Phylogeography and the evolutionary time-scale of passerine radiations in the Sino-Himalayan region (Aves: Passeriformes)[*]

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Abstract

Among several Southeast Asian passerine species groups, highly congruent phylogeographic substructure was detected by molecular studies. In most genera investigated, taxa from the Himalayas were most closely related to the populations from West Chinese provinces and/or adjacent continental SE Asia (Thailand, Vietnam). These Sino-Himalayan genetic clusters are opposed to at least one further SE Asian and/or a further North palearctic cluster. Divergence time estimates for several nodes of the mitochondrial phylogenies were obtained by calibration of a relaxed molecular clock in a non-parametric approach (rate smoothing). Rate-smoothed trees and 95% confidence intervals of time estimates are presented for mitochondrial lineages of crests and kinglets (*Regulus*), Old World warblers (*Phylloscopus*, *Seicercus*), coal tits and allies (*Periparus*) and treecreepers (*Certhia*). Splits between terminal taxa from the Sino-Himalaya were dated to Pliocene/Pleistocene boundary. Several Himalayan species show a recent East/West differentiation, most intra-Himalayan splits between eastern and western populations were dated to the Pleistocene period. Highest genetic differentiation within the Himalayas corresponding to a divergence time at the Pliocene/Pleistocene boundary was found in coal tits between western *Periparus ater melanolophus* and eastern *P. a. aemodius*.
Furthermore, divergence time estimates for larger phylogeographic clusters (Sino-Himalayan vs. Central Asian mountain systems and North Palearctic) are presented. The results are discussed with respect to possible Pleistocene refuges, which caused part of the present-day genetic diversity.

Zusammenfassung:

Anhand molekulargenetischer Studien lassen sich zwischen verschiedenen südostasiatischen Singvogelgruppen mit ähnlichem Verbreitungsmuster auch nahezu deckungsgleiche phylogeographische Muster erkennen. Die im Himalaya beheimateten Taxa sind in den meisten Gattungen nächstverwandt zu den Populationen im angrenzenden West-China und/ oder dem südostasiatischen Festland (Thailand, Vietnam, Teile Yunnans). Diese genetischen Verwandtschafts-Cluster des Sino-Himalaya stehen oftmals wiederum mindestens einer weiteren genetischen Linie aus Südostasien und/ oder der Nord-Paläarktis gegenüber. Es stellt sich somit die Frage, ob solche Muster auf dieselben evolutionären Prozesse und entsprechend auch auf vergleichbare Entstehungszeiträume zurückzuführen sind. Die in dieser Arbeit präsentierten Altersschätzwerte für verschiedene Aufspaltungsereignisse zwischen Vorläufern heutiger Populationen (Subspezies) wurden durch Kalibrierung einer molekularen Uhr für Cytochrom-*b*-Sequenzdaten ermittelt (rate-smoothing Methode). Als Eichpunkte wurden paläogeographische Datierungen zur Entstehung von Vulkaninseln oder der Öffnung von Landbrücken herangezogen. Entsprechend zeitlich kalibrierte Phylogenien und Konfidenzintervalle (95%) für Altersschätzwerte werden angegeben für Goldhähnchen (*Regulus*), Laubsänger (*Phylloscopus*, *Seicercus*), Tannen- und Fichtenmeisen (*Periparus*) sowie Baumläufer (*Certhia*). Aufspaltungen zwischen Schwestertaxa aus dem Himalaya und dem angrenzenden China/ Südostasien wurden fast ausnahmslos auf den Übergang Pliozän/ Pleistozän datiert. Einige Arten des Himalayas zeigen erkennbare subspezifische Ost-West-Differenzierung, die eindeutig eiszeitlichen Ursprungs ist. Innerhalb des Himalayas wurde die höchste genetische Divergenz bei Tannenmeisen zwischen dem westlichen *Periparus ater melanolophus* und dem östlichen *P. a. aemodius* gefunden. Entsprechend ihres Sequenzunterschiedes von etwa 2,3 % (Cytochrom *b*) dürften sich die Vorläufer der östlichen und westlichen Tannenmeisenpopulationen des Himalaja bereits während des Übergangs Pliozän/ Pleistozän voneinander getrennt haben. Weitere Altersschätzwerte wurden für die tieferen Aufspaltungsereignisse der molekularen Stammbäume berechnet (Sino-Himalaja vs. Tien-Shan-Altai-Region und Nord-Paläarktis). Die Ergebnisse werden im Hinblick auf mögliche eiszeitliche Refugialgebiete diskutiert, in denen allerdings nur ein Teil der heutigen genetischen Diversität der untersuchten Vogelgruppen entstanden sein kann. Viele Differenzierungsereignisse selbst innerhalb bislang als gute Arten anerkannter Taxa nahmen höchstwahrscheinlich zu früheren Zeiten deutlich vor dem Beginn des Pleistozän ihren Anfang.

Key words: Passeriformes, Sino-Himalaya, cytochrome *b*, molecular clock, rate-smoothing, Pleistocene, Pliocene

1. Introduction

The Himalayan Mountains and the neighbouring sub-Himalayan Indo-Burmese region are considered an important hotspot of avian biodiversity (Mittermeier et al. 1999, Roselaar et al. 2007). The Himalayan range itself harbours about 8% of the world´s bird species (Price et al. 2003) and though only 15 species are entirely endemic (1.5%), there are four endemic bird areas defined by Bird Life International (EBA, Stattersfield et al. 1998) which largely or partly extend to the Himalayan mountain system. Extensive avifaunal research in the Himalayas has yielded detailed information about distribution and breeding ranges and species composition of the different montane and altomontane vegetation zones (for Nepal see Martens & Eck 1995). As a result of a growing focus on molecular methods in avian systematics (and bioacoustics particularly in passerines, too) several taxa were subject to a recent re-evaluation based on their large intra- and interspecific differentiation (review for Old World warblers: Alström 2006, Rheindt 2007).

