Phylogenetic placement of two enigmatic genera, *Borthwickia* and *Stixis*, based on molecular and pollen data, and the description of a new family of Brassicales, Borthwickiaceae

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Abstract Capparaceae (Brassicales) as traditionally circumscribed is heterogeneous, and several genera have been segregated from it based on molecular and/or morphological data. However, *Borthwickia* and *Stixis*, two Southeast Asian endemic genera of Capparaceae with controversial positions, have not previously been evaluated in a molecular phylogenetic study. Here, we used four plastid DNA regions (*matK*, *ndhF*, *rbcL*, *trnL-trnF*) and pollen data to determine their phylogenetic relationships within core Brassicales. Our results showed that neither *Borthwickia* nor *Stixis* is a member of Capparaceae. The two genera, together with *Forchhammeria*, Gyrostemonaceae, Resedaceae, and *Tirania*, formed a clade with strong support. *Stixis* is closely related to *Tirania*, a relationship that is also supported by morphological characters, such as six sepals and three- or four-locular ovaries. Most interestingly, *Borthwickia* was resolved as sister to the *Forchhammeria*-Resedaceae-*Stixis*-*Tirania* clade with moderate to strong support. However, *Borthwickia* differs markedly from its sister group in having opposite leaves, one indistinct stigma, more than four carpels and locules, a linear ovary with ridges, and pollen grains with perforate exine sculpturing. Thus, we describe a new family, Borthwickiaceae, for the genus.

Keywords *Borthwickia*; Borthwickiaceae; Brassicales; Capparaceae; phylogeny; pollen morphology; *Stixis*

Supplementary Material The alignment file is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

During the past two decades, tremendous progress has been made in understanding relationships among angiosperms by using DNA sequence data (e.g., Soltis & al., 2000, 2011; Wang & al., 2009; Moore & al., 2010). With the basic phylogenetic framework of angiosperms established, a DNA phylogeny-based angiosperm classification system was proposed (APG, 1998) and has been repeatedly updated (APG II, 2003; APG III, 2009). However, due to relatively limited taxon sampling within families, those higher-level phylogenetic analyses of angiosperms did not clarify the circumscriptions of some heterogeneous families. These required more focused work, such as done in Celastraceae (Simmons & al., 2001a, b; Zhang & Simmons, 2006), Capparaceae (Rodman & al., 1993, 1996, 1998; Karol & al., 1999; Chandler & Bayer, 2000; Hall & al., 2002, 2004), Scrophulariaceae (Olmstead & Reeves, 1995; De Pamphilis & al., 1997; Olmstead & al., 2001; Oxelman & al., 2005), and Simaroubaceae (Fernando & al., 1995).

Capparaceae sensu Pax & Hoffmann (1936), comprising ca. 45 genera with 800 species, are primarily restricted to seasonally dry tropical forests. The family has been regarded as a heterogeneous assemblage within Brassicales and has been considered to include many unrelated taxa. Based on phylogenetic analyses, some taxa have been segregated from Capparaceae, including Cleomoideae (Hall & al., 2002), *Calyptrotheca* Gilg (Applequist & Wallace, 2000), *Emblingia* F. Muell. (Chandler & Bayer, 2000), *Forchhammeria* Liebm. (Hall & al., 2002), *Koeberlinia* Zucc. (Rodman & al., 1993), *Pentadiplandra* Baill. (Rodman & al., 1996), *Physena* Noronha ex Thouars (Morton & al., 1997), *Setchellanthus* Brandegee (Karol & al., 1999), and *Tirania* Pierre (Hall & al., 2004). To date, other incertae sedis genera, such as *Borthwickia* W.W. Smith, *Keithia* Spreng., *Neothorelia* Gagnep., *Poilanedora* Gagnep., and *Stixis* Lour., have not been sampled in a molecular phylogenetic study because of the difficulty in obtaining plant material. The systematic positions and allies particularly of *Borthwickia* and *Stixis* have been controversial.

Borthwickia contains one species, *B. trifoliata* (Fig. 1), which is restricted to wet valleys, forests and ravines in southern to southeastern Yunnan (China) and eastern to northern Myanmar (Sun, 1999). Since it was first described by Smith (1911), *Borthwickia* has usually been placed in Capparaceae (e.g., Pax & Hoffmann, 1936; Jacobs, 1968; Brummitt, 1992; Sun, 1999; Wu & al., 2004; Zhang & Tucker, 2008). Smith (1911) considered *Borthwickia* to be close to *Ritchiea* R. Br.

