. 2), although a suitable amount of total genomic DNA was isolated (see Table 1).

Polysaccharides can be seen during DNA extraction procedure as the lysis suspension takes on a blurred viscous consistency. The genetic markers chosen could not be amplified by PCR in our studies when polysaccharides were not removed from the DNA preparation. High salt concentrations during cell lysis help to remove polysaccharides, as they increase their solubility in ethanol (Fang & al., 1992), thus they become dissolved in the lysis buffer but cannot precipitate with the DNA. NaCl concentrations of 1 M, as reported in Fang & al. (1992), did not result in total removal of polysaccharides of most carnivorous plant tissue used. We observed that concentrations of 5 M NaCl added to the suspension in the lysis step did result in the highest yields of total genomic DNA (Lodhi & al., 1994, recommend concentrations of at least 2.5 M NaCl for DNA isolation from grapevines rich in polysaccharides).

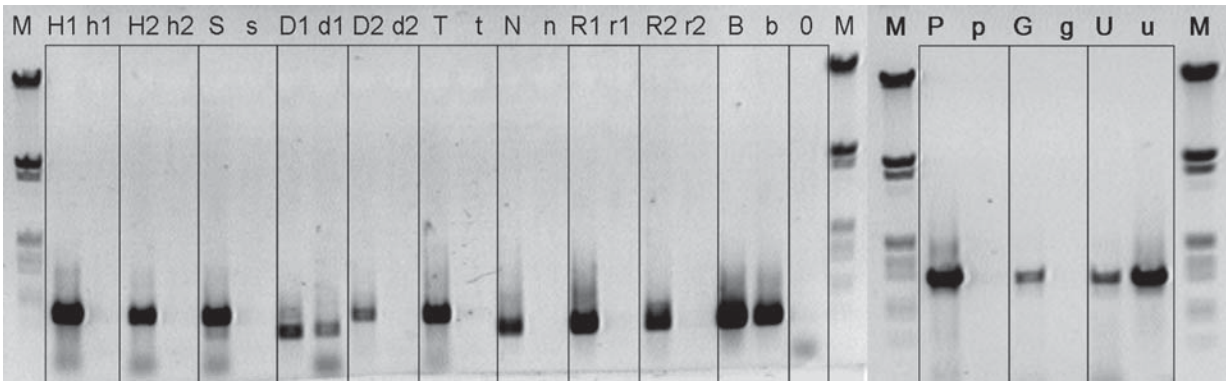


Fig. 2. DNA amplification from carnivorous plant taxa. Ethidium bromide-stained 0.7% agarose gel of PCR products of DNA obtained from standard and modified Macherey-Nagel protocol. M: lambda DNA ladder (Thermo Fisher Scientific). H1/h1: *Heliamphora* sp. 1. H2/h2: *Heliamphora tatei*. S/s: *Sarracenia alata*. D1/d1: *Drosera alba*. D2/d2: *Drosera zeyheri*. T/t: *Triphyophyllum peltatum*. N/n: *Nepenthes mira*. R1/r1: *Roridula dentata*. R2/r2: *Roridula gorgonias*. B/b: *Byblis gigantea*. P/p: *Pinguicula moctezumae*. G/g: *Genlisea aurea*. U/u: *Utricularia benjaminiana*. Vouchers see Table 1. For PCR of H1 to b, the nuclear marker ITS was used, for samples P to u the chloroplast marker *rbc*L. The greyscale of the gel is inverted.