The recent strong efforts made in order to improve the robustness of molecular trees are in contrast to the carelessness displayed with respect to the calibration and application of a molecular clock. A common stopgap in avian systematics is the use of the 2% rule of thumb regardless of marker genes, rate constancy or model-correction of sequence data (reviews in Garcia-Moreno 2004, Lovette 2004). So far, only a few studies dealt with divergence time estimates for SE Asian bird species in the context of phylogeography. Johansson et al. (2007) provided a comprehensive phylogeny of Old World warblers (*Phylloscopus, Seicercus*) including representatives of nearly all Sino-Himalayan and Oriental species. Their age estimates (based on a combination of a rate-smoothed calibration and the 2% rule) suggest Pleistocene speciation events for six sister-species pairs from the Himalayas and adjacent China, and even older divergence times dating back to the Pliocene era for other Sino-Himalayan species pairs. For vicariance-dispersal analysis Johansson et al. (2007)

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distinguished eight biogeographic regions and, strikingly, the phylogeographic branching pattern of some *Phylloscopus* sub-clades is quite similar to patterns found in other forest-dwelling Sino-Himalayan bird species with phylogenetic affiliations to closely related SE Asian and N Palearctic species (review: MARTENS & PÄCKERT 2003). Nonetheless, there is almost no comparative study of a molecular clock calibration for different groups of birds with a similar phylogeographic branching pattern (Paridae: PÄCKERT et al. 2007).

This paper aims to evaluate a time-scale of species splits in the SE Asian region, with particular focus on the Sino-Himalayan taxa, based on cytochrome-*b* sequence data of well studied passerine genera (crests and kinglets: *Regulus*, Old World warblers: *Phylloscopus* and *Seicercus*, coal tits and allies: *Periparus*, treecreepers: *Certhia*; for references to the original studies see Methods!). We provide divergence time estimates for Himalayan species and their close relatives, derived from non-parametric rate-smoothed calibration.

2. Methods

We compared mitochondrial cytochrome-*b* sequence data sets from several previous phylogenetic studies with respect to phylogeographic patterns and age estimates for lineage splits among Sino-Himalayan populations (subspecies) for the following passerine genera: *Regulus* (PÄCKERT et al. 2003, 2008), *Periparus* (MARTENS et al. 2006), *Certhia* (TIETZE et al. 2006) and *Phylloscopus* (HELBIG et al. 1996, MARTENS et al. 2004, OLSSON et al. 2004, 2005, PÄCKERT et al. 2004, in press, MARTENS et al. 2008). Age dating was performed in a non-parametric rate-smoothing approach with the software r8s 1.70 (NPRS algorithm, SANDERSON 2003, 2004). Maximum-likelihood input trees for each passerine genus except *Phylloscopus* were reconstructed with TreePuzzle (SCHMIDT et al. 2004) with each taxon represented by one (the most common) haplotype. Best fit substitution models for phylogenetic tree reconstruction were applied to each dataset according to the settings provided in the original studies. The topologies of input trees are shown in Fig. 1. For calibration of the relaxed molecular clock we assigned from one to six different age constraints to fixed nodes of an input tree. As constraints we chose several paleogeographic age estimates corresponding to the emergence and breakdown of geographical barriers, i. e. ages of volcanic islands or opening and closure of land bridges. Age dating based on paleogeographic events is commonly applied for relatively recent splits at the intra- and interspecific level where the fossil record is either poor or does not allow for species distinction in passerines. Most frequently applied substitution rates for cytochrome *b* resulted from clock calibrations by FLEISCHER et al. (1998, 2006). Age constraints used for clock calibration in this study and the associated references are given in Table 1. For details on non-parametric age dating based on these calibration points compare PÄCKERT et al. (2006, 2007). Confidence intervals (95% CI) were calculated according to the recommended built-in strategy implemented in r8s (SANDERSON 2004) for lineage splits between Himalayan taxa and their close relatives.

Due to a lack of appropriate calibration points in the *Phylloscopus* tree we used a different approach for age dating in Old World warblers. First, the node involving the Canarian

Phylloscopus canariensis lineage was the only calibration point available in this genus, but age estimates with r8s based on this single constraint did not yield reliable results.

Yet JOHANSSON et al. (2007) faced these difficulties when performing age dating for the *Phylloscopus* tree. They calibrated a rate-smoothed tree with branch lengths proportional to time with r8s and equated the depth of that tree to the depth of a clocklike tree obtained from maximum likelihood analysis. The substitution rate of 2% per my applied by JOHANSSON et al. (2007) to their molecular data is an approximation for cytochrome *b* that resulted from various independent calibrations. Since substitution rates might significantly differ even among mitochondrial genes (RUOKONEN & Kvist 2002, PÄCKERT et al. 2006, 2007), and still more between mtDNA and nuclear DNA, the approximative value of 2% per my might not be an appropriate choice for clock calibration based on concatenated mitochondrial and nuclear sequence data (JOHANSSON et al. 2007).

