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Non-monophyletic taxa and cryptic species—Evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*, Aves)

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Abstract

The avian taxa *Cryptigata* and *Acanthopneuste* have been treated either as subgenera within *Phylloscopus* (leaf-warblers), or as a distinct genus and an informal group, respectively. The circumscriptions of these taxa have varied between authors. We estimated the phylogeny, based on the mitochondrial cytochrome *b* and 12S genes and the nuclear myoglobin intron II, of all except two of the species placed in the *Cryptigata* and *Acanthopneuste* groups, as well as two recently described species and representatives of all subgenera and major clades in *Phylloscopus* and *Seicercus* recognized by previous studies. Neither *Cryptigata* nor *Acanthopneuste* is found to be monophyletic. The polytypic species *P. reguloides* and *P. davisoni* show deep divergences between some of their respective subspecies, and the latter species is non-monophyletic. We propose that the former be split into three species and the latter into two species. *Seicercus xanthoschistos* is nested in a clade that includes only *Phylloscopus*, and we recommend that it be placed in *Phylloscopus*. The rate of morphological divergence varies considerably among the taxa in this study. Our results emphasize the importance of dense taxon sampling in intrageneric phylogenetic studies.

Keywords: *Phylloscopus*; *Seicercus*; *Cryptigata*; *Acanthopneuste*; mtDNA; Myoglobin intron II; Phylogeny; Species limits; Morphological evolution; Taxon sampling

1. Introduction

The leaf-warblers, genus *Phylloscopus* (Sylviidae, Aves), comprise more than 130 taxa placed in 45–55 mostly polytypic species (Irwin et al., 2001a; Watson et al., 1986). The different species are renowned for being difficult to distinguish by morphological characters, but are generally more divergent vocally. Much recent taxonomic work has focused on vocalizations, leading to a number of taxonomic re-evaluations and the discovery of new species (review in Irwin et al., 2001a). Several studies based on molecular phylogenies have addressed evolutionary aspects such as speciation, morphological adaptations to different ecological niches, visual signals, and biogeography (Marchetti, 1993; Marchetti et al., 1995; Price and Pavelka, 1996; Price et al., 1997; Richman, 1996; Richman and Price, 1992), as well as taxonomic questions at different levels (Helbig et al., 1993, 1995, 1996; Irwin et al., 2001a). Most phylogenetic studies have used one representative of polytypic species as terminal units. Exceptions are the *P. collybita* (Hansson et al., 2000; Helbig et al., 1993, 1996) and *P. trochiloides* (Irwin et al., 2001b) complexes that have been investigated at subspecific or population levels.

The genus *Phylloscopus* has been difficult to define unambiguously, and species have been moved between

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Phylloscopus, *Cryptigata*, and *Seicercus*, as well as other genera, depending on which morphological characters have been emphasized. Several subgenera have been introduced to accommodate the variation. Ticehurst (1938) treated *Cryptigata* as a separate genus and *Acanthopneuste* as an informal group within *Phylloscopus*, while Watson et al. (1986) considered both *Cryptigata* and *Acanthopneuste* to be subgenera in *Phylloscopus*. However, these two authors differed strongly with regard to which species should be included in the respective group (Fig. 1).

Phylloscopus cantator, *P. coronatus*, *P. davisoni*, *P. occipitalis*, *P. reguloides*, and *P. ricketti* in *Cryptigata* sensu Watson et al. (1986), and two recently described species, *P. emeiensis* (Alström and Olsson, 1995a) and *P. hainanus* (Olsson et al., 1993), share a number of morphological traits: medium to large size; uniformly greenish upperparts; white underparts with a variable amount

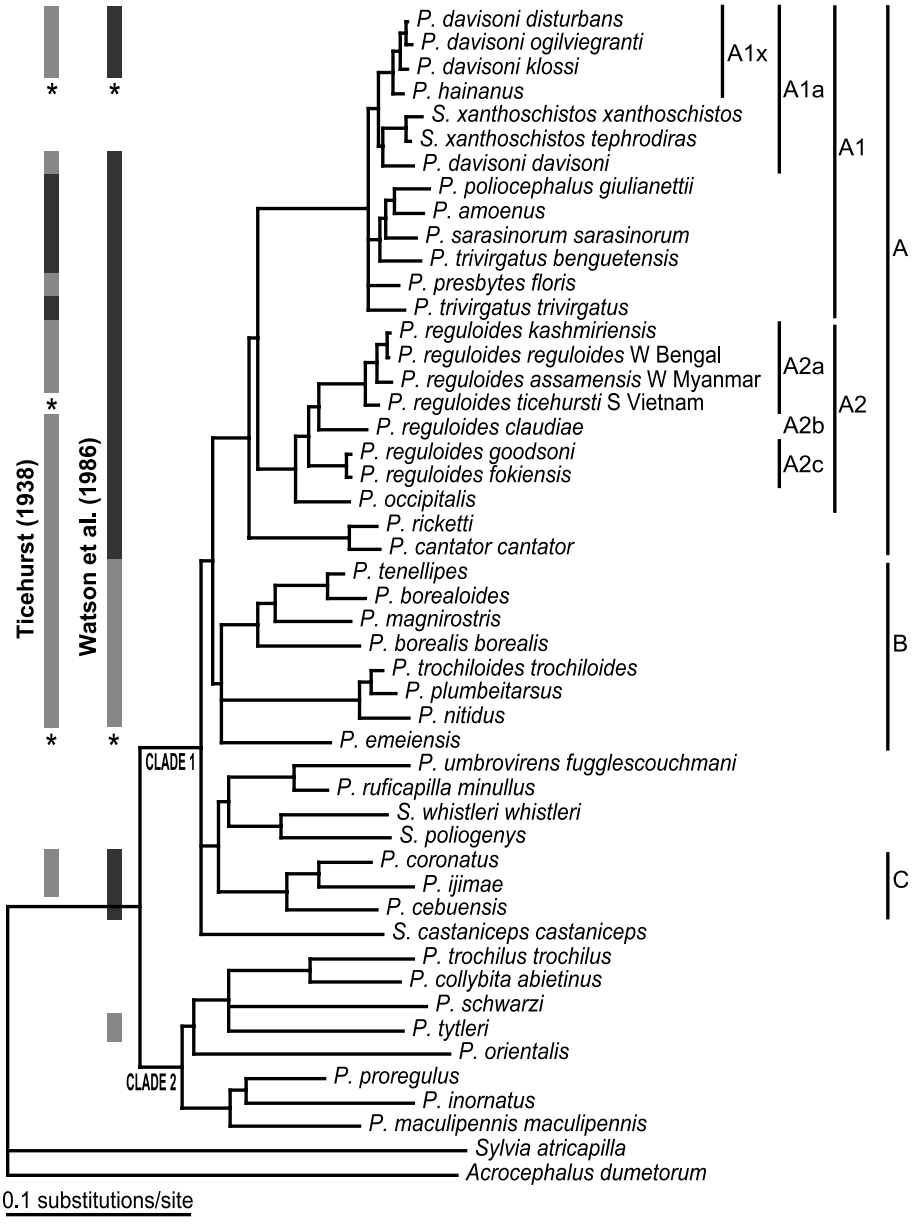

Fig. 1. Tree of *Cryptigata* and *Acanthopneuste* sensu Ticehurst (1938) and Watson et al. (1986), and representatives of all subgenera in *Phylloscopus* recognized by Watson et al., and all main clades in *Phylloscopus* and *Seicercus*, as revealed by previous studies. The tree was estimated by Bayesian analysis of combined cytochrome *b*, 12S, and myoglobin intron II sequences (2172 bp) divided into two partitions analysed under different models (GTR + Γ + I for the mitochondrial genes, GTR + Γ for myoglobin intron II). The bars on the left show *Cryptigata* (black) and *Acanthopneuste* (grey), as circumscribed by Ticehurst (1938) and Watson et al. (1986); asterisks mark the taxa that were not described at the time. The bars on the right refer to clades discussed in the text. All taxa, but only one sample per taxon, were included in this analysis. The names of the taxa in clade A are those of previous authors (e.g., Watson et al., 1986). Nodal support shown in Fig. 2.

of yellow admixed, or entirely yellow underparts; dark crown with a pale median stripe; uniformly coloured wings with one or two pale bars; outer tail-feathers with a variable amount of white; and lower mandible lacking a dark tip; *P. coronatus*, *P. davisoni*, *P. occipitalis*, *P. reguloides*, and *P. emeiensis* are so similar that they are difficult to distinguish between even in the hand (Baker, 1997; Rasmussen and Anderton, 2005; Ticehurst, 1938; Williamson, 1967). These eight species are distributed mainly in the Himalayas, the eastern half of China, Japan, and the northern part of South-east Asia, with up to four species breeding sympatrically. Some of the taxa allocated to the polytypic species *P. poliocephalus*, *P. sarasinorum*, and *P. trivirgatus*, which are also placed in *Cryptigata* by Ticehurst (1938) and Watson et al. (1986), show most of the above character states, notably the pale median crown-stripe (Beehler et al., 1986; Coates and Bishop, 1997; Kennedy et al., 2000; MacKinnon and Phillipps, 1993). These occur allopatrically in peninsular Malaysia, the Philippines, Indonesia, New Guinea, Bismarck Archipelago, and Solomon Islands. The other species in *Cryptigata*, which also occur on Asian islands, are more divergent morphologically.