PCR amplification of DNA obtained from our modified protocol was possible due to the absence of contaminants, which could not be removed by the NucleoSpin Plant standard protocol (see Fig. 2). However, DNA of *Drosera alba* (D1/d1), *Byblis gigantea* (B/b) and *Utricularia benjaminiana* (U/u) did amplify with templates obtained from both protocols. In the first two of these, freshly collected silica dried plant tissue from cultivated plants was used, and the total dry weight was higher than in the samples from herbarium specimens. This might explain a higher yield of total genomic DNA extracted, and therefore a higher content of DNA template for PCR reactions. However, the PCR product yield obtained using the standard protocol was significantly lower in *Drosera alba* compared to the modified protocol (see Table 1 and DNA bands D1 / d1 in Fig. 2), despite the fact that the concentration of total template DNA was slightly higher when using the standard protocol. Thus a major factor for success in PCR-amplification is not the quantity but the quality of the DNA template used. In the case of *Utricularia benjaminiana*, the reason why both protocols worked well may be the fact that the tissues of this aquatic plant are soft and pale green, and therefore do not contain high amounts of secondary plant metabolites which can act as contaminants during DNA preparation.

Existing DNA extraction protocols for the carnivorous plant *Drosera* using the CTAB method (Bekesiova & al., 1999) are unreliable when considering yield and quality of DNA isolated from plant material which was not grown in vitro. Attempts to obtain DNA from the extraction protocol published by Bekesiova & al. (1999) with various species of *Drosera* and other carnivorous plants failed with leaf tissue from fresh, greenhouse grown and field collected plant material, as well as with dried leaves from herbarium specimens. A reason for this might be the fact that Bekesiova & al. (1999) used in vitro-grown plants of *D. spatulata* (Figures 1A and 1B in that paper show *D. spatulata*, not *D. rotundifolia*). Plants grown on axenic media under artificial lights often exhibit a reduced content of secondary metabolites and light induced phytopigments such as anthocyanins. This is also true for many carnivorous plants, which are less vividly coloured when grown in vitro.

A few carnivorous plant genera have not been tested in this study (the monotypic genera *Aldrovanda*, *Cephalotus*, *Darlingtonia*, *Dionaea* and *Drosophyllum*). In all of these, high numbers of polyphenols and polysaccharides have also been found: flavonoids in *Cephalotus* (Nicholls & al., 1985; Jay & Lebreton, 1972) and *Darlingtonia* (Jay & Lebreton, 1972), quinones, phenolics and polysaccharide secretions in *Drosophyllum*, *Dionaea* and *Aldrovanda* (Schlauer & al., 2005; Juniper & al., 1989). Therefore we assume that our protocol will work equally well with these genera to avoid problems caused by secondary plant metabolites in DNA purification or amplification. This protocol has also been tested successfully with some non-carnivorous plants that have high contents of polysaccharides and polyphenols, *Mabea* (Euphorbiaceae), *Oxalis* (Oxalidaceae) and *Echinopsis* (Cactaceae).

Acknowledgements

The authors would like to thank Fernando Rivadavia, Brazil, and Dr Alastair Robinson, England, for providing plant material from herbarium specimens and Dr Andreas Wistuba, Germany, for providing in vitro grown plant material from tissue culture for this study. The help of Michael Miller of the Zoologische Staatssammlung Munich in determining the DNA amount is acknowledged. Thanks to Tanja Ernst and Florian Turini for technical assistance, to Delsy Trujillo-Chavez for help with the Spanish abstract and to two anonymous reviewers for useful suggestions on the manuscript.

References

Bekesiova, I., Nap, J.P. & Mlynarova, L. 1999. Isolation of high quality DNA and RNA from leaves of the carnivorous plant Drosera rotundifolia. *Plant Molecular Biology Reporter* 17: 269-277.

Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical Bulletin* 19: 11-15.

Fang, G., Hammar, S. & Grumet, R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA. *BioTechniques* 13: 52-56.

Hasebe, M., Omori, T., Nakazawa, M., Sano, T., Kato, M. & Iwatsuki, K. 1994. RbcL gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proceedings of the National Academy of Sciences of the USA* 91: 5730-5734.

Jay, M. & Lebreton, P. 1972. Chemotaxonomic research on vascular plants 26. The flavonoids of the Sarraceniaceae, Nepenthaceae, Droseraceae and Cephalotaceae; a critical study of the order Sarraceniales. *Naturaliste Canadienne* 99: 607-613.

Juniper, B.E., Robins, R.J. & Joel, D.M. 1989. *The Carnivorous Plants*. Academic Press. London.