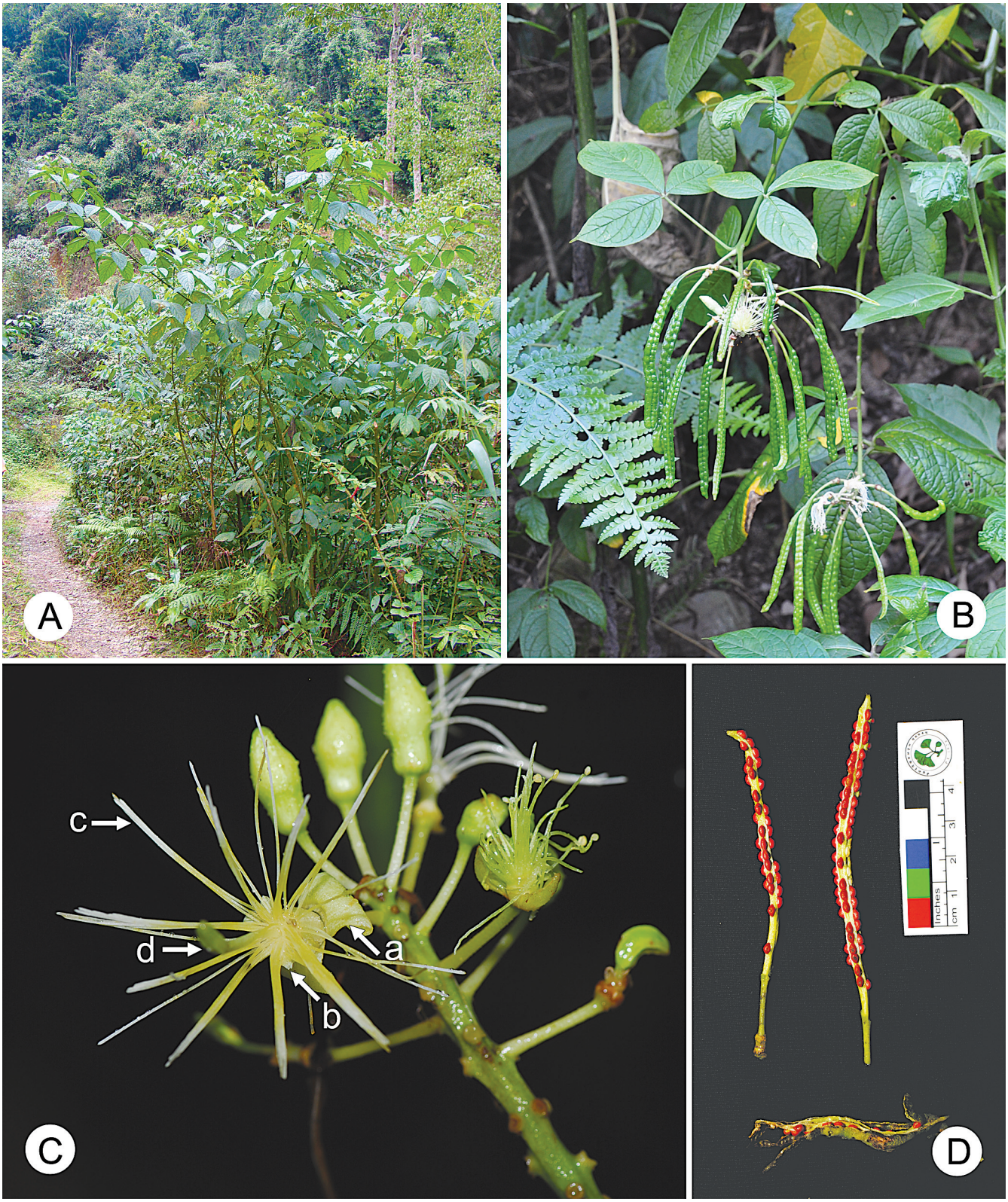

Fig. 1. *Borthwickia trifoliata* in Yunnan, China. **A,** Habitat; **B,** plant with leaves, inflorescences, and fruits; **C,** flower with sepal (a), petal (b), stamen (c), and ovary (d); **D,** fruits and seeds. — Photos: A, Jun-Xia Su; B, Jiang-Hai He; C, Xiao-Hua Jin; D, Tuo Yang.

ex G. Don. Nevertheless, Jacobs (1968) first described the fruit characters of *Borthwickia* and suggested that the genus had an affinity with *Maerua* Forssk. based on the presence of an androgynophore, many stamens, and a torulose fruit (Fig. 1B–D). Based on its unique morphological characters, such as a flower with two sepals and six petals, Pax & Hoffmann (1936) questioned the position of *Borthwickia* within Capparaceae. Kers (2003) further suggested that *Borthwickia* does not belong to Capparaceae, an idea that was followed by Mabberley (2008). Nevertheless, neither Kers (2003) nor Mabberley (2008) assigned the genus to any family.

Stixis, consisting of seven species, occurs from Sikkim (India) to Malaysia, with the Indochinese peninsula as a primary center of diversity (Jacobs, 1963; Hutchinson, 1967; Chen & al., 2003). It was described as a member of Capparaceae by De Loureiro (1790: 295), but was subsequently placed in different tribes of the family by different authors. Pax & Hoffmann (1936) placed *Stixis* together with *Forchhammeria*, *Neothorelia*, *Physena*, and *Tirania* in tribe Stixeae based on receptacle and flower characters. Hutchinson (1967) placed *Stixis* in tribe Cadabeae together with *Bachmannia* Pax, *Boscia* Lam., *Buchholzia* Engl., *Cadaba* Forssk., *Courbonia* Brongn., *Hypselandra* Pax & K. Hoffm., *Maerua*, and *Thylachium* Lour., based on their bisexual flowers without petals. Wu & al. (2004) placed *Stixis* together with *Forchhammeria* in tribe Stixeae and *Tirania* in tribe Cappareae, because petals are present in the former two genera, but absent in *Tirania*. However, Kers (2003) excluded tribe Stixeae of Pax & Hoffmann (1936) from Capparaceae; this treatment was followed by Mabberley (2008) and supported by recent molecular phylogenetic analyses (by sampling *Forchhammeria* and *Tirania*; Hall & al., 2002, 2004; Hall, 2008; Martín-Bravo & al., 2007, 2009, 2010).

Pollen characters are potentially of systematic significance in Brassicales because different families sometimes have different pollen characters, such as Capparaceae (Mitra, 1975), Resedaceae (Perveen & Qaiser, 2001; El Naggar, 2002), and Gyrostemonaceae (Tobe & Takahashi, 1995). So far, the pollen morphology of most families of Brassicales has been investigated (e.g., Erdtman, 1952; Mitra, 1975; Perveen & Qaiser, 2001), but that of *Borthwickia* has never been surveyed. Additionally, the pollen characters of *Stixis* have not been used to explore its systematic position although they are different from those of some other members of Capparaceae (Mitra, 1975).

Previous studies have placed Capparaceae, *Forchhammeria*, and *Tirania* in the core Brassicales, which also contain Brassicaceae, Cleomaceae, Emblingiaceae, Gyrostemonaceae, Pentadiplandraceae, Resedaceae, and Tovariaceae (Hall & al., 2002, 2004). The aim of our study was to investigate the phylogenetic position of *Borthwickia* and *Stixis* in the core Brassicales using evidence from molecular analysis and pollen morphology.

■ MATERIALS AND METHODS

Taxon sampling and molecular data. — Our molecular analysis included four plastid DNA markers (*matK*, *ndhF*, *rbcL*, *trnL-trnF*) which were previously used in phylogenetic studies of Brassicales (e.g., Hall & al., 2002, 2004; Hall, 2008; Martín-Bravo & al., 2007, 2009, 2010). In this study, the delimitation of Capparaceae followed the treatments of Hall (2008) and Iltis & al. (2011). A total of 66 samples of 57 species from 39 genera in 12 families, representing all families of the core Brassicales and four orphan genera of uncertain position (*Borthwickia*, *Forchhammeria*, *Stixis*, *Tirania*), were included. Three individuals from two populations of monotypic *Borthwickia* and ten individuals representing three of seven species of *Stixis* were newly sampled in this study. Based on previous studies (Rodman & al., 1993, 1996, 1998; Hall & al., 2002, 2004), we selected Bataceae (*Batis maritima*), Caricaceae (*Carica papaya*), Koeberliniaceae (*Koeberlinia spinosa*), and Tropaeolaceae (*Tropaeolum majus*) as outgroups because all of these fall outside core Brassicales. Taxa and GenBank accession numbers for all samples included in this study and voucher information for newly generated sequences are listed in the Appendix.