In order to estimate a range of divergence times between major *Phylloscopus* cyt-*b* lineages, we first computed linearized NJ trees with MEGA 3.1 (KUMAR et al. 2004) by using two substitution rate estimates of 0.008 and 0.0125 substitutions per site per lineage per my by FLEISCHER et al. (1998, 2006). The tree resulting from a calibration based on the lower rate estimate is shown in Fig. 2 (left: age ranges of nodes referring to the higher rate estimate are indicated for several subclades). Divergence time ranges of SE Asian lineage splits corresponded best to a scenario postulating the early Pliocene opening of the land bridge between the Malaysian Peninsula and Borneo at about 5 my BP and the land bridge between the Malaysian Peninsula and Sumatra at 2 my BP, respectively (NISHIMURA 2002, INGER 2005). In addition to the Messinian constraint applied to the *P. canariensis* node, these two age constraints were assigned to a) the initial split between larger SE Indomalayan subclades and b) the basal split of the *Seicercus montis*/*S. grammiceps*/*S. castaniceps* clade (Fig. 2: symbols indicated at corresponding nodes). Furthermore, two Mediterranean/Canarian age constraints were applied to lineage splits between Mediterranean/Canarian lineages of the outgroup taxa *Sylvia melanocephala* and *S. mystacea* (sequence data by DIETZEN et al. 2008; outgroup clade not shown in Fig. 2). For comparison we performed a second run with r8s 1.70, applying an earlier and broader age range to the basal split between Indomalayan and continental SE Asian warbler lineages (divergence time estimate for split between SE insular Indomalayan tits of *Periparus* (*Pardaliparus*) and continental SE Asian *Periparus*, compare Fig. 1). Confidence search with r8s 1.70 failed for several recent splits involving Himalayan populations (no cross-over point was found for these nodes). Therefore 95% CIs were available only for some *Phylloscopus* species splits. In the linearized tree and in Table 1 only upper and lower range of age estimates from the standard clock calibration are indicated (Fig. 2, left).

Glossary: Biogeographic regions (slightly modified after JOHANSSON et al. 2007)

- East Himalaya: Eastern Himalayas from parts of Sikkim eastwards to the mountains of the Indo-Burmese region including Chin Hills (MITTERMEIER et al. 1999) (part of region B: JOHANSSON et al. 2007)

- West-Himalaya: Western Himalayas from about Sikkim westwards to Hindukush in the West (part of region B: JOHANSSON et al. 2007)
- Sino-Himalaya: entire Himalayan chain and adjacent W Chinese mountains, mainly C/N Yunnan, Sichuan, Gansu, parts of Qinghai and Shaanxi – an area of often closely related species or/and subspecies.
- SE Asia: continental Thailand, Vietnam, Laos, Cambodia and S parts of Yunnan (part of region D, including SE China: JOHANSSON et al. 2007)
- W China: mountainous and forested areas of C/N Yunnan, Sichuan, Gansu, parts of Qinghai and Shaanxi (region C: JOHANSSON et al. 2007)
- Central Asia; the Tian Shan-Altai mountain system, including parts of Pamir (part of region A: JOHANSSON et al. 2007)
- N Palearctic: in this context the Siberian taiga belt southward to forested areas of Mongolia and NE China.

3. 1. Results

In all passerine groups investigated (whole genera and subclades of the *Phylloscopus* tree) the Sino-Himalayan populations appear as strongly differentiated genetic lineages (Fig. 1). The kinglets comprise a single Sino-Himalayan lineage (*Regulus regulus himalayensis* incl. ssp. *sikkimensis*) which is sister to all remaining Eurasian Goldcrests. The treecreeper and the coal tit trees (*Periparus* and *Certhia*) each comprise two deeply split Sino-Himalayan clades. In most species studied, genetic differentiation among **Himalayan populations** is relatively slight, but in several species a bipartition into distinct Eastern and Western genetic clusters was found. Most recent lineage splits between sister taxa from the Eastern *vs*. the Western Himalayas and adjacent Myanmar were dated to the mid to late Pleistocene era in Rufous-vented tits (*Periparus rubidiventris*) and for several lineages of *Phylloscopus* warblers (Table 1). Highest genetic distances of 2.3% (cytochrome *b*) within the Himalayan region were found between western *Periparus ater melanolophus* and *P. a. aemodius*, corresponding to an age estimate of 2.4 to 3.7 mya for the respective lineage split (Table 1, Fig. 3). Similarly high genetic differentiation was found between Blyth's Leaf-warblers from the Himalayas (*Phylloscopus reguloides reguloides*) and those from adjacent Myanmar (*P. r. assamensis*, Table 1, Fig. 2). Only in *Certhia hodgsoni* are the Himalayan populations not monphyletic: the eastern Himalayan populations (ssp. *mandellii*) are sister to the adjacent Chinese populations from Sichuan (ssp. *khamensis*) while one lineage from Kashmir, western Himalayas (nominate ssp. *hodgsoni*) is sister to the latter clade (Fig. 1). These three taxa, however, form a well-supported Sino-Himalayan cluster. The interior Himalayan split within *C. hodgsoni* was accordingly dated 1.9 to 2.5 mya. For E-W differentiation of Himalayan *Bradypterus thoracicus* see ALSTRÖM et al. (2008).

The phylogeographic pattern and differences in genetic diversity of Sino-Himalayan vicariants are exemplarily shown in the cyt-*b* haplotype network for *Phylloscopus reguloides* (Fig. 4). Three distinct haplotype clusters correspond to traditional subspecies and are strongly separated by 10 and 11 substitutions, respectively. The Himalayan cluster (subspecies *reguloides*) shows a starlike structure with all haplotypes derived from the most common haplotype in the centre. Compared to subspecies *ticehursti* from Yunnan, Thailand and Vietnam haplotype and nucleotide diversities of the Himalayan population are low, and significantly negative Tajima´s D indicates a recent population expansion of *P. r. reguloides* within the Himalayan chain.

Age estimates for lineage splits between **central Asian populations** and N Palearctic sister clades were similarly dated to recent Pleistocene events: *Regulus regulus tristis* vs. *R. r. japonensis* , *Periparus ater rufipectus* vs. *P. a. ater* and *Certhia familiaris tianshanica* vs. *C. f. familiaris*. Haplotypes may even be identical between populations of these two areas. Older age estimates were obtained for splits between sister clades from C Asia and the Himalayas, e.g. *Periparus rufonuchalis* vs. *P. rubidiventris* and *P. griseolus* vs. *P. affinis*. These splits were dated to the Miocene/Pliocene boundary.