Katterman, F.R.H. & Shattuck, V.I. 1983. An effective method of DNA isolation from the mature leaves of Gossypium species that contain large amounts of phenolic terpenoids and tannins. *Preparative Biochemistry* 13: 347-359.

Lloyd, F.E. 1934. Is Roridula carnivorous? *Canadian Journal of Research* 10: 780-786.

Lodhi, M.A., Ye, G.N., Weeden, N.F. & Reisch, B.I. 1994. A simple and efficient method for DNA extraction from grapevine cultivars, Vitis species and Ampelopsis. *Plant Molecular Biology Reporter* 12: 6-13.

Loomis, W.D. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. *Methods in Enzymology* 31(Pt A): 528-544.

Macherey-Nagel. 2007. *NucleoSpin®Plant: Genomic DNA from Plant User manual, June 2007/Rev. 6*. Macherey-Nagel GmbH & Co. KG. Düren.

Maliyakal, E.J. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Research* 20: 2381.

Müller, K., Borsch, T., Legendre, L., Porembski, S., Theisen, I. & Barthlott, W. 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. *Plant Biology* 6: 477-490.

Nicholls, K.W., Bohm, B.A. & Ornduff, R. 1985. Flavonoids and affinities of the Cephalotaceae. *Biochemical Systematics and Ecology* 13: 261-263.

Porebski, S., Bailey, L.G. & Baum, B.R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter* 15: 8-15.

Richards, E. 1988. Preparation of genomic DNA from plant tissue. In: Ausubel, F.M. & al. (eds.), *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience. New York.

Rost, K. & Schauer, R. 1977. Physical and chemical properties of the mucin secreted by Drosera capensis. *Phytochemistry* 16: 1365-1368.

Schlauer, J., Budzianowski, J., Kukulczanka, K. & Ratajczack, L. 2004. Acetoside and related phenylethanoid glycosides in Byblis liniflora Salisb. plants propagated in vitro and its systematic significance. *Acta Societatis Botanicorum Poloniae* 73: 9-15.

Schlauer, J., Nerz, J. & Rischer, H. 2005. Carnivorous plant chemistry. *Acta Botanica Gallica* 152: 187-195.

Sheridan, P.M. & Griesbach, R.J. 2001. Anthocyanidins of Sarracenia L. Flowers and Leaves. *Hortscience* 36: 384.

Shioda, M. & Marakami-Muofushi, K. 1987. Selective inhibition of DNA polymerase by a polysaccharide purified from slime of Physarum polycephalum. *Biochemical and Biophysical Research Communications* 146: 61-66.

Stange, C., Prehn, D. & Arce-Johnson, P. 1998. Isolation of Pinus radiata Genomic DNA Suitable for RAPD Analysis. *Plant Molecular Biology Reporter* 16: 1-8.

Tel-Zur, N., Abbo, S., Myslabodski, D. & Mizrahi, Y. 1999. Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera Hylocereus and Selenicereus (Cactaceae). *Plant Molecular Biology Reporter* 17: 249-254.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A. & al. (eds.), *PCR Protocols - a guide to methods and applications, United Kingdom edition*: 315-322. Academic Press. San Diego.

Wollenweber, E. 2007. Flavonoids occurring in the sticky resin on Roridula dentata and Roridula gorgonias (Roridulaceae). *Carnivorous Plant Newsletter* 36: 77-80.

Associate Editor: J. Diéguez
Received: 24-VII-2008
Accepted: 18-V-2009

Curriculum vitae

Name: Fleischmann
Surname: Andreas Stefan
Born: 12.06.1980, Landsberg am Lech, Germany

Education and scientific work

1991 – 2000	Dominikus-Zimmermann-Gymnasium, Landsberg
2000 – 2006	Studies of Biology: Ludwig-Maximilians-Universität, Munich, Germany (Systematic Botany, Zoology, Paleontology, Ecology)
2005 – 2006	Diploma Thesis: „Molekulare Phylogenie, Biogeographie und morphologische Diversität der Gattung *Heliamphora* Benth. (Sarraceniaceae)“, supervisor: Prof. Dr. G. Heubl (LMU, Systematic Botany)
Since June 2006	Scientific work at LMU, Biology, Systematic Botany and Mycology, research assistant, various publications
Since May 2008	PhD Thesis: „Phylogenetic relationships, systematics, and biology of carnivorous Lamiales, with special focus on the genus *Genlisea* (Lentibulariaceae)”, supervisor: Prof. Dr. G. Heubl (LMU, Systematic Botany)

Scholarships

May 2008 – April 2011	Graduate funding of the Universität Bayern eV.