DNA extraction, PCR amplification, and sequencing. — Total genomic DNA was extracted from silica gel-dried leaf material or herbarium specimens using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's protocol. The *matK*, *rbcL*, and *trnL-trnF* regions were amplified following the PCR protocols described by Li & al. (2004), and the *ndhF* region was amplified following that of Bohs & Olmstead (1997). The *matK* region was amplified with the primer pair AF/8R of Ooi & al. (1995), the *ndhF* region with the 972F/2110R primers of Olmstead & al. (1993), the *rbcL* region with the 1F/1494R primers of Chen & al. (1998), and the *trnL-trnF* region with the c/f primers of Taberlet & al. (1991). PCR products were purified using the Tian quick Midi Purification Kit (Tiangen Biotech) following the manufacturer's protocol and were directly sequenced. Additional primers 06F forward (Li & al., 2004) and 06R reverse (Xiang, 2010) for *matK* and 991R for *rbcL* (Chen & al., 1998) were used for sequencing. Sequencing reactions were conducted using the ABI Prism BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Foster City, California, U.S.A.). Sequences were analyzed using an ABI 3730xl DNA sequencer. Fifty-one sequences were newly obtained from four plastid regions (12 *matK*, 13 *ndhF*, 13 *rbcL*, 13 *trnL-trnF*).

Phylogenetic analysis. — Sequences were aligned using Clustal X v.1.83 (Thompson & al., 1997) and manually adjusted with BioEdit v.5.0.9 (Hall, 1999). Two difficult-to-align regions in the *trnL-trnF* region, representing 89 nucleotides, were removed from the analyses. Gap characters were scored using "simple indel coding rules" (Simmons & Ochoterena, 2000) with GapCoder (Young & Healy, 2003). Phylogenetic analyses were initially performed for individual regions using maximum parsimony (MP). Because no strongly supported conflicting nodes were found among the trees from different regions, the combined dataset of four loci was analyzed with MP and Bayesian inference (BI) methods as implemented in PAUP* v.4.0b10 (Swofford, 2003) and MrBayes v.3.0b4 (Ronquist & Huelsenbeck, 2003), respectively.

For the MP analysis, heuristic searches were performed with 1000 random-addition replicates, with one tree held at each step during stepwise addition, and tree-bisection-reconnection

branch swapping. Multrees was in effect and steepest descent off. Nonparametric bootstrap support (BS; Felsenstein, 1985) for each clade was estimated from 1000 replicates with 10 random taxon additions and heuristic search options.

For the BI analysis, the best-fitting substitution model for each DNA region (*matK*, *ndhF*, *rbcL*, *trnL-trnF*) was selected by ModelTest v.3.8 (Posada & Crandall, 1998) and using the Akaike information criterion (AIC). The chosen models were TVM+G (nst = 6, rate = gamma) for both *matK* and *trnL-trnF*, and TVM+I+G (nst = 6, rate = gamma) for both *ndhF* and *rbcL*. Indel characters of four DNA regions were divided into four independent partitions. The indel data were run under the datatype = standard option. Two independent Markov Chain Monte Carlo (MCMC) runs were conducted simultaneously, each with four linked chains, for 10,000,000 generations, sampling one tree every 1000 generations. Convergence of runs was indicated when the average standard deviation of split frequencies dropped below 0.01 after 2,730,000 generations. In addition, the log-likelihood values of the cold chain of the two simultaneous runs and graphical plots of −lnL generated by the sump command were checked to confirm that stationarity had been reached. For each run, the first 3000 trees were discarded as burn-in; the consensus tree and posterior probability (PP) values were calculated using the remaining 7001 trees.

Pollen morphology. — Pollen samples of two species, *Borthwickia trifoliata* (*Jin & al. YNET125*, Qimaba, Lüchun, Honghe, Yunnan, China) and *Stixis suaveolens* (*Qinghua Li 737*, XTBG, Menglun, Mengla, Xishuangbanna, Yunnan, China), were collected from herbarium specimens in PE. In addition, pollen samples of *Borthwickia trifoliata* were also taken from flower specimens pickled in FAA of two individuals collected in Qimaba, Lüchun, Yunnan, China, on 22 May 2011. The material was acetolyzed for light microscopy according to Erdtman (1960). The acetolyzed pollen grains were mounted on glass slides in silicone oil. Observations and measurements were made with a Zeiss Imager A1 microscope. Pollen grain size was based on the average of 20 measurements. For Scanning Electron Microscope (SEM), acetolyzed pollen grains were suspended in a drop of pure alcohol and directly transferred with a fine pipette to a clean stub with double-sided tape. The stubs were coated with gold palladium for 100–130 s in a Hitachi Ion Sputter E-1010 (Hitachi Science Systems, Tokyo, Japan) at 15 mA. Pollen grains were observed and photographed using a Hitachi S-4800 SEM at 10.0 kV. The terminology used here for the description of pollen followed Punt & al. (2007), which is available online (http://www3.bio.uu.nl/palaeo/glossary/glos-int.htm). The pollen morphology of *Borthwickia* and *Stixis* was compared with that of other Brassicales taxa.

■ RESULTS

Molecular phylogenetics. — The aligned *matK*, *ndhF*, *rbcL*, and *trnL-trnF* datasets had 1346, 940, 1270, and 1297 characters, respectively. Table 1 summarizes the number of variable and parsimony-informative sites and tree statistics for the various datasets. The combined dataset consisted of 4853 characters, of which 2043 were variable and 1240 were potentially parsimony-informative. The MP analysis generated 528 equally parsimonious trees of 4441 steps with a consistency index (CI) of 0.63 and a retention index (RI) of 0.83. The trees from the BI analyses had similar topologies as the MP strict consensus tree (Fig. 2).

Our analyses indicate that the core Brassicales comprise three monotypic families (Emblingiaceae, Pentadiplandraceae, Tovariaceae) and two clades, i.e., Brassicaceae-Capparaceae-Cleomaceae and GRFT (sensu Hall & al., 2004; represented by Gyrostemonaceae, Resedaceae, *Forchhammeria*, and *Tirania*). Within the GRFT clade, Gyrostemonaceae is the earliest-diverging lineage, followed by *Borthwickia* and a well-supported *Forchhammeria*-Resedaceae-*Stixis*-*Tirania* clade (MP-BS = 100%, PP = 100%). *Borthwickia* is resolved as sister to the latter clade with moderate to strong support (MP-BS = 82%, PP = 100%). *Stixis* is monophyletic (MP-BS = 100%, PP = 100%) and sister to *Tirania* (MP-BS = 100%, PP = 100%).

Pollen morphology. — Pollen grains of *Stixis suaveolens* are tricolporate, mainly spheroidal in equatorial view, and trilobate-circular in polar view. Endoapertures are subcircular. Grains are 14.8 (12.9–17.0) × 11.0 (9.5–13.6) µm. Exine sculpturing is coarsely reticulate with heterobrochus. Colpi with distinct margins are nearly equal to the polar axis in length and taper toward the poles. Colpus membranes are psilate (Fig. 3A–C).