Splits between **Sino-Himalayan** sister clades were dated to the Pliocene-Pleistocene boundary in treecreepers, coal tits and several lineages of the *Phylloscopus* tree; however, in the latter group older age estimates were found for some Sino-Himayalan splits, too (Figs 2, 3, Table 1). The phylogeographic pattern of the Goldcrests differs in that there is a complete lack of genetic differentiation between Himalayan (ssp. *himalayensis*) and continental Chinese populations (samples from Gansu, ssp. *sikkimensis*). The only SE Asian relative, the endemic Taiwan Firecrest (*R. goodfellowi*), was shown to be the sister species to a monophyletic clade of all goldcrest subspecies, and the split between the Taiwan lineage and ancestors of *R. regulus* was dated to 3.0 - 3.1 mya (Figs 1, 3). A widespread phylogenetic pattern in *Phylloscopus* and *Seicercus* warblers is a sister group relationship of a Sino-Himalayan clade and a sister clade from (**south-) eastern China** and/or adjacent continental SE Asia (Thailand, Vietnam, etc; Fig. 2: yellow bifurcations). In treecreepers this pattern is represented by the split between the Sino-Himalayan *C. nipalensis*/ *C. tianquanensis* clade and the SE Asian *C. discolor*/ *C. manipurensis* clade. According to our age estimates these lineages diverged during the early to mid Pliocene in *Phylloscopus* and during the late Miocene in *Certhia*.

Most lineage splits between **N Palearctic** taxa and Sino-Himalayan relatives were dated to the late Pliocene era in all genera. Most recent time estimates (Pliocene-Pleistocene boundary) were obtained for the phylogeographic pattern of one widely distributed NE Palearctic taxon and its sister taxon with a restricted W Chinese breeding range: *Phylloscopus fuscatus fuscatus* vs. *P. f. robustus*, *P. proregulus* vs. *P. kansuensis*, *Certhia familiaris familiaris* vs. *C. f. bianchii* (Figs 2, 3).

Oldest intrageneric splits in *Certhia* and *Periparus* correspond to a basal bifurcation that involves two separate Himalayan lineages: *Certhia nipalensis*/ *C. hodgsoni*, *P. a. melanolophus*/ *P. r. rubidiventris* (West-Himalaya), *P. a. aemodius*/ *P. r. beavani* (East Himalaya). In both genera the basal split between these two Sino-Himalayan lineages was dated to the Miocene period at 7 to 10 my BP (Fig. 3).

3.2 Discussion

Reliability of molecular clocks

The increasing number of molecular clock calibrations in avian systematics and the development of more sophisticated tools of data analysis resumed the live controversy on the reliability of molecular clocks in general and the 2% rule of thumb in particular (reviews in Arbogast et al. 2002, Garcia-Moreno 2004, Lovette 2004, Ho & Larson 2006). Though a large number of molecular clock calibrations in avian molecular systematics were performed for cytochrome *b* alone, serious doubts were cast on age estimates inferred from single gene trees. It is generally questionable whether single gene trees do reflect the true phylogenetic relationships, because conflicting topologies can result from different sequence data sets (especially mitochondrial *vs.* nuclear DNA; for conflicting phylogenetic patterns in *Phylloscopus*, see Bensch et al 2006). As a matter of fact, the choice of calibration points, age constraints and substitution model settings also have a great impact on clock calibrations (Päckert et al. 2006). For instance, several of the named uncertainties occurred in the *Periparus* data set, as follows. i) The basal polytomy of the *Periparus ater* clade in the ML input tree yielded the same age estimates for all lineage splits included (Sino-Himalaya *vs.* China *vs.* N Palearctic), whereas topologies resulting from NJ and Bayesian reconstruction differ and lead to different estimates (compare Päckert et al. 2006). ii) The Messinian salinity crisis is a rather poor paleogeographic reference for the avifaunal interchange between continental Europe and N Africa (Griswold & Baker 2002, Godoy et al. 2004) and most nodes referred to the according maximum age constraint were actually dated much younger (Päckert et al. 2006). Since the N African *P. a. atlas* clade was also included in the basal polytomy, we did not use the Messinian calibration point in *Periparus*. iii) Since no further age constraint could reasonably be assigned to an ingroup node, four calibration points were chosen in two closely related outgroup genera (*Cyanistes* and *Poecile*). However, the use of external calibration points might lead to a considerable underestimation of ingroup node ages (Pereira & Baker 2006, Ho & Larson 2006).

Generally, comparison of rate and time estimates inferred from different clock calibrations should be done with particular care. In the strict sense, the calibrations for each of the four genera differ with respect to the number and age constraints of calibration points applied here, and so do the clock calibrations for the *Phylloscopus* tree performed in this study and in the study by Johansson et al. (2007). Compared to the latter study and further age dating performed with *Phylloscopus* sequence data (Price et al. 2000, Irwin et al. 2001), the root of our linearized trees is considerably younger and time estimates based on the older calibration point for the Indo-Malayan land bridge fit the previously evaluated time scenarios best. Nevertheless, even the calibration based on the older calibration point (with a rather broad maximum and minimum age constraint) corroborates the time scenario described below as demonstrated for the *Phylloscopus reguloides* clade.

This has to be considered in a tentative reconstruction of an evolutionary timescale of passerine lineages in the Sino-Himalayan region, as is tentatively done in the following.