Teaching

Since April 2003	Tutorials for students of biology, LMU („Artenvielfaltkurs”); botanical student excursions; Plant collections for student courses
April 2005 – June 2007	Botanical tutorials for students of pharmacy, Institut für Pharmazie, LMU Munich.

Field work (botanical expeditions, collection of specimens, ecological observations)

Nov – Dec 2004	Venezuela, Estado Bolívar, La Gran Sabana, flora of the summits of Roraima- and Kunenán-tepui
Sept – Oct 2005	Thailand, Botanical excursion of LMU, Munich
Aug – Sept 2006	Sierra Leone, South Africa, Zambia; South Africa; field Work for “Monograph of the genus *Genlisea*”

Sept – Oct 2008	Perth, Western Australia, Australia; studies on Droseraceae and Lentibulariaceae of Australia (assosciated scientist: Allen Lowrie, Perth)
Jan – Feb 2009	Venezuela, Estado Bolívar, La Gran Sabana, flora of the summits of the Chimantá Massif, Auyán-tepui, Ptari-tepui and Roraima-tepui; collecting specimens for PhD thesis; Field work for "Monograph of the genus *Genlisea*" (associated scientist: Dr. Otto Huber, Caracas Herbarium)
Apr 2010	Brazil, São Paulo, Minas Gerais, Goias and Bahia states. Droseraceae and Lentibulariaceae of Brazil (associated scientists: Dr. Rafael Oliveira, Unicamp, Campinas, São Paulo; Paulo M. Gonella, Herbarium SPF, São Paulo); Field work for "Monograph of the genus *Genlisea*"

Talks

17.11.2010	"Evolution of carnivory in the plant kingdom". Evolution and Systematics of Plants & Fungi, Systematic Botany, Munich.
23.01.2009	"El género *Heliamphora* (Sarraceniaceae) - la morfología y datos moleculares". Fundacíon Instituto Botánico de Venezuela, Caracas.
10.12.2008	"Relationships of and within Lentibulariaceae". Evolution and Systematics of Plants & Fungi, Systematic Botany, Munich.
27.09.2008	"Carnivorous Plants of the Cape Province, South Africa". International Carnivorous Plant Conference 2008, Sydney, Australia
20.09.2008	"*Drosera* species of South America" and "Carnivorous Plants in tropical Africa (Zambia and Sierra Leone)". European Carnivorous Plants Exhibition and Exchange 2008, Mira, Italy.
08.04.2008	"Phylogeny of the genus *Heliamphora* (Sarraceniaceae) with special focus on biogeography and character evolution". Systematics 2008, Göttingen.
25.03.2007	"Karnivoren des tropischen Afrika". Meeting of the German Carnivorous Plant Society G.F.P. eV 2007, Bonn.

Posters

Grande, J., Fleischmann, A., Una nueva variedad de *Heliamphora minor* de la cumbre del Auyán-tepui. XIX Congreso Venezolano de Botánica, 17-19 May 2011, Maracay.

Olano-Marín, C., Fleischmann, A., Bräuchler, C., Heubl, G., Molecular phylogeny, biogeography and character evolution in the tribe Rhinantheae (family Orobanchaceae). 21-27 February 2001, Biosystematics 2011, Berlin.

Further publications in peer-reviewed journals and books:

Fleischmann, A., Grande, J.R., 2011. Taxonomía de *Heliamphora minor* Gleason (Sarraceniaceae) en el Auyán-tepui, incluyendo una nueva variedad. Acta Botánica Venezuelica. (in press).