Our pollen morphological observations of three individuals from two populations of *Borthwickia trifoliata* indicated

Table 1. Statistics from maximum parsimony analyses of the various datasets.

Dataset	No. taxa	Total length	Variable characters (incl. gaps)	Informative characters (incl. gaps)	Gaps scored	No. trees	Length of trees	CI	RI	RC
matK	62	1346	712	447	26	14,160	1684	0.61	0.83	0.51
ndhF	60	940	468	303	17	14,985	1183	0.60	0.82	0.49
rbcL	53	1270	301	179	0	184	610	0.62	0.79	0.49
trnL-F	54	1297	562	311	175	13,710	941	0.74	0.87	0.65
Four loci	66	4853	2043	1240	218	528	4441	0.63	0.83	0.53

Abbreviations: CI, consistency index; RI, retention index; RC, rescaled consistency index.

that pollen grains of the species are tricolporate, subprolate in equatorial view, and trilobate-circular in polar view. Endoapertures are circular. Grains are 29.7 (26.6–33.2) × 22.3 (18.4–25.1) µm. Exine sculpturing is perforate. Colpi are nearly equal to the polar axis in length and have inconspicuous margins and acute ends. Colpus membranes have small tubercles and holes (Fig. 3D–F).

■ DISCUSSION

Neither *Stixis* nor *Borthwickia* is a member of Capparaceae. — Our analyses indicate that the core Brassicales include three monotypic families (Emblingiaceae, Pentadiplandraceae, Tovariaceae) and two clades, Brassicaceae-Capparaceae-Cleomaceae and GRFT, which is congruent with previous molecular studies (e.g., Rodman & al., 1993, 1996, 1998; Hall & al., 2002, 2004; Martín-Bravo & al., 2009, 2010). Traditionally, *Borthwickia* and *Stixis* have been placed in Capparaceae sensu Pax & Hoffmann (1936), and have been considered to have a close relationship with *Ritchiea* or *Maerua* (Smith, 1911; Jacobs, 1968) and tribe Cadabeae (Hutchinson, 1967), respectively. Our results indicate that both *Borthwickia* and *Stixis* were distantly related to Capparaceae, supported by having one 5-bp insertion (indel V) in the *trnL-trnF* region (vs. absent) and lacking one 6-bp insertion (indel VI) in the *ndhF* gene (vs. present) (Fig. 2). These two genera also differ markedly from Capparaceae in some morphological characters, including axile placentation (vs. parietal), five or more sepals (vs. usually four), and three or more carpels (vs. usually two) (Table 2). Importantly, our observations in *Stixis suaveolens* indicate that pollen grains are spheroidal and less than 15 µm in polar axis diameter, which is similar to that of the other five species of *Stixis*, whereas pollen grains of Capparaceae are

Table 2. A comparison of nine morphological characters of *Borthwickia* and *Stixis* and their putatively related families or genera.

Taxon	Phyllotaxis	No. sepals	No. petals	No. carpels	No. locules	No. stamens	No. stigmas	Exine sculpture	Placentation
Capparaceae	Alternate	(3–)4(–7)	(0–)4(–8)	2(–8)	1(–2)	4–8(>10)	1	Perforate, reticulate, scabrate, etc.	Parietal
Boscia	Alternate	4	0	2	1	>10	1	Echinulate	Parietal
Buchholzia	Alternate	4	0	2	1	>10	1	Reticulate	Parietal
Cadaba	Alternate	4	2–4(0)	2(4)	1–2	4–8	1	Reticulate, striate	Parietal
Maerua	Alternate	4	0, 4	2(3)	1–2	>10	1	Reticulate, perforate	Parietal
Ritchiea	Alternate	4	4	2–4	1	>6	1	Columella, reticulate, rugulose	Parietal
Thylachium	Alternate	4	0	6–10	1	>10	1	?	Parietal
Gyrostemonaceae	Alternate	4–8	0	≥1	≥1	>10	≥2	Scabrate-spinulate	Axile
Borthwickia	Opposite	5–8	5–8	4–6	4–6	>10	1	Perforate	Axile
Resedaceae	Alternate	(2–)4–6(–8)	(2–)4–6(–8)	(2–)3–8	1	>10	≥2	Reticulate, rugulate, striate	Axile (basal-central) marginal, parietal
Forchhammeria	Alternate	4–8	0	2	2	>10	≥2	Reticulate	Axile
Neothorelia	Alternate	6	6	3	3	>10	≥2	Reticulate	Axile
Stixis	Alternate	6(5)	0	3(4)	3	>10	≥2	Reticulate	Axile
Tirania	Alternate	6	6	4	4	>10	≥2	?	Axile

Characters for Capparaceae and six genera of Capparaceae (*Boscia*, *Buchholzia*, *Cadaba*, *Maerua*, *Ritchiea*, *Thylachium*) from Erdtman (1952), Hutchinson (1967), Mitra (1975), and Kers (2003); for Gyrostemonaceae from Tobe & Takahashi (1995) and George (2003); for Resedaceae from Erdtman (1952), Abdallah & de Wit (1978), Perveen & Qaiser (2001), El Naggar (2002), Kubitzki (2003), Martín-Bravo & al. (2007), and Heywood & al. (2007); for *Borthwickia* and *Stixis* from Sun (1999), Wu & al. (2004), Zhang & Tucker (2008), and this study; for *Forchhammeria* from Erdtman (1952) and Hansen (1977); and for *Neothorelia* from Mitra (1975).
Note: Both Smith (1911) and Pax & Hoffmann (1936) considered that flowers of *Borthwickia* had two sepals and six petals, but sepals and petals were each five to eight based on our observations as well as the description of Sun (1999) and Zhang & Tucker (2008).