Only 14 *Acanthopneuste* and *Cryptigata* taxa sensu Ticehurst (1938) and Watson et al. (1986) have been included in previous phylogenetic analyses (Olsson et al., 2004; Price et al., 1997; Richman, 1996; Richman and Price, 1992). We here use two mitochondrial genes (cytochrome *b* and 12S) and a single-copy nuclear intron (myoglobin intron II) to test the validity of *Acanthopneuste* and *Cryptigata* and their relationships to other leaf-warblers and to the genus *Seicercus*. We also discuss species limits in the polytypic species, the evolution of morphological characters, and the importance of dense taxon sampling in intrageneric phylogenetic studies.

2. Materials and methods

2.1. Study group

We obtained tissue, blood, and/or feathers from live individuals of all currently recognized species of *Acanthopneuste* and *Cryptigata*, except *P. makirensis* and *P. olivaceus*, and from the recently described *P. emeiensis* and *P. hainanus* and representatives of all subgenera recognized by Watson et al. (1986) and all major clades in *Phylloscopus* and *Seicercus* as shown in Olsson et al. (2004), Price et al. (1997), Richman (1996), and Richman and Price (1992) (Appendix A). *Acrocephalus dumetorum* and *Sylvia atricapilla* were chosen as outgroups; *Acrocephalus* is believed to be one of the closest relatives of *Phylloscopus*, while *Sylvia* is further away (Cibois, 2003; Sibley and Ahlquist, 1990). The cytochrome *b* sequences of *S. atricapilla*, *A. dumetorum*, and a few *Phylloscopus* were obtained from GenBank (Appendix A), but most were sequenced originally for this study.

2.2. DNA extraction and sequencing

DNA was extracted from blood either by a slightly modified standard phenol–chloroform extraction or by using QIAgen Blood Kit according to the manufacturer's recommendations. Feathers were extracted either with QIAamp Mini Kit or QIAamp DNEasy Kit (Qiagen), following the manufacturer's recommendations, with the exception that 30 µl of 0.1% DTT was added to the first incubation step in order to dissolve the feathers and increase the DNA yield. For amplification of the cytochrome *b* gene we used the primers L-14995 and H-16065 (Helbig et al., 1995) and the PCR cycling parameters 2.5 min at 95 °C; 60 cycles of 30 s at 95 °C, 30 s at 45 °C, and 2 min at 72 °C; terminated by 7 min at 72 °C. The primers L-1091 and H-1478 were used for the 12S gene (Kocher et al., 1989); the PCR cycling parameters were the same as for cytochrome *b*, except that the annealing temperature was 50 °C. The myoglobin intron II was amplified in a first step by primers Myo 2 and 3 (Heslewood et al., 1998; Slade et al., 1993) for 5 min at 95 °C; followed by 20 cycles of 40 s at 95 °C, 40 s at 62 °C, and 1 min at 72 °C; terminated by 8 min at 72 °C. This was followed by a second amplification of PCR products from the first step by primers Myo 2 and 3F (Heslewood et al., 1998; Slade et al., 1993), with the same settings, except that the annealing temperature was 59 °C and the number of cycles was 40. Amplified products were purified by QIAquick PCR Purification Kit (Qiagen).

Sequencing was performed on an ALF-Express (Pharmacia), a CEQ 8000 (Beckman Coulter), or ABI 3100 (Amersham Biosciences). Sequencing reactions on the ALF-Express were performed using Cy5-labelled primers and ThermoSequenase sequencing kit (Amersham) with a two-step cycle: 2 min denaturation at 96 °C, followed by 20 cycles of 30 s at 95 °C and 40 s at respective annealing/extension temperature. On the CEQ 8000 and ABI 3100, the sequencing reactions were carried out as a three-step cycle according to the manufacturer's recommendations. The sequencing primers were H-15557 (Richman and Price, 1992), H-15298, L-15320, and L-15722 (Helbig et al., 1995) for cytochrome *b*, and Myo 2, 3F, Myoint.c, and Myoint.nc for the myoglobin intron II (Heslewood et al., 1998; Irestedt et al., 2002; Slade et al., 1993); 12S was sequenced using the PCR primers. The sequences are deposited in GenBank.

2.3. Phylogenetic analyses

Sequences were aligned using MegAlign 4.03 in the DNASTAR package (DNAstar); some manual adjustment was necessary for the 12S and myoglobin sequences. Molecular phylogenies were estimated by

Bayesian inference using MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001, 2003). The choice of model (GTR + Γ_4 + I for cytochrome *b* and GTR + Γ_4 for myoglobin) was determined based on the Akaike Information Criterion (Akaike, 1973) and a hierarchical likelihood ratio test (Posada and Crandall, 1998), both calculated in MrModeltest (Nylander, 2004). Posterior probabilities were calculated for cytochrome *b* and myoglobin separately, and for the concatenated cytochrome *b* and 12S sequences, as well as for the combined mitochondrial and nuclear sequences. The combined mitochondrial and nuclear data were divided into two partitions (one for the mitochondrial and one for the nuclear sequences), using rate multipliers to allow different rates for the different partitions (Nylander et al., 2004; Ronquist and Huelsenbeck, 2003). All analyses of mitochondrial genes were calculated under a general time-reversible (GTR) model (Lanave et al., 1984; Rodríguez et al., 1990; Tavaré, 1986), assuming rate variation across sites according to a discrete gamma distribution with four rate categories (Γ_4; Yang, 1994) and an estimated proportion of invariant sites (I; Gu et al., 1995), while the myoglobin intron II was analysed under a GTR + Γ_4 model. Default priors were used. Four Metropolis-coupled MCMC chains were run for 3–5×10^6 generations and sampled every 100 generations; the temperature was set to 0.05 and 0.1, respectively, in some runs to improve the mixing of the chains, if that was found to be poor at the default temperature 0.2. The first 300,000 generations, before the chain reached apparent stationarity (burn-in), were discarded and the posterior probability estimated for the remaining generations. Every analysis was repeated three to six times, starting from random trees, and the results compared to ascertain that the chains had reached the same target distributions (as suggested by Huelsenbeck et al., 2002). The samples from the stationary phases of the independent runs were pooled to obtain the final results.

Clade support for the unweighted data set was also assessed by parsimony bootstrapping in PAUP* 4.08b (Swofford, 2001), under the following settings: heuristic search strategy, starting trees obtained via random stepwise addition, 10 replicates, followed by TBR branch swapping, MulTrees option not in effect (only one tree saved per replicate), gaps treated as missing data, 1000 replicates; five parsimony-informative indels in the myoglobin intron II and three in the 12S gene were coded as binary characters (present/absent).

To estimate the posterior probability for monophyly of certain taxa, we used PAUP* to calculate the number of trees in which these taxa were monophyletic among the total number of trees sampled from the stationary phases of the pooled independent Bayesian inference runs.

Pairwise sequence divergences for cytochrome *b* and myoglobin intron II were calculated in PAUP*, under the maximum likelihood criterion and the same models as in the Bayesian analyses (i.e., GTR + Γ_4 + I for cytochrome *b* and GTR + Γ_4 for myoglobin); the parameter values were taken from the pooled Bayesian analyses of the respective regions.

3. Results

DNA sequences comprising a contiguous 1038 base pair portion of the cytochrome *b* gene were obtained for 68 individuals from 55 populations of *Phylloscopus* and *Seicercus* and two outgroups (Appendix A). In addition, a 395–396 base pair portion of the 12S gene, and a 710–724 base pair stretch of the myoglobin gene including the complete intron II and flanking regions of exons 2 (13 bp) and 3 (10 bp) were obtained for representatives of 47 and 44 *Phylloscopus* and *Seicercus* taxa, respectively, and the two outgroups. The cytochrome *b* sequence was amplified as one fragment to minimize the risk of amplifying nuclear pseudocopies of the gene (e.g., Arctander, 1995; Quinn, 1997; Quinn and White, 1987; Sorensen and Quinn, 1998). No unexpected start, stop or nonsense codons, that could indicate the presence of a nuclear copy, were observed in the cytochrome *b* sequences.