Fleischmann, A., 2011. Do we have any evidence that any plants have given up carnivory? Carnivorous Plant Newsletter 40: 37.

Fleischmann, A., Robinson, A.S., McPherson, S., Heinrich, V., Gironella, E., Madulid, D.A., 2011. *Drosera ultramafica* (Droseraceae), a new sundew species of the ultramafic flora of the Malesian highlands. Blumea 56: 10-15.

McPherson, S., Wistuba, A., **Fleischmann, A.**, Nerz. J., 2011. Sarraceniaceae of South America. Redfern Natural History Publications, Dorset. 562 pp.

McPherson, S., Bourke, G., **Fleischmann, A.**, Robinson, A.S., Jaunzems, M., 2011. A new pitcher plant from Palawan: *Nepenthes leonardoi*. Carniflora Australis 8: 4-9.

Merckx, V., Stöckel, M., **Fleischmann, A.**, Bruns, T.D., Gebauer, G., 2010. 15N and 13C natural abundance of two mycoheterotrophic and a putative partially mycoheterotrophic species associated with arbuscular mycorrhizal fungi. New Phytologist 188: 590-596.

Fleischmann, A., 2010. Evolution of Carnivorous Plants. pp. 68-123, In: McPherson, S.: Carnivorous Plants and their Habitats (eds. Fleischmann, A., Robinson, A.S.), Redfern, Dorset.

Fleischmann, A., 2010. Corkscrew plants: *Genlisea*. pp. 1104-1141 in: McPherson, S.: Carnivorous Plants and their Habitats (eds. Fleischmann, A., Robinson, A.S.), Redfern, Dorset.

Fleischmann, A., 2010. Bladderworts: *Utricularia*. pp. 1142-1227 in: McPherson, S.: Carnivorous Plants and their Habitats (eds. Fleischmann, A., Robinson, A.S.). Redfern, Dorset.

Fleischmann, A., McPherson, S., 2010. Some ecological notes on *Heliamphora* (Sarraceniaceae) from Ptari-tepui. Carniflora Australis 7: 19-31.

Fleischmann, A., Wistuba, A., Nerz, J., 2009. Three new species of *Heliamphora* (Sarraceniaceae) from the Guayana Highlands of Venezuela. Willdenowia 39: 273-283.

Robinson, A.S., **Fleischmann, A.**, McPherson, S., Heinrich, V., Gironella, E.P., Pena, C.Q., 2009. A spectacular new species of *Nepenthes* L. (Nepenthaceae) pitcher plant from central Palawan, Philippines. Botanical Journal of the Linnean Society 159: 195-202.

Rivadavia, F., Vicentini, A., **Fleischmann, A.**, 2009. A new species of sundew (*Drosera*, Droseraceae), with water-dispersed seed, from the floodplains of the northern Amazon basin, Brazil. Ecotropica 15: 13-21.

Fleischmann, A., Lee, C.C., 2009. A new variety of *Drosera spatulata* (Droseraceae) from Sarawak, Borneo. Carnivorous Plant Newsletter 38:4-9.

Fleischmann, A., Gibson, R., Rivadavia, F., 2008. *Drosera ericgreenii* (Droseraceae), a new species from the fynbos of South Africa. Bothalia 38: 141-144.

Fleischmann, A., Wistuba, A., McPherson, S., 2007. *Drosera solaris* (Droseraceae), a new sundew from the Guayana Highlands. Willdenowia 37: 551-555.

Wistuba, A., Nerz, J., **Fleischmann, A.**, 2007. *Nepenthes flava*, a new species of Nepenthaceae from the northern part of Sumatra. Blumea 52: 159-163.

Fleischmann, A., Krings, M., Mayr, H., Agerer, R., 2007. Structurally preserved polypores from the Neogene of North Africa: Ganodermites lybicus gen. et sp. nov. (Polyporales, Ganodermataceae). Review of Palaeobotany and Palynology 145: 159-172.