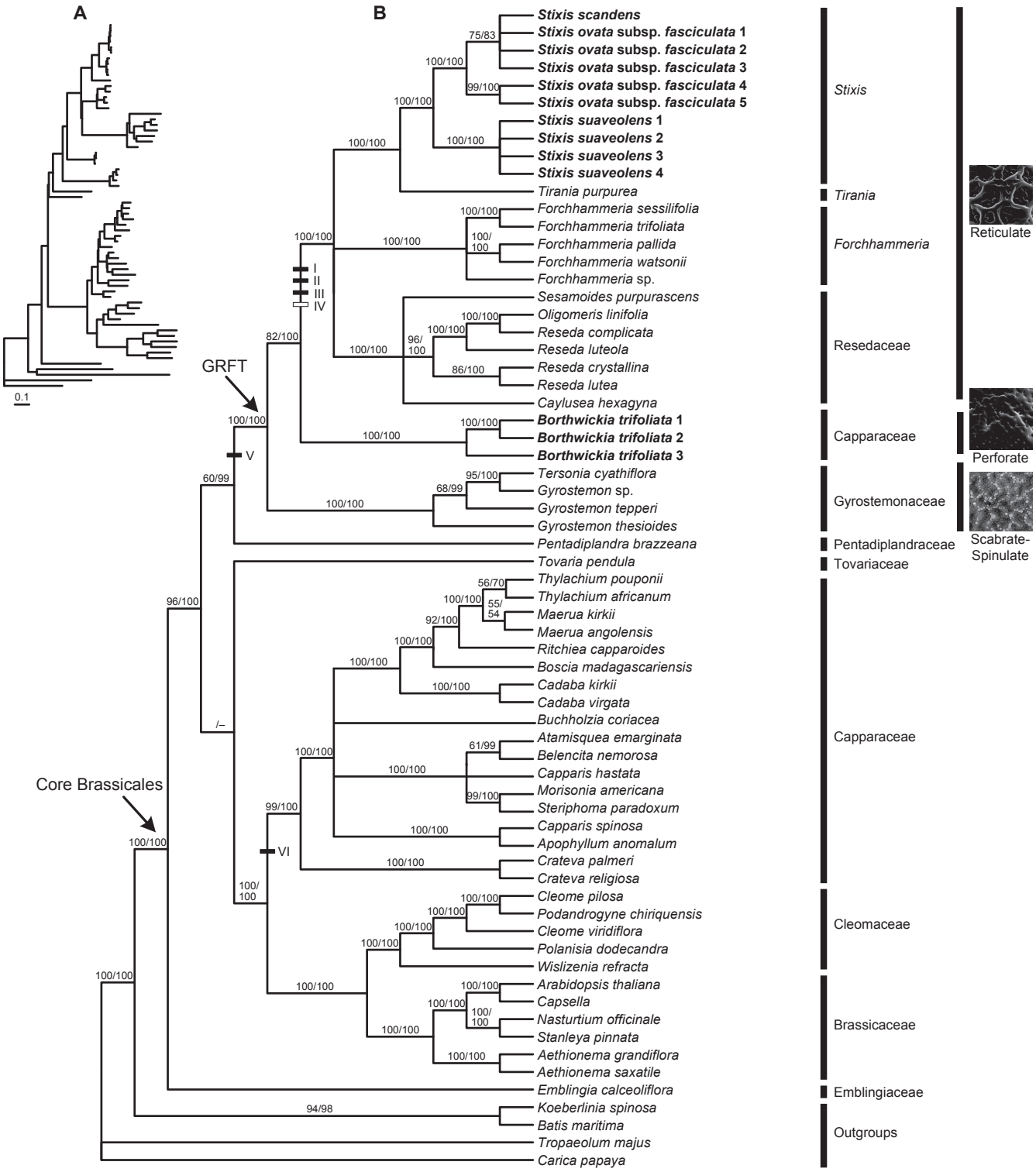

Fig. 2. Phylogenetic relationships of the core Brassicales based on the combined *matK*, *ndhF*, *rbcL*, and *trnL-trnF* datasets. **A,** Bayesian phylogram; **B,** strict consensus tree of 528 most parsimonious trees. — Numbers above branches are MP bootstrap values and Bayesian posterior probabilities as percentages (>50%). The dash (–) indicates a node that does not appear in the BI trees. Molecular synapomorphies (indels) are indicated by boxes on the branches and Roman numerals. Filled boxes represent non-homoplasious synapomorphies, and empty boxes indicate homoplasious changes. Familial classification is based on APG III (2009). The delimitation of Capparaceae is based on Hall (2008) and Iltis & al. (2011).