The phylogenetic analyses of the concatenated sequences of the light strand of the cytochrome *b* gene and the 12S gene fragments contained 1438 characters, of which 395 (27.5%) were parsimony informative; cytochrome *b* comprised 1038 characters, 351 (33.8%) of which were parsimony informative. The phylogenetic analyses of the myoglobin intron II data set contained 734 characters, of which 75 (10.2%) were parsimony informative. The combined 12S, cytochrome *b*, and myoglobin data set contained 2172 characters, of which 470 (21.6%) were parsimony informative.

The tree resulting from the analysis of the combined mitochondrial and nuclear sequences is shown in Figs. 1 and 2. Most clades are well supported; only two of the clades with 95% posterior probability have $\leqslant$70% bootstrap support. The mitochondrial tree (Fig. 3) is better resolved than the nuclear tree (Fig. 4), especially terminally. However, there are few topological conflicts between these two trees, and only one of the inconsistencies, namely the position of *P. occipitalis* (see below), has a posterior probability of 80% in both trees.

All of the taxa allocated to *Cryptigata* and *Acanthopneuste* by Ticehurst (1938) and Watson et al. (1986) are part of one of the two main clades that has also been recovered in previous phylogenetic analyses of the genus *Phylloscopus* (Olsson et al., 2004; Price et al., 1997; Richman, 1996; Richman and Price, 1992), i.e., clade 1 in Fig. 1. However, the classifications of Ticehurst (1938) and Watson et al. (1986) are not corroborated, since our analyses do not support monophyly of either *Acanthopneuste* or *Cryptigata* (Figs. 1–4).

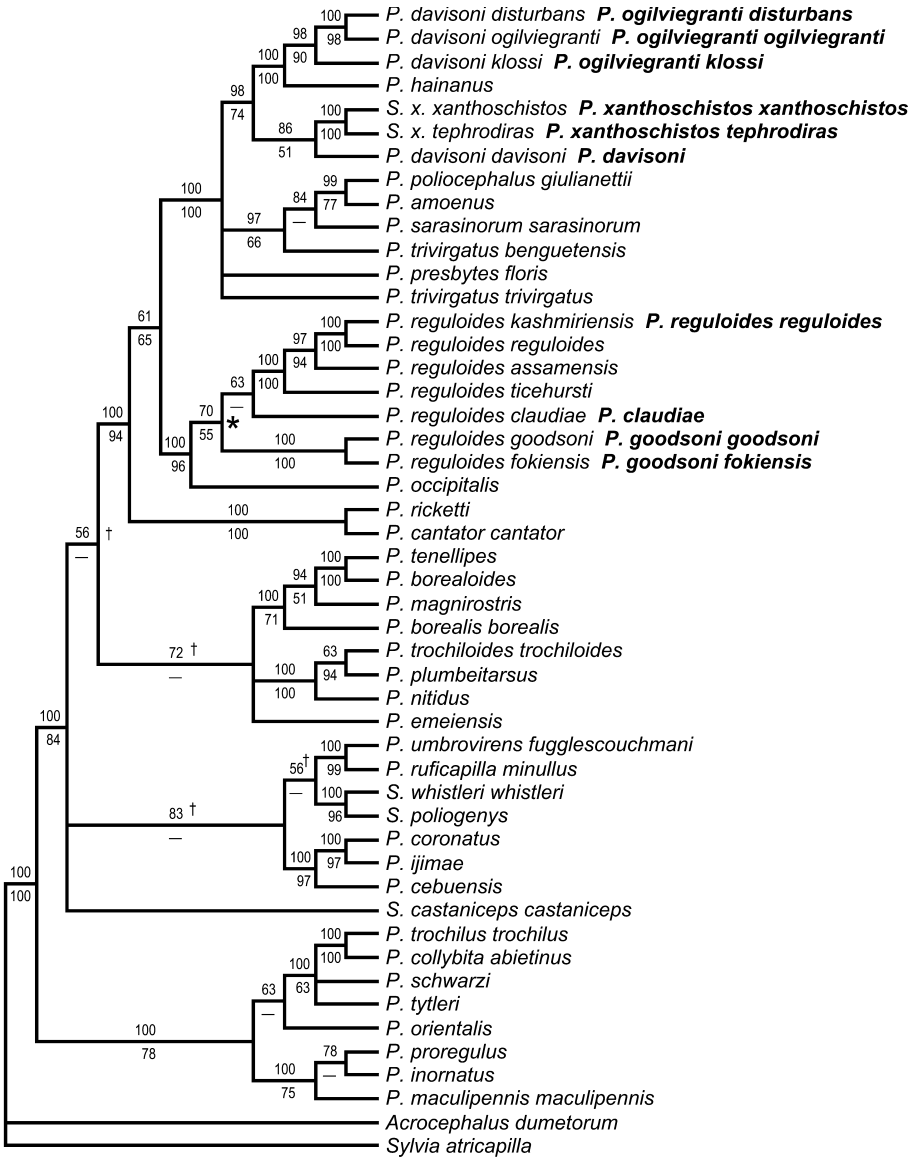

Fig. 2. Tree resulting from the same analysis as in Fig. 1. Posterior probabilities (⩾50%; 162,000 trees) are indicated above the nodes and parsimony bootstrap values (⩾50%; 1000 replicates) below the nodes. New taxonomic arrangements proposed here are shown in bold. The asterisk denotes a node where mitochondrial and nuclear data result in conflicting hypotheses (cf. Figs. 3–5), and the daggers denote poorly supported clades that affect the positions of *P. emeiensis* and *P. coronatus*/*P. ijimae*/*P.cebuensis*.

The morphologically similar *P. cantator*, *P. coronatus*, *P. davisoni*, *P. emeiensis*, *P. hainanus*, *P. occipitalis*, *P. reguloides*, and *P. ricketti* do not form a monophyletic group (Figs. 1–4); all except *P. coronatus* and *P. emeiensis* are part of a clade (A in Fig. 1) that also includes the six tropical islands taxa and *S. xanthoschistos*. Within this clade, the tropical islands taxa and *S. xanthoschistos* form a strongly supported clade together with *P. davisoni* and *P. hainanus* (A1 in Fig. 1). *P. cantator* and *P. ricketti* are each other's nearest relatives in all analyses, with high support, although their sister relation to the others in clade A is poorly supported by the data. The precise position of *P. emeiensis* is uncertain, since the support for four internal nodes affecting its placement is poor (marked by daggers in Fig. 2). However, the posterior probability that it is part of clade A is only 0.001 (183 out of the162,000 sampled trees). *P. coronatus* is firmly placed in a clade together with *P. ijimae* and *P. cebuensis* (clade C in Fig. 1), although the position of this clade is not safely established as a result of poor support for four internal nodes (marked by daggers in Fig. 2). However, the inclusion within clade A of *P. coronatus*, either on its own or together with the two other taxa in clade C, receives 0 posterior probability (0 out of the 162,000 sampled trees).

The close relationship between *P. occipitalis* and *P. reguloides* is corroborated in all analyses (Figs. 1–5). The Bayesian analysis of the cytochrome *b*/12S sequences lends high support to a sister relation between *P. occipitalis* and *P. reguloides goodsonil/fokiensis*, while