Timing of passerine speciation in the Sino-Himalayan region

With respect to Himalayan Old World warblers Johanssen et al. (2007) pointed out that except for two Himalayan sister species pairs, "all of the Himalayan species have their closest relative outside the Himalayas". In fact that phylogeographic pattern is widespread not only in *Phylloscopus* but also in the other passerine genera studied here: Himalayan and W Chinese (or Indo-Burmese) clades are sister taxa and the respective **Sino-Himalayan clade** is opposed to another deeply split sister clade from S China and/ or adjacent Thailand and Vietnam (for a similar topology in Sino-Himalayan coal tits instead of a basal polytomy compare Gill et al. 2004, Martens et al. 2006). Similar branching patterns resulted from phylogenetic studies of Yellow-bellied Bush-warblers, *Cettia acanthizoides* (Alström et al. 2007). According to our molecular clock calibration, most lineage splits between Himalayan and Chinese sister clades were dated to the Pliocene-Pleistocene boundary or even younger (in accordance with estimates by Johansson et al. [2007] for Himalayan endemics and parapatric sister species). Taking into account an onset of first cold cycles even before 2.4 my BP (first glaciation of Northeast Asia was suggested from 2.75 my BP onwards by Tiedemann et al. 1994, Maslin et al. 1996), speciation processes in the Sino-Himalayan region might have been largely triggered by climate and vegetation changes at the end of the Pliocene. However, there are a few exceptional cases of strikingly high genetic differentiation between vicariant populations from the Himalayas and adjacent China at the intraspecific level in *Seicercus poliogenys* (see Olsson et al. 2004, del Hoyo et al. 2006) and *Phylloscopus affinis*. Divergence times between the vicariant taxa date back until the mid Pliocene, and high genetic distance values as well as clade topology of *P. affinis* and *P. griseolus* indicate species status of the taxa involved. When further morphological and bioacoustic character analyses were employed, a description of new taxa within the presently monotypic *Phylloscopus affinis* was presented by Martens et al. (2008).

Genetic differentiation **within the Himalayas** is comparably low in several analysed cases, although Eastern and Western populations of several passerine species are genetically distinct - and mostly correspond to traditional subspecies. With respect to the lack of Pleistocene divergences between Himalayan Old World warbler species Johansson et al. (2007) argued that "habitats along the range may have been largely unsuitable for warblers" during the Pleistocene. Firstly, it should be pointed out that the taxon sampling by Johansson et al. (2007) did not encompass intraspecific differentiation - the taxonomic level at which East-West differentiation was actually found in several species studied here. Second, a complete lack of suitable Pleistocene forest habitats in the Himalaya is certainly not generally accepted wisdom. At present, the cloud forests of the southern slopes of nearly the entire Himalayan chain harbour an overwhelmingly rich fauna of soil-dwelling arthropods, and most of these species are dependent on permanent humidity which can only be provided by forest cover. Moreover, these arthropod genera possess only weak dispersal abilities and are represented by large numbers of species with often small to minute areas. Apparently these species developed more or

less at the places in which they still live today, with minor altitudinal changes. A complete retreat of forest cover along the Himalayan chain at any time would have been disastrous for this sophisticated fauna, as is the case for man-made forest reduction at present. A few examples are mentioned here: Diplopoda (ENGHOFF 1987), Opiliones (MARTENS 1978), sparassid spiders (JÄGER 2001), carabid beetles (SCHMIDT 2007) and many others do exist. Passerine bird W-E diversification needs other explanations. One should not become set on the few examples analyzed so far and which are striking. MARTENS & ECK (1995: 51) identified suture zones of eastern and western subspecies in the Nepal Himalayas and fixed four: Karnali catchment transition zone, Dhaulagiri transition zone, Kathmandu transition zone, Arun catchment transition zone. More than 50 species were analyzed, demonstrating that the Himalayan system harbours populations which differentiated in that area or immigrated at various periods. It may well be that many of the subspecific-level taxa do not represent molecular clades/lineages of their own but doubtlessly the Himalayan system is an area of constant and heavy though recent diversification.

Though reconstructions of potential SE Asian forest refuges during the last glacial maximum are contradictory and do not lead to a consistent scenario (NAZARENKO 1988, RAY & ADAMS 2001), intraspecific East-West differentiation of Himalayan passerines found here suggests a number of isolated glacial refuges in that region. Moreover, as exemplarily shown for Himalayan Blyth's Warbler population (*P. r. reguloides*) there is evidence from genetic variation and phylogeographic pattern in other warbler species that Himalayan Pleistocene refuges must have harboured quite small populations which unlike their SE Asian relatives underwent a decrease of genetic variation through repeated bottlenecks and later a rapid postglacial range expansion (PÄCKERT et al. 2004, in press).

According to our age estimates, Eurasian Treecreeper, Goldcrest and Coal Tit populations from the **Central Asian mountain system** are of especially young Pleistocene origin, too. The closest relatives of all three taxa occupy large North Palearctic distributional areas, and Pleistocene dispersal to C Asia presumably originated from Siberian stem populations that moved southward with the onset of continental glaciation. In contrast, invasion of C Asian Rufous-naped Tits (*P. rufonuchalis*) and Sulphur-bellied Warblers (*P. griseolus*) to the Tian Shan and Altai mountains was dated much earlier to the Miocene-Pliocene boundary. Unlike the N Palearctic founders, closest relatives of the latter two taxa are Sino-Himalyan species, and founder populations of *P. rufonuchalis* and *P. griseolus* presumably dispersed westward from a Himalayan ancestral population. Similarly, youngest lineage splits between **N Palearctic** and W Chinese sister taxa were dated back to the Pleistocene, suggesting a southward dispersal of precursors of present Chinese *Phylloscopus fuscatus robustus* and *P. kansuensis*. Divergence times between N Palearctic and S Chinese and/or Sino-Himalayan taxa (or subspecies groups as in *R. regulus*) were dated much older to the mid to late Pliocene era (oldest estimate for *Certhia hodgsoni* vs. *C. familiaris*).

Unlike climate-induced Pleistocene radiations that led to a vicariant distribution pattern corresponding to only slight intraspecific differentiation as described above, the oldest intrageneric lineage splits dating back to the Miocene period

led to a pattern of present-day sympatry (and syntopy) of three *Periparus* species (*P. rufonuchalis*, *P. rubidiventris*, *P. ater*), four *Certhia* species (*C. hodgsoni*, *C. himalayana*, *C. nipalensis*, *C. discolor*, three of them even in syntopy; MARTENS & ECK 1995) and up to even eleven warbler species of *Phylloscopus*/ *Seicercus* in the Himalayan regions (DEL HOYO et al. 2006, for warblers) which in W China locally concentrate to up to 15 breeding species (MARTENS, unpublished data). In all three genera local sympatry in the Himalayas goes along with ecological differentiation, altitudinal parapatry in several cases and striking morphological and bioacoustic differences (for Nepal see MARTENS & ECK, 1995; for *Phylloscopus* see PRICE 1991, PRICE et al. 2000, 2003).