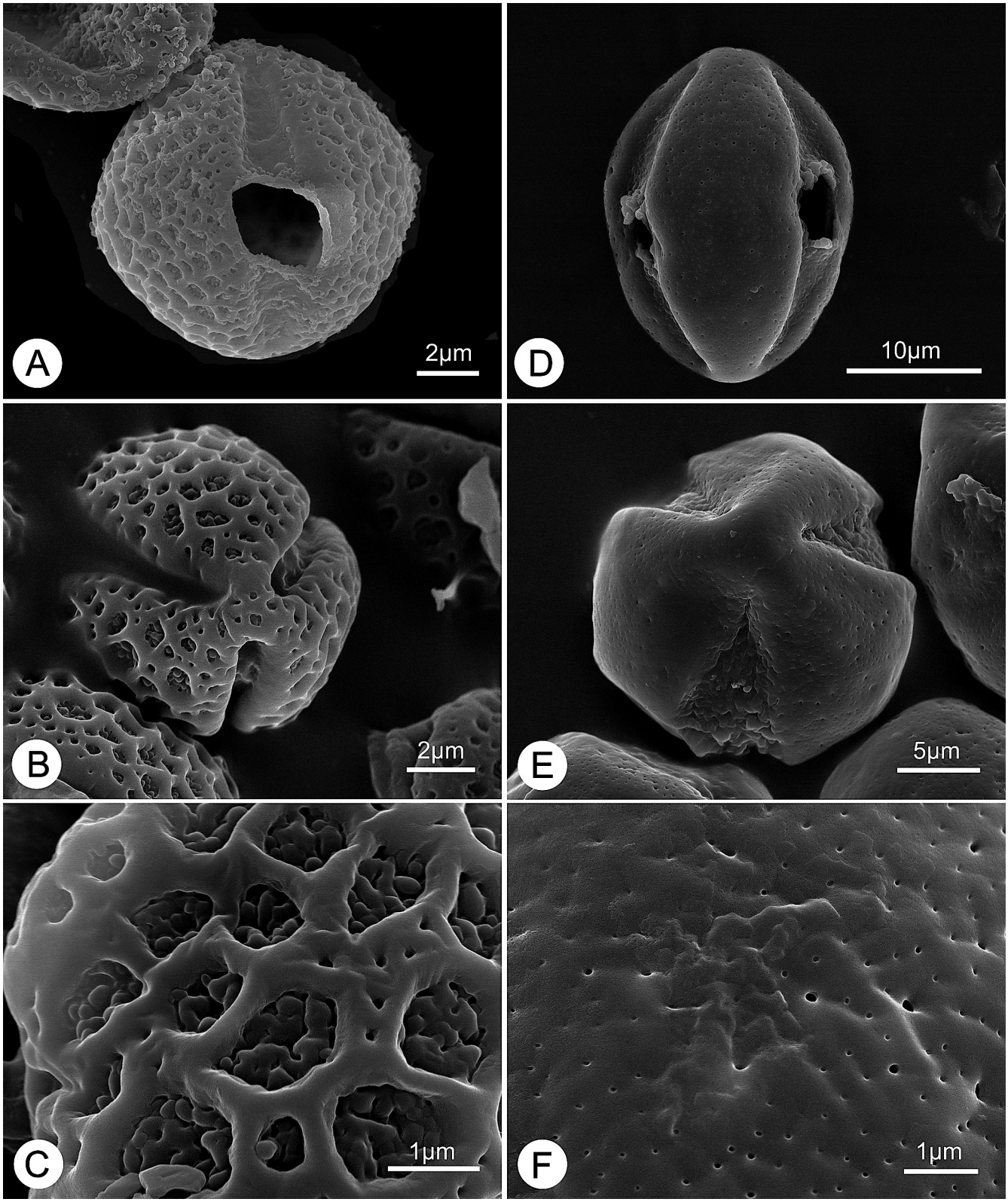

Fig. 3. SEM photographs of pollen grains. **A–C:** *Stixis suaveolens*: **A,** equatorial view; **B,** polar view; **C,** exine pattern. **D–F:** *Borthwickia trifoliata*: **D,** equatorial view; **E,** polar view; **F,** exine pattern.

usually prolate and more than 20 µm in polar axis diameter (Erdtman, 1952; Mitra, 1975) (Fig. 3).

Our results show that both *Borthwickia* and *Stixis* are members of the GRFT clade, which is characterized by an exclusive 5-bp insertion (indel V) in the *trnL-trnF* region (Fig. 2; also see Hall & al., 2002). In addition, *Borthwickia* and *Stixis* also shared several morphological characters, such as an irregular number of sepals, numerous stamens, and axile placentation, with some other members of the GRFT clade (Table 2), providing further support for the new positions of *Borthwickia* and *Stixis*.

***Stixis* is closely related to *Tirania*. —** *Stixis* was placed in tribe Stixeae of Capparaceae, with *Forchhammeria*, *Neothorelia*, and *Tirania*, and was considered close to *Forchhammeria* based on having flowers without petals (Pax & Hoffmann, 1936). However, *Stixis* is remarkably different from *Forchhammeria* in having bisexual flowers (vs. dioecious; Hansen, 1977) and an ovary usually with three carpels and locules (vs. an ovary with two carpels and locules; Table 2). Additionally, *Stixis* is endemic to Southeast Asia, but *Forchhammeria* is limited to Mexico, Central America, and the West Indies (Hansen, 1977).

Our results strongly support that *Stixis* is sister to *Tirania*, and the two genera together with *Forchhammeria* and Resedaceae form a monophyletic group within the GRFT clade. This group is characterized by one 9-bp deletion (indel I) in the *matK* gene, two 8-bp deletions (indels II, III) in the *trnL-trnF* region, and one 3-bp deletion (indel IV) in the *ndhF* gene (Fig. 2). The close relationship between *Stixis* and *Tirania* is supported by morphological characters, such as the presence of six sepals and an ovary with more than two locules (Table 2). Although closely related to *Tirania*, *Stixis* differs from *Tirania* in having racemes and panicles (vs. solitary flowers) and lacking stipules and petals (vs. present; Hutchinson, 1967; Sun, 1999; Zhang & Tucker, 2008). Therefore, our data support retaining *Stixis* and *Tirania* as separate genera.

Forchhammeria, *Neothorelia*, *Stixis*, and *Tirania* were placed in tribe Stixeae by Pax & Hoffmann (1936). Doweld & Reveal (2008) proposed a familial rank for the tribe, Stixaceae. However, it did not be accepted or used by subsequent authors (APG III, 2009; Martín-Bravo & al., 2009, 2010). To date, three of these four genera have been sampled in molecular phylogenetic analyses, but the monophyly of this group (represented by *Forchhammeria*, *Stixis*, and *Tirania*) remains not to be resolved (Fig. 2). *Neothorelia* shares several morphological features with *Stixis* and *Tirania*, such as six-merous flowers and an ovary with more than two locules (Table 2). Moreover, *Neothorelia* has pollen grains almost identical to those of *Stixis* (Mitra, 1975; this study). Thus, *Neothorelia* seems to be closely related to *Stixis* and *Tirania*, but needs to be evaluated in a phylogenetic context. However, the family assignments of members of tribe Stixeae are still premature, for which further study by sampling more markers and more taxa, especially from *Neothorelia*, is needed.

***Borthwickia* belongs to a monotypic family. —** In our phylogenetic tree (Fig. 2), *Borthwickia* was segregated from Capparaceae and embedded within the GRFT clade with strong support. *Borthwickia* is morphologically distinct from both other members of the GRFT clade and Capparaceae in having opposite leaves (vs. alternate) and an ovary with more than four locules (vs. less than four; Fig. 1A, B; Table 2).

Our analyses resolved *Borthwickia* as sister to the *Forchhammeria*-Resedaceae-*Stixis*-*Tirania* clade, but *Borthwickia* is remarkably different from all members of this clade in having a single stigma (vs. more than one; Table 2) and a linear ovary with ridges (vs. globose, ovoid, or elliptic; Hutchinson, 1967; Kers, 2003; Kubitzki, 2003). Significantly, our pollen observations showed that the exine sculpture of *Borthwickia* pollen grains is perforate, unlike the reticulate ornamentation in *Forchhammeria*, Resedaceae, and *Stixis* (Fig. 3; Table 2). Additionally, *Borthwickia* is distinguished from its sister clade in lacking one 9-bp deletion in the *matK* dataset, two deletions (8-bp and 9-bp) in the *trnL-trnF* dataset, and one 3-bp deletion in the *ndhF* dataset (Fig. 2). Because *Borthwickia* has the autapomorphies and other distinct morphological and molecular characters mentioned above, we propose a new monotypic family for it and describe Borthwickiaceae below.

■ TAXONOMIC TREATMENT

Borthwickiaceae J.X. Su, Wei Wang, Li Bing Zhang & Z.D. Chen, **fam. nov.** – Type: *Borthwickia* W.W. Smith in Trans. & Proc. Bot. Soc. Edinburgh 24: 175. 1911.

Frutex vel arbuscula. Rami juniores quadrangulati, vetustiores cylindrici. Folia trifoliata, opposita. Inflorescentiae terminales, racemosae. Flores bisexuales. Sepala 5–8, omnino connata, in bilobos rumpentia sub anthesi. Petala 5–8, distincta, sepali tubo manifesto breviora. Stamina 60–70, in androgynophori brevis crassi apice inserta; pollinis granula tricolpora, exiniis punctis. Nectarium conicum, androgynophorum cingens. Ovarium lineare porcatum, 4–6 loculis, placentis axialibus. Stigma simplex sessile obsoletum indivisum. Capsula torulosa, ab basi ad apicem dehiscens in siccitate. Semina reniformia; embryo flexus.