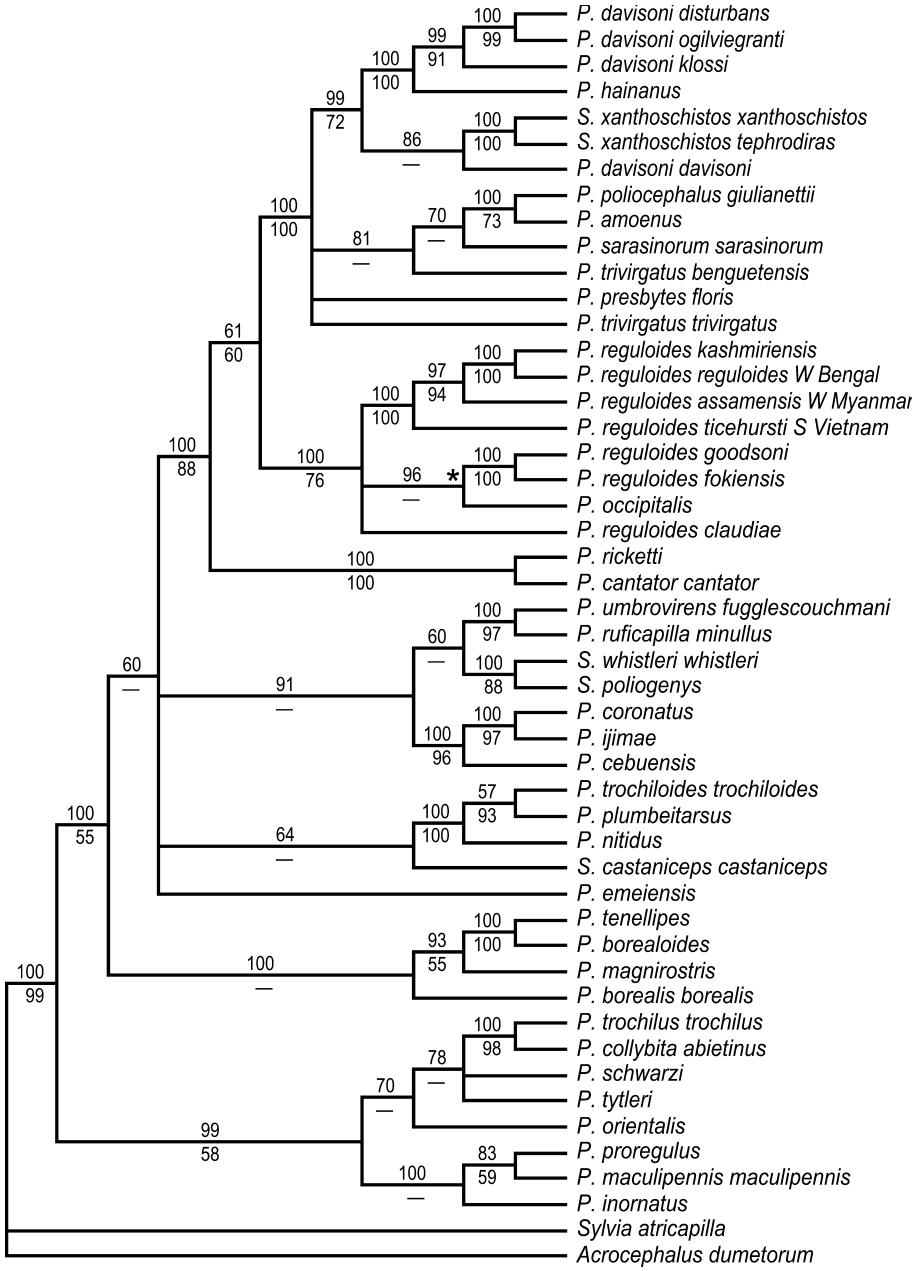

Fig. 3. Relationships of the same taxa as in Figs. 1 and 2, based on concatenated cytochrome *b* and 12S sequences (1438 bp) analysed by Bayesian inference under the GTR + Γ + I model. Posterior probabilities (⩾50%; 141,000 trees) are indicated above the nodes and parsimony bootstrap values (⩾50%; 1000 replicates) below the nodes. The asterisk denotes a node where mitochondrial and nuclear data result in conflicting hypotheses (cf. Figs. 2 and 4).

the parsimony bootstrap support for this relationship is lacking (Fig. 3). The analysis of cytochrome *b* for all of our samples fails to resolve the position of *P. occipitalis* in relation to *P. reguloides* (Fig. 5), while the nuclear data place *P. occipitalis* as sister to all *P. reguloides* taxa, with moderate support (Fig. 4). The same is found in the combined analysis, although the support is lower (Fig. 2). The seven taxa allocated to *P. reguloides* are divided into three main clades, which are highly divergent and well supported, both in the cytochrome *b*/12S and myoglobin trees (Figs. 1–6): (1) a Himalayan to South-east Asian clade comprising *kashmiriensis*, *reguloides*, *assamensis*, and *ticehursti* (clade A2a in Fig. 1); (2) the central Chinese *claudiae* (A2b); and (3) the south-east Chinese *goodsoni* and *fokiensis* (A2c). The cytochrome *b* divergences between these three clades are 6.2–8.8%, which is just below or at the lower end of the differences between sympatric species in our data set (e.g., *P. reguloides kashmiriensis* and *P. occipitalis*), and well within the range of allopatric taxa that are unanimously treated as separate species (Figs. 6 and 7). The Himalayan/South-east Asian clade is further divided into three subclades: (1)

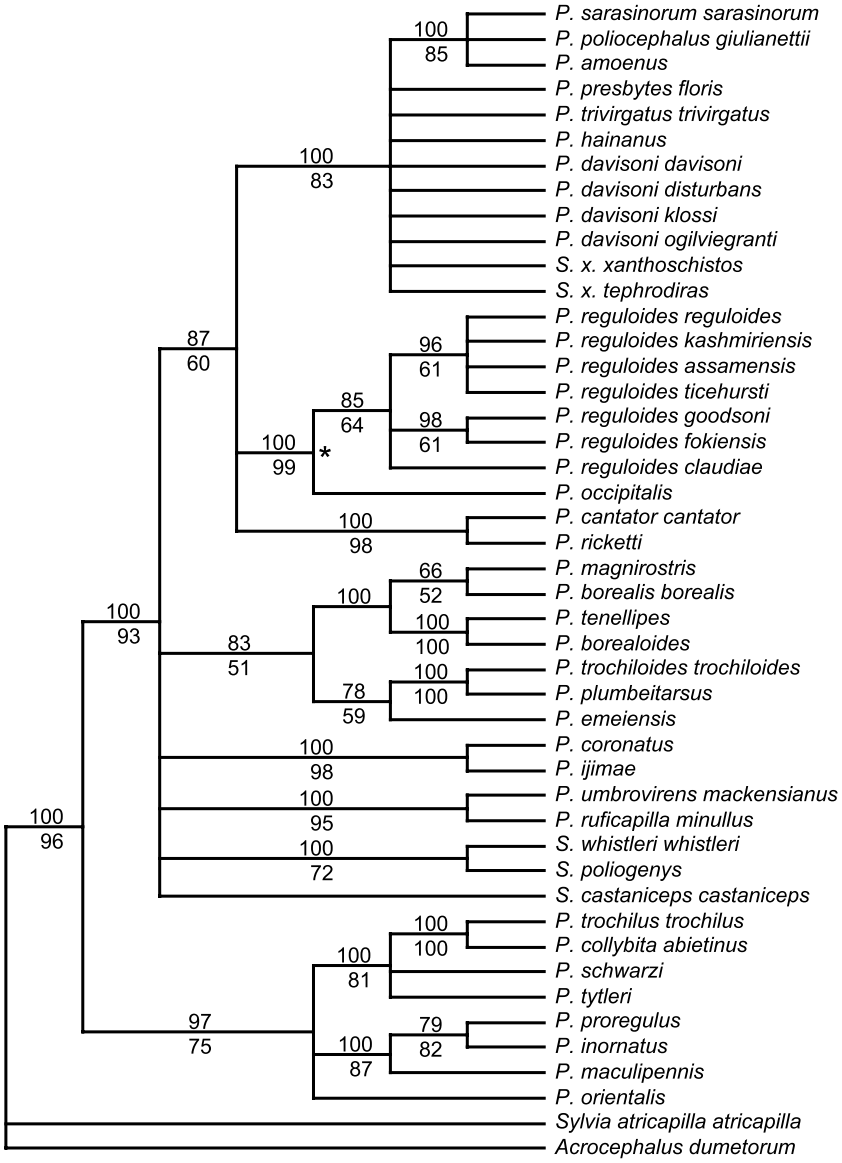

Fig. 4. Relationships of the same taxa as in Figs. 1–3, except *P. trivirgatus benguetensis*, *P. cebuensis*, and *P. nitidus*, based on myoglobin intron II sequences (734 bp) analysed by Bayesian inference under the GTR + Γ model. Posterior probabilities (⩾50%; 101,000 trees) are indicated above the nodes and parsimony bootstrap values (⩾50%; 1000 replicates) below the nodes. The asterisk denotes a node where mitochondrial and nuclear data result in conflicting hypotheses (cf. Figs. 2 and 3).

kashmiriensis and *reguloides* from the Himalayas, (2) *assamensis* (sensu stricto, hereafter s.s.) from western Myanmar, and (3) *assamensis* from north-western Thailand (sensu Alström and Olsson, 1993) and Yunnan (south central China), an unassigned population from north-western Vietnam, and *ticehursti* from southern Vietnam (Figs. 5 and 6). The divergences in cytochrome *b* between these clades (1.8–3.3%) are considerably lower than between the three main clades (Figs. 6 and 7).