Acknowledgements

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Table 1: Age constraints assigned to given nodes of the molecular input trees for rate and age estimates with "r8s"; nodes correspond to major geographic lineage splits; age estimates for paleogeographic events (volcanic island formation, ISL; opening and closing of land bridges LB) given in my.

| node	event	split	max_age	min_age	reference
1	LB	Nearctic/ Palearctic	5.5	4.8	Glandenkov et al. 2002, Marincovich et al. 2002
2	ISL	Continent/ Madeira	4.6	0.7	Geldmacher et al. 2000
3	ISL	Continent/ Canary Isls	4.0	1.9	Ancochea et al. 1990
4	ISL	East/ West Canary Isls	1.77	-	Ancochea et al. 1990
5	ISL	Continent/ Azores	0.88	0.78	Johnson et al. 1998
6	LB	Europe/ N Africa	5.32	-	Krijgsman et al. 1999 ("Messinian crisis")
7	LB	Malaysia / Borneo	5.0	-	Inger 2005
8	LB	Malaysia / Sumatra	3.0	-	Nishimura 2002

Table 2: Genetic differentiation and age estimates for mitochondrial lineage splits between vicariant Goldcrest, Coal Tit and Old World warbler taxa a) within the Himalayan chain b) Himalayan and Chinese sister taxa.