Shrubs or small trees, 1–6 m tall, unarmed, evergreen. Twigs quadrangular with dense, short, white pubescence, later glabrescent; larger branches cylindrical. Stipules absent. Leaves opposite, palmately ternate compound; petiole (3–)5–13(–20) cm, petiolules ca. 1 cm; leaflet blade membranous, margins entire, abaxially with white short pubescence on veins, adaxially glabrous, lateral veins 7–9 pairs, reticulate veins impressed adaxially and prominent abaxially; terminal leaflet oblong, elliptic-lanceolate, sometimes obovate-lanceolate, (5–)8–20(–30) × (1.5–)4–10(–16) cm; lateral leaflets ovate-lanceolate, slightly smaller than terminal one, base asymmetric. Racemes terminal, 8–20 cm, sessile, rachis with dense short white pubescence; bracts 1.0–1.5 cm, usually linear, deciduous. Flower parts spirally arranged on receptacle; pedicel 1.2–1.5 cm, trichomes like those on axis. Sepals 5–8, whitish, connected into a galericulate tube with short white pubescence on both surfaces, splitting into two lobes at anthesis, deciduous. Petals 5–8, whitish, oblong or spatulate, 1.5–1.8 cm, 1/3–1/2 as long as calyx tube, subequal, distinct, erect, proximally valvate and distally

imbricate, membranous and glabrous above middle, thick and with dense pubescence abaxially and sparse pubescence adaxially below middle. Androgynophore ca. 5 mm. Stamens 60–70, 1.4–2.0 cm, fertile, free, at summit of androgynophore; anthers ovate, 2-locular, longitudinally dehiscent, dorsifixed; pollen grains 29.7 (26.6–33.2) × 22.3 (18.4–25.1) µm, tricolporate, subprolate, exine perforate. Nectary conical, ascending from petal base to stamen base, surrounding androgynophore. Ovary linear, 1.0–1.5 cm, distally with 4–6 vertical grooves and ridges, 4–6-locular, with axile placentation, each locule with ovules in two rows; stigma indistinct, sessile. Fruit a capsule, 6–9 cm × 4–6 mm, linear, terete, torulose, base attenuate, apex with a 3–5 mm beak; dehiscent along ventral suture from base to apex leaving a persistent axis with 4–6 ridges; pericarp thin, black to brown after drying. Seeds numerous, 2–3 mm, red when fresh and reddish brown after drying, reniform; embryo bent, scarcely differentiated. Fl. Apr.–Jun., fr. Jul.–Oct. (Smith, 1911; Sun, 1999; Zhang & Tucker, 2008; own observations)

Etymology. – *Borthwickia* was named in honor of A.W. Borthwick, an English doctor of science and botanist (Smith, 1911).

Distribution and habitat. – *Borthwickia* contains one species: *B. trifoliata* W.W. Smith. It occurs in wet valleys, forests, and ravines at altitudes of 300–1400 m in southern to southeastern Yunnan, China, and eastern to northern Myanmar.

Representative specimens examined. – BURMA. Shan State, 15 May 1909, *MacGregor 714* (E), alt. 500 m, habitat not specified. CHINA. Yunnan: Honghe, Hekou, Laohuazhai, 19 Aug. 1993 (young fruit), *Shui & al. 003469* (PE), alt. 1160 m, in forests; Honghe, Hekou, the way from Dawei Mt. to Yaoshan, 09 Oct. 1999 (mature fruit), *Shui & al. 11932* (KUN), alt. 900 m, in forests; Honghe, Lüchun, Qimaba, 25 Jun. 2009 (flower, young fruit), *Jin & al. YNET125* (PE), alt. 400 m, in forests; Honghe, Jinping, Mengla, Tuomazhai, 28 Jun. 2009 (young fruit), *Jin & al. YNET381* (PE), alt. 900 m, in forests; Xishuangbanna, Mengla, Yiwu, Guafengzhai, *Ren & Su RS002* (PE), 17 Jan. 2011, alt. 800 m, in ravines; Honghe, Jinping, Mengla, Tuomazhai, 09 Oct. 2011, *Yang & al. 00499* (PE), alt. 963 m, in ravines; Honghe, Lüchun, the way from Huanglian Mt. to Qimaba, 10 Oct. 2011 (mature fruit), *Yang & al. 00536* (PE), alt. 1074 m, in forests. — DNA sequences of *Borthwickia* were extracted from three specimens (*YNET125*, *YNET381*, *RS002*).

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Appendix. Taxa and GenBank accession numbers for all samples included in this study; voucher information is given for newly generated sequences. An asterisk after the accession number indicates sequences reported here for the first time.

Taxon, voucher, accession number of *matK*, *ndhF*, *rbcL*, *trnL-trnF*. – missing data; * newly generated sequences.