Phylloscopus davisoni is non-monophyletic, since three of its subspecies form a strongly supported clade together with *P. hainanus* (Fig. 1, clade A1x; Figs. 2–4). Moreover, the Bayesian analyses of the mitochondrial and combined data sets identify a sister relation between *davisoni* (s.s.) and *S. xanthoschistos* sspp. However, the support for this is not strong, and lacking in the bootstrap analyses; the myoglobin tree is unresolved in this respect. The cytochrome *b* divergences between *davisoni* and *ogilviegrantildisturbanslklossi* (4.0–4.7%) are similar to or greater than among several other allopatric taxa that are unanimously treated as separate species, e.g., between any one of these and *S. xanthoschistos* sspp. (4.0–5.3%) (Fig. 7). The same pattern is apparent in the myoglobin data, although the differences are smaller. The clade comprising *ogilviegranti*, *disturbans*, and *klossi* is strongly supported by the mitochondrial data. The pairwise cytochrome *b* divergences between them (0.3–1.2%) are considerably smaller than between any one of

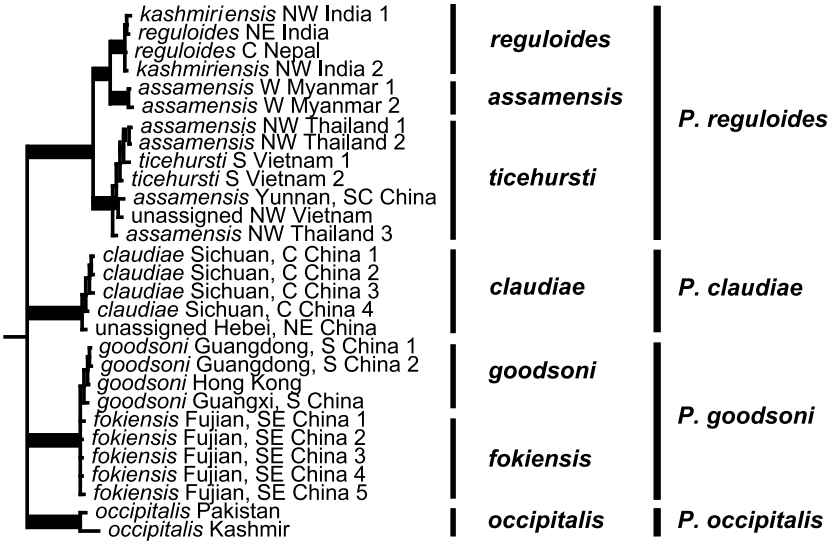

Fig. 5. Relationships of all our samples of *P. reguloides* (sensu lato) and *P. occipitalis*, based on cytochrome *b* sequences (1038 bp) analysed by Bayesian inference under the GTR + Γ + I model (part of a tree including all taxa in Figs. 1, 2). Clades with ⩾95% posterior probabilities (81,000 trees) and parsimony bootstrap (1000 replicates) are indicated by thick branches. New classification proposed here shown by bars (subspecies to the left, species to the right).

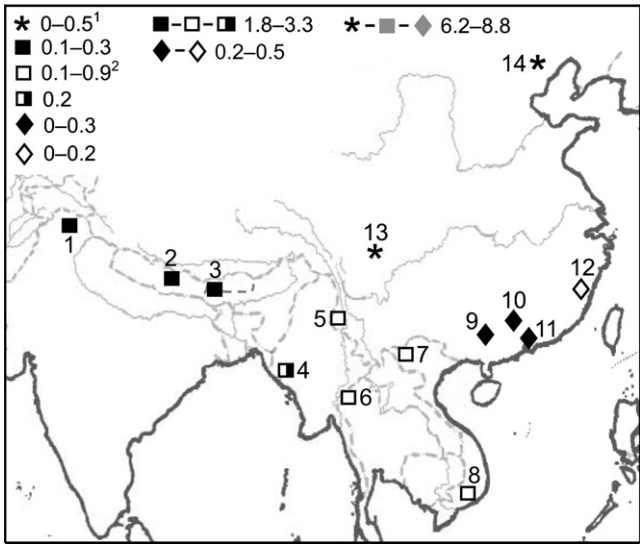

Fig. 6. Origin, pairwise cytochrome *b* divergences, and proposed new classification of our samples of *P. reguloides* sensu lato. Filled square: *reguloides*, (1) Himachal Pradesh, India ($n = 2$); (2) central Nepal ($n = 1$); (3) West Bengal, India ($n = 1$); half-filled square: *assamensis* (4) west Myanmar ($n = 2$); open square: *ticehursti*, (5) Yunnan, China ($n = 1$); (6) north-western Thailand ($n = 3$); (7) north-western Vietnam ($n = 1$); (8) southern Vietnam ($n = 2$); filled diamond: *goodsoni*, (9) Guangxi, China ($n = 1$), (10) Guangdong, China ($n = 2$), (11) Hong Kong ($n = 1$); open diamond: *fokiensis*, (12) Fujian, China ($n = 5$); star: *claudiae*, (13) Sichuan, China ($n = 4$), (14) Hebei, China ($n = 1$). The values in the upper part of the figure represent pairwise cytochrome *b* divergences in % between different populations: left column: within least-inclusive taxa; middle column: between the taxa in the *P. reguloides* (sensu stricto) and *P. goodsoni* clades; and right column: between the *P. claudiae*, *P. reguloides* (sensu stricto) and *P. goodsoni* clades.[1]The smallest genetic distance between the samples from localities 13 and 14 is 0.2%. [2]The largest distance between individuals at one locality is 0.7% (locality 6).

them and *davisoni* s.s. (Fig. 7). In fact, the difference between the two former (0.3%) is comparable with the variation found at a single locality in some other taxa (Fig. 7). *P. hainanus* differs only slightly from its sister taxa *ogilviegranti*, *disturbans*, and *klossi*: 1.5–1.9% in cytochrome *b* (Fig. 7) and not at all in myoglobin.

Four of the tropical islands taxa in clade A1 (Fig. 1) form a clade. However, this is not unanimously supported, and within this clade only the sister relationship between *P. poliocephalus giulianettii* and *P. amoenus* is well supported in all analyses. The positions of *P. presbytes floris* and *P. trivirgatus trivirgatus* are unresolved within clade A1.

4. Discussion

4.1. Evaluation of Cryptigata and Acanthopneuste, and relationships of S. xanthoschistos

Ticehurst (1938) treated all of the tropical islands leaf-warblers in this study, except *P. presbytes*, as *Cryptigata* (though *cebuensis* was apparently not studied). All of these, including *P. presbytes floris*, but excluding *P. cebuensis*, correspond to a part of clade A1, for which monophyly is not supported. The name *Cryptigata* could be applied to clade A in Fig. 1, which is strongly supported in the present analysis, and includes the type of *Cryptigata* (*giulianettii*). This agrees with Watson et al.'s (1986) circumscription of this group, except that these authors also included *P. coronatus*, *P. ijimae*, and *P. cebuensis* and placed *xanthoschistos* (sensu lato, hereafter s.l.) in *Seicercus*.

Watson et al.'s (1986) circumscription of *Acanthopneuste* pertains to a group of warblers that lack crown patterns, and agrees with clade B, except that *P. tytleri* is excluded from this clade; moreover, *P. emeiensis* is also part of the clade. However, this clade is only weakly or moderately supported in the Bayesian analyses of the nuclear and combined mitochondrial and nuclear data sets, and receives no support in the other analyses. Should future studies lend further support to clade B, the name *Acanthopneuste* could be applied to it.

Olsson et al. (2004) point out that *Seicercus* appears to be nested within *Phylloscopus*, and conclude that *Seicercus* may be synonymized with *Phylloscopus*, alternatively that *Phylloscopus* may be divided into a number of genera. The present study strongly corroborates this, but we await a more complete phylogeny of the genus before proposing any taxonomic changes. However, we support the transfer of *xanthoschistos* (s.l.) from *Seicercus* to *Phylloscopus* recommended by Olsson et al. (2004).

4.2. Phylogeny and species limits in the Phylloscopus reguloides/occipitalis complex

The close relationship between *P. reguloides* and *P. occipitalis* suggested by DNA is corroborated by a non-molecular character: males of all taxa in clade A2 in Fig. 1 flick their wings, one at a time, when excited in the breeding season (Alström and Olsson, 1993; Rasmussen and Anderton, 2005; own observations). This behaviour is not shown by any other taxon dealt with in this study, or by any other warbler known to us. The precise position of *P. occipitalis* within this clade is, however, uncertain, as there is conflict between the different data sets as well as between different analyses.