West Himalaya/Kashmir	East Himalaya/ NE India/ Myanmar	gendist. in %	age [CI 95] my
Periparus ater melanolophus	*P. a. aemodius*	2.3	[2.44 – 3.68]
Periparus rubidiventris rubidiventris	*P. r. beavani*	1.6	0.48
Regulus regulus himalayensis	*R. r. sikkimensis*	< 0.2	< 0.1
Certhia hodgsoni hodgsoni	*C. h. mandelli*	2.9 – 3.9	[1.90 – 2.51]
Certhia himalayana	*Certhia himalayana*	0.7	< 0.5
Phylloscopus reguloides reguloides	*Ph. r. assamensis*	2.2	0.73 - 1.13
Seicercus xanthoschistos xanthoschistos	*S. x. tephrodiras*	0.8	0.31 - 0.48
Seicercus whistleri whistleri	*S. w. nemoralis*	1.6	0.62 - 0.97
Himalaya	**China**	**gendist. in %**	**age [CI 95] my**
Periparus melanolophus/ P. a. aemodius	*P. ater eckodedicatus*	2.3 – 2.5	[2.44 – 3.68]
Periparus r. rubidiventris/ beavani	*P. r. whistleri*	2.5	[2.20 – 4.10]
Certhia h. mandelli	*C. h. khamensis*	2.1	[0.97 – 1.45]
Phylloscopus r. reguloides / assamensis	*P. r. ticehursti*	3.0 – 3.2	0.94 - 1.47
Seicercus xanthoschistos	*Phylloscopus ogilviegranti*	5.2 – 5.6	1.37 - 2.13
Seicercus whistleri	*S. valentini*	8.7 – 10.1	2.37 - 3.70
Seicercus poliogenys	*S. poliogenys*	7.5	2.13 - 3.33
Regulus regulus	*Regulus goodfellowi*	6.1	[3.38 - 3.58]
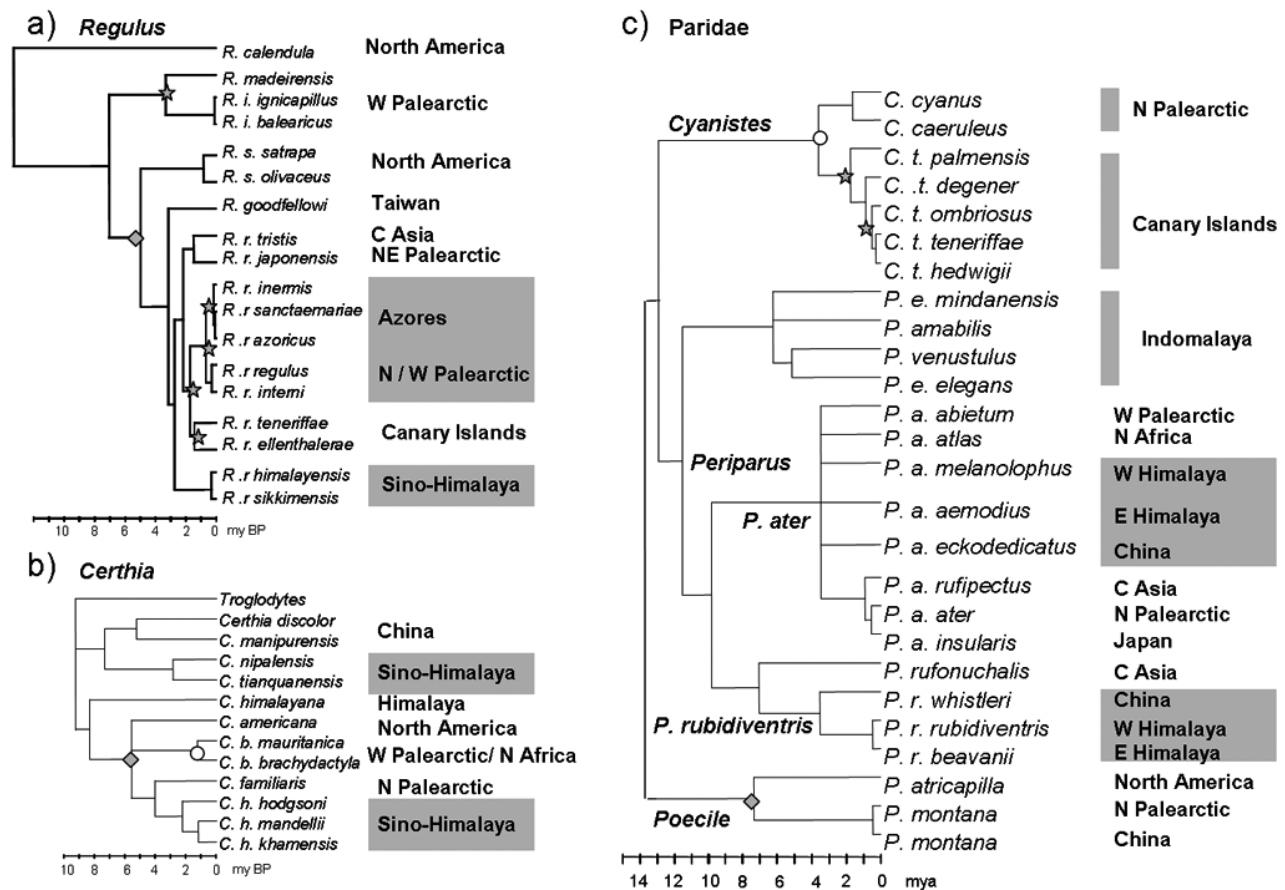
Fig. 1: Maximum likelihood clock trees as calibrated with r8s 1.70 based on partial cytochrome-*b* sequences of *Regulus* (585 bp), *Periparus* (633 bp) and *Certhia* (512 bp); calibration points used as maximum age constraint (islands) or maximum and minimum age constraints (land bridges) indicated by symbols at the associated nodes: star= volcanic ages of Atlantic Islands (Canary Isl., Azores), diamond= opening of Bering Strait, circle= Messinian salinity crisis.
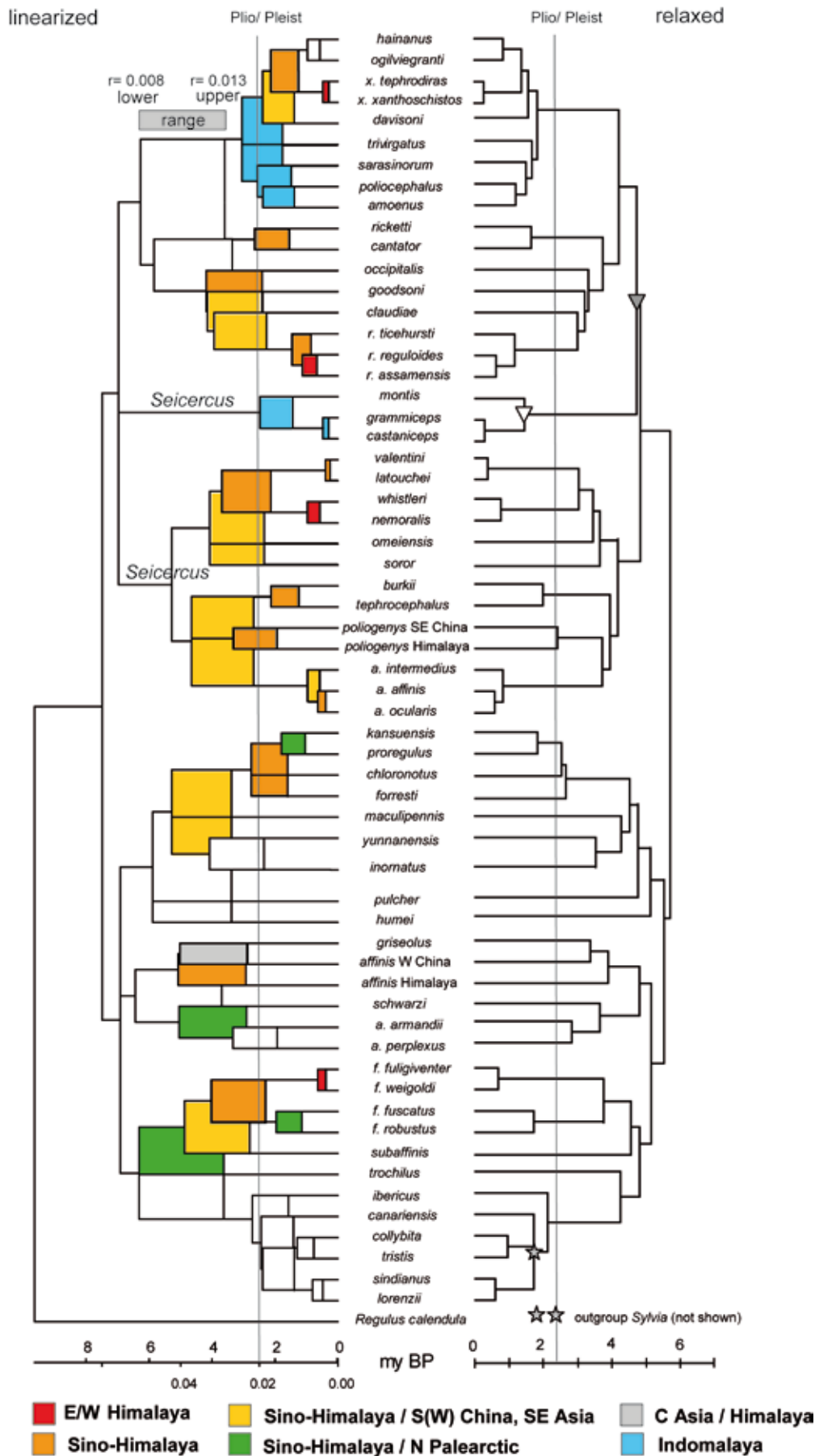