INGROUPS: ***Aethionema grandiflora*** L., AF144354, AF064657, AY167983, –. ***Aethionema saxatile*** R. Br., EU371817, AY483250, AY483262, AY122451. ***Apophyllum anomalum*** F. Muell., AY483227, AY122356, AY483264, AY122409. ***Arabidopsis thaliana*** (L.) Heynh., AF144348, AY122394, D88901, AY122452. ***Atamisquea emarginata*** Miers, EU371745, AY122357, –, AY122410. ***Belencita nemorosa*** (Jacq.) Dugand, EU371746, AY122358, –, AY122411. ***Borthwickia trifoliata*** 1 W.W. Smith, China, Yunnan, Honghe, Lüchun, Qimaba, *Jin & al. YNET 125* (PE), JQ733089*, JQ733101*, JQ733114*, JQ733127*. ***B. trifoliata*** 2 W.W. Smith, China, Yunnan, Honghe, Jinping, Mengla, Tuomazhai, *Jin & al. YNET 381* (PE), JQ733091*, JQ733103*, JQ733116*, JQ733129*. ***B. trifoliata*** 3 W.W. Smith, China, Yunnan, Xishuangbanna, Mengla, Yiwu, Guafengzhai, *Ren B.Q. & Su J.X.* RS002 (PE), JQ733090*, JQ733102*, JQ733115*, JQ733128*. ***Boscia madagascariensis*** (DC.) Hadj-Moust., EU371749, AY122359, –, AY122412. ***Buchholzia coriacea*** Engl., EU371750, AY122360, –, AY122413. ***Cadaba kirkii*** Oliv., EU371752, AY122361, –, AY122414. ***Cadaba virgata*** Bojer, EU371753, AY122362, –, AY122415. ***Capparis hastata*** Jacq., AY483228, AY122366, M95754, AY122420. ***Capparis spinosa*** L., EU371772, EU373694, AY167985, AY122422. ***Capsella bursa-astoris*** (L.) Medic., –, AY122396, D88904, AY122454. ***Capsella rubella*** Reut., AF144334, –, –, –. ***Caylusea hexagyna*** (Forssk.) M.L. Green, FJ212207, –, FJ212220, DQ987069. ***Cleome pilosa*** Benth., AY483231, AY122385, AY483267, –. ***Cleome viridiflora*** Schreb., AY483232, AY122386, AY483268, AY122441. ***Crateva palmeri*** Rose, AY483229, AY122370, AY483265, AY122427. ***Crateva religiosa*** G. Forst., EU371780, AY122371, –, AY122428. ***Emblingia calceoliflora*** F. Muell., –, AY483256, AF146014, –. ***Forchhammeria pallida*** Liebm., –, AY122381, AY483274, AY122437. ***Forchhammeria sessilifolia*** Standl., AY483243, AY483257, AY483275, –. ***Forchhammeria*** **sp.,** AY483244, AY483258, AY483276, –. ***Forchhammeria trifoliata*** Radlk., AY483245, AY483259, AY483277, –. ***Forchhammeria watsonii*** Rose, AY483246, AY483260, AY483278, –. ***Gyrostemon*** **sp.,** AY483236, AY483252, L22439, –. ***Gyrostemon tepperi*** (F. Muell. ex H. Walter) A.S. George, AY483237, AY483253, L22440, –. ***Gyrostemon thesioides*** (Hook. f.) A.S. George, FJ212199, –, FJ212210, DQ986975. ***Maerua angolensis*** DC., EU371783, AY122377, –, AY122433. ***Maerua kirkii*** (Oliv.) F. White, AY483230, AY122378, AY483266, AY122434. ***Morisonia americana*** L., EU371784, AY122374, –, AY122430. ***Nasturtium officinale*** R. Br., AY483225, AY122399, AF020325, AY122457. ***Oligomeris linifolia*** (Vahl) J.F. Macbr., AY483240, AY483255, AY483272, FJ212256. ***Pentadiplandra brazzeana*** Baill., AY483239, AY483254, U38533, AY122463. ***Podandrogyne chiriquensis*** (Standl.) Woodson, AY483233, AY122393, AY483269, AY122450. ***Polanisia dodecandra*** DC., AY483234, AY483251, AY167984, AY122447. ***Reseda complicata*** Bory, FJ212205, –, FJ212218, DQ987046. ***Reseda crystallina*** Webb & Berthel., FJ212200, –, FJ212212, FJ212283. ***Reseda lutea*** L., AY483241, AY122406, AY483273, AY122464. ***Reseda luteola*** L., FJ212206, –, FJ212219, DQ987050. ***Ritchiea capparoides*** (Andr.) Britten, EU371785, AY122375, –, AY122431. ***Sesamoides purpurascens*** (L.) G. López., FJ212208, –, FJ212221, DQ987064. ***Stanleya pinnata*** (Pursh) Britton, AY483226, AY122401, AY483263, AY122459. ***Steriphoma paradoxum*** Endl., EU371786, AY122376, –, AY122432. ***Stixis ovata*** subsp. ***fasciculata*** 4 (King) Jacobs, China, Yunnan, Xishuangbanna, Mengla, *Ren B.Q. & Su J.X. RS001* (PE), JQ733093*, JQ733105*, JQ733118*, JQ733134*. ***S. ovata*** subsp. ***fasciculata*** 5 (King) Jacobs, China, Yunnan, Xishuangbanna, Mengla, Gongbing Mt., *Ren B.Q. & Su J.X. RS013* (PE), JQ733095*, JQ733107*, JQ733120*, JQ733135*. ***S. ovata*** subsp. ***fasciculata*** 1 (King) Jacobs, China, Yunnan, Honghe, Hekou, Yaoshan, Taiyangzhai, Baiquanchong, *Ren B.Q. & Su J.X. RS017* (PE), JQ733097*, JQ733109*, JQ733122*, JQ733136*. ***S. ovata*** subsp. ***fasciculata*** 2 (King) Jacobs, China, Yunnan, Honghe, Hekou, Yaoshan, Liangzizhai, *Ren B.Q. & Su J.X. RS019* (PE), JQ733098*, JQ733110*, JQ733123*, JQ733137*. ***S. ovata*** subsp. ***fasciculata*** 3 (King) Jacobs, China, Yunnan, Honghe, Hekou, Yaoshan, Geniao, *Yang Z.G. 008* (KUN), –, JQ733112*, JQ733125*, JQ733139*. ***S. scandens*** Lour. China, Yunnan, Honghe, Hekou, Yaoshan, Taiyangzhai, Baiquanchong, *Shui Y.M. & al. 12443* (KUN), JQ733099*, JQ733111*, JQ733124*, JQ733138*. ***S. suaveolens*** 1 (Roxb.) Pierre, China, Hainan, *Chen Z.D. HN171* (PE), JQ733100*, JQ733113*, JQ733126*, JQ733133*. ***S. suaveolens*** 3 (Roxb.) Pierre, China, Yunnan, Xishuangbanna, Mengla, Menglun, XTBG, *Guo C.C. BN04* (PE), JQ733092*, JQ733104*, JQ733117*, JQ733130*. ***S. suaveolens*** 4 (Roxb.) Pierre, China, Yunnan, Xishuangbanna, Mengla, Menglun, XTBG, *Ren B.Q. & Su J.X. RS012* (PE), JQ733094*, JQ733106*, JQ733119*, JQ733131*. ***S. suaveolens*** 2 (Roxb.) Pierre, China, Yunnan, Honghe, Hekou, Yaoshan, Taiyangzhai, Dudian, *Ren B.Q. & Su J.X. RS015* (PE), JQ733096*, JQ733108*, JQ733121*, JQ733132*. ***Tersonia cyathiflora*** (Fenzl) A.S. George ex J.W. Green, AY483238, AY122404, L22441, AY122462. ***Thylachium africanum*** Lour., EU371788, AY122379, –, AY122435. ***Thylachium pouponii*** Aubrév. & Pellegr., EU371789, AY122380, –, AY122436. ***Tirania purpurea*** Pierre, –, AY483261, AY483279, –. ***Tovaria pendula*** Ruiz & Pav., AY483242, AY122407, M95758, AY122465. ***Wislizenia refracta*** Engelm., AY483235, AY122391, AY483271, AY122448. **OUTGROUPS:** ***Batis maritima*** L., AY483219, AY122403, L22438, –. ***Carica papaya*** L., AY483221, AY483248, M95671, DQ061124. ***Koeberlinia spinosa*** Zucc., AY483222, AY483249, L14600, –. ***Tropaeolum majus*** L., AY483224, AY122408, L14706, AB043665.