The breeding ranges of the taxa allocated to *P. reguloides* are insufficiently known, and it is not known for certain whether any of the three main clades are in contact (cf. Fig. 6). However, the pronounced genetic divergences and the concordant genealogies of the unlinked mitochondrial and nuclear loci between the three main clades suggest that the gene flow between them has been restricted for considerable time, and that they may therefore be regarded as separate evolutionary lineages (cf. Baum and Shaw, 1995). We propose that they be treated as separate species: *P. reguloides* (with the subspecies *reguloides*, *assamensis*, and *ticehursti*), *P. claudiae* (monotypic), and *P. goodsoni* (with the subspecies *goodsoni* and *fokiensis*) (Fig. 5, Table 1).

The morphological differences between these three species are very slight (Baker, 1997; Ticehurst, 1938; Williamson, 1967), and their songs and calls are presently considered indistinguishable. Although different species of *Phylloscopus* usually differ markedly in voice (e.g., Irwin et al., 2001a; Martens, 1980), this is not always the case. For example, *P. occipitalis* and *P. reguloides reguloides/kashmiriensis*, which are undoubtedly separate species under any species concept, as they breed sympatrically with no signs of interbreeding (e.g., they differ by 8.2–8.9% in cytochrome *b*), have exceptionally similar vocalizations (Martens, 1980; Rasmussen and Anderton, 2005).

The cytochrome *b* divergence between the taxa *reguloides* (s.s.), *assamensis* (s.s.) and *ticehursti* is substantial,

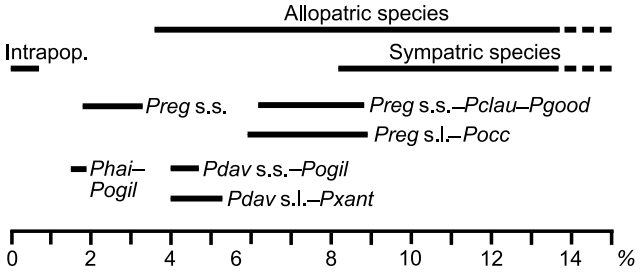

Fig. 7. Pairwise distances in the cytochrome *b* gene among the taxa in the present study, estimated by maximum likelihood under the GTR + Γ + I model. Taxonomy of the allopatric and sympatric species according to Dickinson (2003) and Watson et al. (1986), as shown in Fig. 1; upper limits of these divergences not marked (48.5 and 38.8%, respectively). Intrapop. refers to samples of the same taxon from a single locality. The bar labelled *Preg* s.s. refers to the divergences between *reguloides*, *assamensis*, and *ticehursti* according to the classification proposed here. *Pclau* is *P. claudiae*; *Pgood* is *P. goodsoni*; *Pogil* is *P. ogilviegranti*; *Pdav* s.s. is *P. davisoni* sensu stricto; and *Pxant* is *Pxanthoschistos*; all according to the classification proposed here. *Pdav* s.l. is *P. davisoni* sensu lato, i.e., including *ogilviegranti*, *disturbans*, and *klossi*; *Pocc* is *P. occipitalis*; and *Phai* is *P. hainanus*.

Table 1
Summary of the taxonomic changes proposed here

Watson et al. (1986)	Proposed new taxonomy
P. davisoni	
davisoni (Oates, 1889)	*P. davisoni* monotypic
	P. ogilviegranti
disturbans (La Touche, 1922)	*P. o. disturbans*
klossi (Riley, 1922)	*P. o. klossi*
ogilviegranti (La Touche, 1922)	*P. o. ogilviegranti*
P. reguloides	*P. reguloides*
reguloides (Blyth, 1842)	*P. r. reguloides*
kashmiriensis Ticehurst, 1933	*P. r. reguloides*
assamensis Hartert, 1921	*P. r. assamensis*
ticehursti Delacour & Greenway, 1939	*P. r. ticehursti*
claudiae (La Touche, 1922)	*P. claudiae* monotypic
	P. goodsoni
goodsoni Hartert, 1910	*P. g. goodsoni*
fokiensis Hartert, 1917	*P. g. fokiensis*
Seicercus xanthoschistos	*Phylloscopus xanthoschistos*
xanthoschistos (Gray and Gray, 1846)	*P. x. xanthoschistos*
tephrodiras Sick, 1939	*P. x. tephrodiras*

We suggest that *P. davisoni* be treated as two species, *P. davisoni* and *P. ogilviegranti*; *P. reguloides* as three species, *P. reguloides*, *P. claudiae*, and *P. goodsoni*; and that *Seicercus xanthoschistos* be placed in the genus *Phylloscopus*. The taxa *jerdoni* (Brooks, 1871) and *flavogularis* (Godwin-Austen, 1877) are treated as subspecies of *Seicercus xanthoschistos* by Watson et al. (1986), but were not included in the present analysis.

and suggests ongoing differentiation as a result of barriers to gene flow (it is uncertain whether these taxa are presently in contact; cf. Fig. 6). The separation is, however, apparently too recent to be evident in the myoglobin data. We prefer to treat them as conspecific, though admitting that their ranking is subjective, as is generally the case with allopatric taxa at the early stages of divergence.

Our samples of *reguloides* and *kashmiriensis* do not segregate into separate clades (Fig. 5), and the divergence between them (0.1–0.3%) is comparable to that within populations (Figs. 6 and 7). In combination with the fact that these two taxa are doubtfully separable from each other by morphological characters, we consider them to represent the same lineage, referred to as *reguloides* (by priority: International Commission on Zoological Nomenclature, 1999; Table 1). The population from western Myanmar has previously been allocated to *assamensis* (Ticehurst, 1938; Watson et al., 1986), an arrangement that we provisionally follow, in the absence of samples of topotypical *assamensis* from the nearby Khasi (Khasia) Hills, Meghalaya, India. Compared to *reguloides* s.s., it differs chiefly in showing more white on the outer tail-feathers (Rasmussen and Anderton, 2005; Ticehurst, 1938; Williamson, 1967). Our samples from southern Vietnam (topotypical *ticehursti*), northern Vietnam (no previous allocation to subspecies), western Yunnan (*assamensis* according to Watson et al., 1986), and north-western Thailand (*assamensis* according to Alström and Olsson, 1993) do not fall into geographically separate clades. Moreover, pairwise cytochrome *b* divergences among different localities do not exceed that from a single locality (north-western Thailand, up to 0.7%) (Figs. 6 and 7). The populations from Yunnan, north-western Thailand, and north-western Vietnam appear to be indistinguishable morphologically from topotypical *assamensis*, while topotypical *ticehursti* are more yellow below (Alström and Olsson, 1993; Baker, 1997; Ticehurst, 1938; Williamson, 1967). We tentatively unite all these populations under the name *ticehursti* (by priority: International Commission on Zoological Nomenclature, 1999; Table 1).

Our sample from Hebei, north-eastern China represents a recently discovered breeding population far north of the previously known range of *P. reguloides* (s.l.) (Cheng, 1987). In the present analysis, this sample is firmly placed in the *claudiae* clade, and differs in cytochrome *b* from the others in that clade by only 0.2–0.5%, which is within the range of intrapopulational variation (Figs. 6 and 7). We have not found any diagnostic morphological characters separating it from *claudiae* from Sichuan, and therefore consider it to belong to *claudiae*.

The taxon *goodsoni* was formerly treated as a subspecies of *P. ricketti*, but Alström and Olsson (1995a) proposed that it be recognized as a subspecies of *P. reguloides* (s.l.). That is supported by the present analysis, in which *goodsoni* and *fokiensis* are in the same strongly supported clade, well separated from *P. ricketti*. The breeding range of *goodsoni* was previously unknown, but was suspected to be in south China (Alström et al., 1995b). Three of our samples are from two recently discovered breeding populations in Guangdong and Guangxi provinces, south China, which match *goodsoni* in plumage (Paul J. Leader and Geoff J. Carey, in litt.). Our fourth sample that fits *goodsoni* in plumage is from Hong Kong in the non-breeding season (Paul J. Leader and Geoff J. Carey, in litt.). These four samples differ by 0–0.3% in cytochrome *b*. Our five topotypical *fokiensis* samples differ from each other by 0–0.2%, and from our *goodsoni* samples by 0.2–0.5%. The divergences between *fokiensis* and *goodsoni* are within the range of individual variation (Fig. 7). However, the plumage differences between *goodsoni* and *fokiensis* noted by Alström and Olsson (1995a) are so pronounced that we tentatively recognize both taxa.