Fig. 2: NJ clock trees for Old World warblers of genera *Phylloscopus* and *Seicercus* based on 856 bp cytochrome *b* (sequence data from HELBIG et al. 1996, OLSSON et al. 2004, 2005, PÄCKERT et al. 2004, 2008, MARTENS et al. 2004, 2008); left: linearized tree calibration based on a substitution rate of 0.008 and 0.0125 substitutions/ site/ lineage/ my, respectively (younger estimate indicated for several nodes); right: clock tree calibrated with r8s 1.70, calibration points used as age constraints indicated by symbols at the associated nodes: star= volcanic ages of Atlantic Islands (Canary Isl., Azores), triangle (grey)= opening of land bridge between Borneo and Malaysian Peninsula, triangle (open)= opening of land bridge between Malaysian Peninsula and Sumatra.
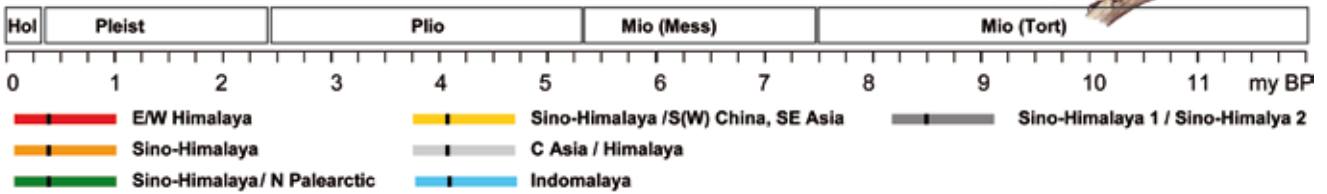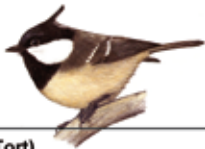
Fig. 3: Age estimates for mitochondrial lineage splits between allopatric populations from the Sino-Himalaya and adjacent regions, 95% confidence intervals and medians as calculated with r8s 1.70; CIs for *Phylloscopus* resulted from a calibration using the older age constraint of the continental Malaysian/Indomalayan split; open bars: no cross-over point found for the upper limit; drawings: *Seicercus omeiensis*, *Regulus regulus* (head pattern), *Certhia himalayana*, *Periparus ater aemodius*, all by K. Rehbinder.
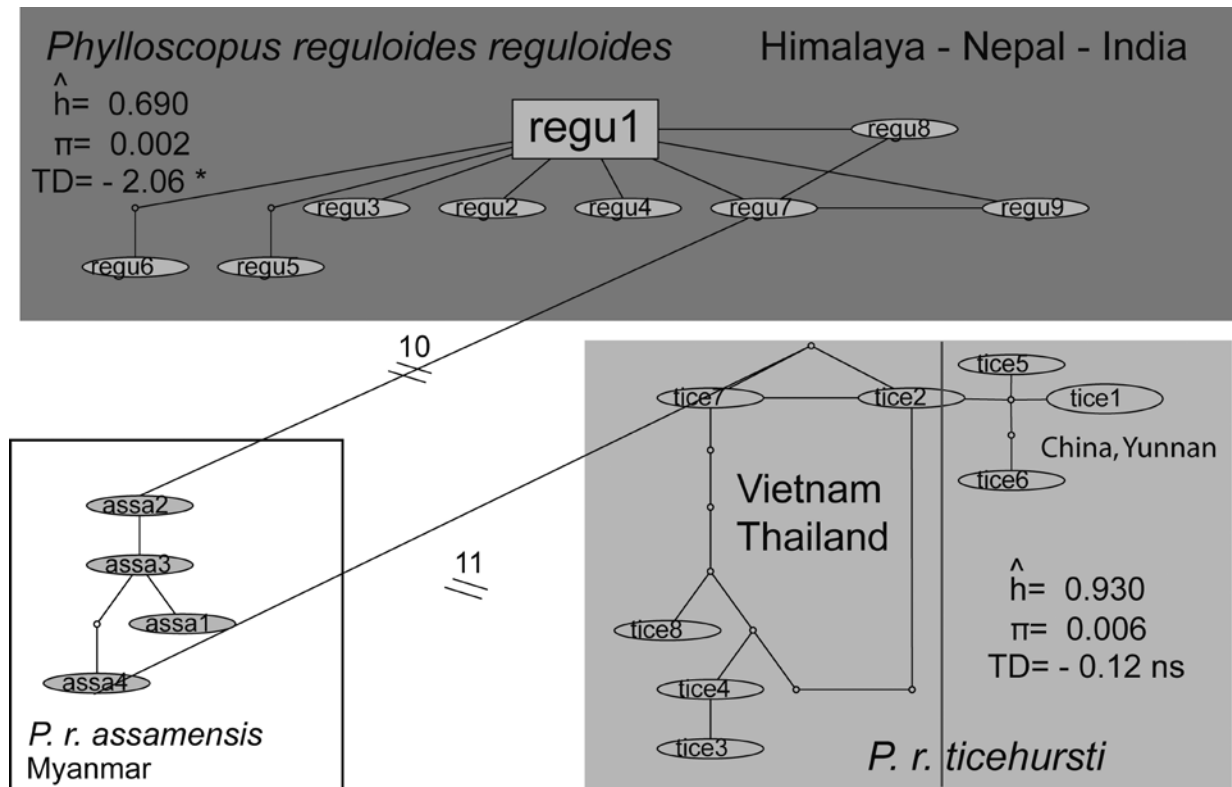

Fig. 4: Haplotype network (cytochrome *b*) for species taxa of the Blyth´s Leaf Warbler complex (*Phylloscopus reguloides* s.l.; sequence data from OLSSON et al. 2005, PÄCKERT et al. in press); haplotype and nucleotide diversities (ĥ and π) and Tajima´s D values (TD) are given for genetic clusters of *P. r. reguloides* and *P. r. ticehursti*.