Williamson (1967) suggested that *assamensis* is a hybrid swarm of *davisoni* and *reguloides*, as it is intermediate morphologically (notably in tail pattern) and geographically between these. That hypothesis is not corroborated by our data, as the cytochrome *b* haplotypes of all of our six samples of *assamensis* sensu Williamson (i.e., from Yunnan, Thailand, and Myanmar) fall in the *P. reguloides* clade. In addition, the myoglobin sequence from Myanmar is identical to the two Himalayan *reguloides*. In spite of this, we cannot exclude the possibility that unidirectional introgression from *davisoni* to *reguloides* of autosomal genes coding for tail pattern may have occurred.

4.3. Phylogeny and species limits in the *P. davisoni* complex

We suggest that *P. davisoni* be split into two species, *P. davisoni* and *P. ogilviegranti* (Fig. 2, Table 1). The non-monophyly of *P. davisoni* s.l. is strongly supported by the mitochondrial data. Moreover, the pronounced cytochrome *b* divergence between *davisoni* s.s. and *ogilviegranti*/*disturbans*/*klossi* indicates long-standing lack of gene flow between them. It is uncertain whether the range of *davisoni* meets any of the others. As with the three main *P. reguloides* s.l. clades, which we suggest to be considered separate species, the morphological differences between *P. davisoni* s.s. and *P. ogilviegranti* are very slight (Baker, 1997; Ticehurst, 1938; Williamson, 1967). Also their songs and calls are extremely similar, with no known differences (own observations).

The mitochondrial divergence between *ogilviegranti*, *disturbans*, and *klossi* is very slight, and it is possible that a larger sample might reveal a mixing of haplotypes among these taxa as a result of incomplete lineage sorting or introgression (though it is uncertain whether their ranges are presently in contact). However, there are slight plumage differences between all three taxa (Baker, 1997; Ticehurst, 1938; Williamson, 1967) supporting their recognition. The poor genetic and morphological differentiations endorse

their treatment as conspecific. The names *ogilviegranti*, *klossi*, and *disturbans* were all published in 1922. La Touche described both *ogilviegranti* and *disturbans* in the Bulletin of the British Ornithologists' Club, but the former name appeared on 3 January in issue 269, while the latter was published on 27 October in issue 271. The name *klossi* was published in the October–December issue of Auk in 1922. Deignan (1961) dates this name to 3 October 1922. Accordingly, the name *ogilviegranti* has priority over *klossi* and *disturbans* under the rules of the International Commission on Zoological Nomenclature (1999).

We favour continued treatment of *P. hainanus* as a species, despite that the genetic differentiation from its sister taxa *ogilviegranti*, *disturbans*, and *klossi* is considerably lower than all of the other pairwise comparisons in the present study of taxa that are unanimously treated as separate species (Fig. 7). However, the plumage differences between *hainanus* and *ogilviegranti/disturbans/klossi* are much more pronounced than the differences between the sympatric species pairs *P. ogilviegranti klossi–P. reguloides ticehursti*, *P. ogilviegranti disturbans–P. claudiae*, *P. ogilviegranti ogilviegranti–P. goodsoni fokiensis*, and *P. reguloides reguloides–P. occipitalis* (cf. Baker, 1997; Olsson et al., 1993; Rasmussen and Anderton, 2005; Ticehurst, 1938; Williamson, 1967). Moreover, being endemic to Hainan island, *hainanus* is geographically isolated from the others.

4.4. Phylogeny and species limits in the *P. trivirgatus* superspecies

Watson et al. (1986) considered *P. makirensis*, *P. poliocephalus*, *P. presbytes*, *P. sarasinorum*, and *P. trivirgatus* to form a superspecies. The present study does not support this, mainly because *P. poliocephalus giulianettii* is sister to *P. amoenus* in all analyses (*P. makirensis* was not studied). Moreover, the positions of *P. trivirgatus trivirgatus* and *P. presbytes floris* are unresolved within clade A1 in Fig. 1.

The suggested non-monophyly of *trivirgatus* and *benguetensis* (although not strongly supported), in combination with their cytochrome *b* divergence of 6.5%, which exceeds all other comparisons between *trivirgatus* s.s. and the other tropical islands taxa, indicate that *trivirgatus* and *benguetensis* are better considered separate species. However, more taxa need to be studied to establish the taxonomic position of the latter.

We predict that all the taxa allocated by Watson et al. (1986) to *P. trivirgatus*, *P. sarasinorum*, *P. presbytes*, and *P. poliocephalus*, and also *P. makirensis*, belong in clade A1. The taxonomy of these are in great need of revision.

4.5. Evolution of morphological traits

The rate heterogeneity in the divergence of morphological traits among the taxa in the present study is pronounced. The sympatric species *P. emeiensis–P. claudiae–P. ogilviegranti disturbans*, *P. goodsoni fokiensis–P. ogilviegranti ogilviegranti*, *P. reguloides ticehursti–P. ogilviegranti klossi*, and *P. regulodies tiechursti–P. davisoni* are exceedingly similar (Alström and Olsson, 1993, 1995a; Baker, 1997; Ticehurst, 1938; Williamson, 1967), and *P. coronatus* is so alike the allopatric *P. occipitalis* that these two were previously considered conspecific (Ali and Ripley, 1997; Vaurie, 1959). None of these are very closely related, and the pairwise cytochrome *b* divergences between them are 11.4–18%. In contrast, clade A1 in Fig. 1 contains several morphologically highly divergent taxa, while the cytochrome *b* divergences within this clade do not exceed 6.8%. *P. xanthoschistos* stands out, as it has diverged so much in plumage that all previous authors have placed it in the genus *Seicercus* (e.g., Baker, 1997; Dickinson, 2003; Inskipp et al., 1996; Sibley and Monroe, 1990; Watson et al., 1986). *P. hainanus* also shows an unusually high degree of plumage differentiation in relation to its small genetic divergence from its nearest relatives. In a different part of the tree, the plumage of *P. coronatus* is markedly different from its closest relative, *P. ijimae* (Baker, 1997; Ticehurst, 1938; Williamson, 1967), from which it differs by 9.7% in cytochrome *b*. These observations support the suggestion by Price and Pavelka (1996) that similar colour patterns can, once evolved, rather easily disappear and re-appear independently in leaf-warblers.

4.6. Taxon sampling in intrageneric phylogenetic studies

Most intrageneric phylogenies to date have dealt mainly with taxa treated as species, and have not strived to include most or all taxa treated as subspecies of polytypic species. Our data comprise all species and all except two of the subspecies, and several additional geographic subpopulations of the continental *Cryptigata* sensu Watson et al. (1986). They indicate cases of non-monophyly and the existence of previously unrecognized species. This underscores the importance of dense taxon sampling in intrageneric phylogenetic studies. Similar views have also been expressed by Omland et al. (1999) and Olsson et al. (2004), and agree with the recent interest in phylogeography (reviewed by Avise, 2000). Underestimating the variation will obscure our understanding of evolutionary processes, biogeography, biodiversity, and conservation biology.

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Appendix A

List of samples (in alphabetical order), with geographic origin and museum reference numbers. Acronyms are: AMNH = American Museum of Natural History, New York, USA; MAR: collection of Jochen Martens, Johannes Gutenberg-Universität, Mainz, Germany; MV: Museum Victoria, Melbourne, Australia; NRM: Swedish Museum of Natural History, Stockholm, Sweden; ZMUC: Zoological Museum of the University of Copenhagen, Copenhagen, Denmark. Taxonomy is the one proposed here. In the status field, individuals sampled on their breeding grounds in the breeding season (and in all but a few cases showing territorial behaviour) are denoted *b*, while individuals sampled on migration or in their winter quarters are denoted *m*.

Taxon	Locality	Status	Regions	Museum number
Acrocephalus dumetorum	Russia?	b	Cyt*b*	
	Punjab, India (ca. 31N, 75E)	m	12S	NRM 569279
			Myo	
Phylloscopus amoenus	Kolombangara, Solomon Islands (ca. 8S, 157E)	b	Cyt*b*	AMNH PRS2727
			12S	
			Myo	
Phylloscopus borealoides	Hong Kong (ca. 22.5N, 114E)	m	Cyt*b*	NRM 569280
			12S	
			Myo	
Phylloscopus borealis borealis	Hebei, NE China (ca. 39.5N, 119E)	m	Cyt*b*	NRM 20036962
			12S	
			Myo	
P. cantator cantator	E Nepal (ca. 27.5N, 87.5E)	b	Cyt*b*	MAR 2781
			12S	
			Myo	
P. cebuensis	Philippines	b	Cyt*b*	Richman & Price 1992
	Luzon, Philippines (ca. 17N, 121E)		12S	ZMUC 02047
P. collybita abietinus	Sweden	b	Cyt*b*	
	N Sweden (ca. 63.5N, 20.5E)	b	12S	NRM 20036964
			Myo	
P. claudiae 1	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036966
P. claudiae 2	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036968
			12S	
			Myo	
P. claudiae 3	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036967
P. claudiae 4	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036965
P. claudiae (prev. unassigned)	Hebei, NE China (ca. 39.5N, 119E)	b	Cyt*b*	NRM 20036969
P. coronatus	Japan (ca. 36N, 140.5E)	b	Cyt*b*	NRM 20036970
			12S	
	Hebei, NE China (ca. 39.5N, 119E)	m	Myo	
P. davisoni	NW Thailand (ca. 18.5N, 98.5E)	b	Cyt*b*	NRM 20036971
			12S	
			Myo	
P. emeiensis	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036975
			12S	
			Myo	
P. goodsoni goodsoni 1	Guangdong, S China (ca. 25N, 113E)	b	Cyt*b*	NRM 20036977
P. goodsoni goodsoni 2	Guangdong, S China (ca. 25N, 113E)	b	Cyt*b*	NRM 20036976
			12S	
P. goodsoni goodsoni	Guangxi, S China (ca. 24N, 109.5E)	b	Cyt*b*	NRM 20036978
			Myo	
P. goodsoni goodsoni	Hong Kong (ca. 22.5N, 114E)	m	Cyt*b*	NRM 20036979
P. goodsoni fokiensis 1	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036980

Appendix A (*continued*)

Taxon	Locality	Status	Regions	Museum number
P. goodsoni fokiensis 2	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036983
P. goodsoni fokiensis 3	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036982
P. goodsoni fokiensis 4	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036981
P. goodsoni fokiensis 5	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036984
			12S	
			Myo	
P. hainanus	Hainan Island, S China (ca. 18.5N, 108.5E)	b	Cyt*b*	NRM 20036985
			12S	
			Myo	
P. ijimae	Japan	b	Cyt*b*	
	Japan (ca. 34N, 139E)	b	12S	NRM 20036986
			Myo	
P. inornatus	Hebei, NE China (ca. 39.5N, 119E)	m	Cyt*b*	NRM 20036987
			12S	
			Myo	
P. maculipennis	West Bengal, NE India (ca. 27N, 89E)	b	Cyt*b*	NRM 569281
			12S	
			Myo	
P. magnirostris	Sichuan, C China (ca. 33N, 104E)	b	Cyt*b*	NRM 20036988
	S India (ca. 12N, 77E)	m	12S	
	Sichuan, C China (ca. 33N, 104E)	b	Myo	
P. nitidus	E Turkey	b	Cyt*b*	
	E Turkey (ca. 41N, 41,5E)	b	12S	NRM 569282
P. occipitalis	NW Frontier Prov., N Pakistan (ca. 34.5N, 73.5E)	b	Cyt*b*	NRM 20036989
			12S	
			Myo	
P. occipitalis	Kashmir, India?	b	Cyt*b*	
P. ogilviegranti klossi	South Annam, S Vietnam (ca. 11.5N, 108E)	b	Cyt*b*	NRM 20036972
			12S	
			Myo	
P. ogilviegranti disturbans	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20036973
			12S	
			Myo	
P. ogilviegranti ogilviegranti	Fujian, SE China (ca. 27.5N, 117.5E)	b	Cyt*b*	NRM 20036974
			12S	
			Myo	
P. orientalis	Bulgaria (ca. 42N, 27E)	b	Cyt*b*	NRM 569283
			12S	
			Myo	
P. plumbeitarsus	Hebei, NE China (ca. 39.5N, 119E)	m	Cyt*b*	NRM 20036990
			12S	
			Myo	
P. poliocephalus giulianettii	Papua New Guinea (ca. 8S, 146E)	b	Cyt*b*	MV E374
			12S	
			Myo	
P. presbytes floris	Flores, Indonesia (ca. 8.5S, 120.5E)	b	Cyt*b*	NRM 20036991
			12S	
			Myo	
P. proregulus	Hebei, NE China (ca. 39.5N, 119E)	m	Cyt*b*	NRM 20036992
			12S	
			Myo	
P. reguloides reguloides 1	Himachal Pradesh, NW India (ca. 32N, 77E)	b	Cyt*b*	NRM 20036993
			Myo	
P. reguloides reguloides 2	Himachal Pradesh, NW India (ca. 32N, 77E)	b	Cyt*b*	NRM 20036994
P. reguloides reguloides	C Nepal (ca. 27.5N, 85E)	b	Cyt*b*	NRM 20036995
			12S	
P. reguloides reguloides	West Bengal, NE India (ca. 27N, 89E)	b	Cyt*b*	NRM 20036996
			Myo	
P. reguloides assamensis 1	W Myanmar (ca. 21N, 93E)	b	Cyt*b*	NRM 20036998
			12S	
			Myo	
P. reguloides assamensis 2	W Myanmar (ca. 21N, 93E)	b	Cyt*b*	NRM 20036997

(*continued on next page*)

Appendix A (*continued*)

Taxon	Locality	Status	Regions	Museum number
P. reguloides ticehursti ("*assamensis* " 1)	NW Thailand (ca. 18.5N, 98.5E)	b	Cyt*b*	NRM 20037000
P. reguloides ticehursti ("*assamensis* " 2)	NW Thailand (ca. 18.5N, 98.5E)	b	Cyt*b*	NRM 20036999
P. reguloides ticehursti ("*assamensis* " 3)	NW Thailand (ca. 18.5N, 98.5E)	b	Cyt*b*	NRM 20037001
			12S	
P. reguloides ticehursti ("*assamensis* ")	Yunnan, S C China (ca. 25N, 98E)	b	Cyt*b*	NRM 20037002
P. reguloides ticehursti (prev. unassigned)	N Vietnam (ca. 22N, 103.5E)	b	Cyt*b*	NRM 20037003
P. reguloides ticehursti 1	S Vietnam (ca. 11.5N, 108E)	b	Cyt *b*	NRM 20037004
P. reguloides ticehursti 2	S Vietnam (ca. 11.5N, 108E)	b	Cyt *b*	NRM 20037005
			12S	
			Myo	
P. ricketti	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	NRM 20037006
			12S	
			Myo	
P. ruficapilla minullus	Tanzania (ca. 5S, 38E)	b	Cyt*b*	ZMUC 119378
			12S	
			Myo	
P. sarasinorum sarasinorum	S Sulawesi, Indonesia (ca. 5S, 119.5E)	b	Cyt*b*	NRM 20037007
			12S	
			Myo	
P. schwarzi	Hebei, NE China (ca. 39.5N, 119E)	m	Cyt*b*	NRM 569284
			12S	
			Myo	
P. tenellipes	Hong Kong (ca. 22.5N, 114E)	m	Cyt*b*	NRM 569285
	Heilongjiang, NE China (ca. 45N, 127.5E)	b	12S	NRM 20037008
	Hong Kong (ca. 22.5N, 114E)	m	Myo	NRM 569285
P. trivirgatus benguetensis	N Luzon, Philippines (ca. 16.5N, 121E)	b	Cyt*b*	ZMUC 02115
			12S	
P. trivirgatus trivirgatus	W Java, Indonesia (ca. 6S, 106.5E)	b	Cyt*b*	NRM 20037009
			12S	
			Myo	
P. tytleri	NW Frontier Prov., N Pakistan (ca. 34.5N, 73.5E)	b	Cyt*b*	
			12S	NRM 569286
			Myo	
P. trochilus trochilus	Sweden	b	Cyt*b*	
	S Sweden (ca. 57.5N, 12E)	b	12S	NRM 20037010
			Myo	
P. trochiloides trochiloides	Sichuan, C China (ca. 29.5N, 103E)	b	Cyt*b*	
			12S	NRM 569286
			Myo	
P. umbrovirens fugglescouchmani	Tanzania (ca. 8S, 37E)	b	Cyt *b*	ZMUC 120713
			12S	
			Myo	
P. xanthoschistos xanthoschistos	NW Frontier Prov., N Pakistan (ca. 34.5N, 73.5E)	b	Cyt*b*	NRM 20037012
			12S	
			Myo	
P. xanthoschistos tephrodiras	W Myanmar (ca. 21N, 93E)	b	Cyt *b*	NRM 20037013
			12S	
			Myo	
Sylvia atricapilla atricapilla	Germany	b	Cyt*b*	
	Spain (ca. 49.5, 0E)	b	12S	NRM 20037014
			Myo	
Seicercus castaniceps castaniceps	W Myanmar (ca. 21N, 93E)	b	Cyt *b*	NRM 569288
			12S	
			Myo	
S. poliogenys	West Bengal, NE India (ca. 27N, 89E)	b	Cyt*b*	NRM 20037016
			12S	
			Myo	
S. whistleri whistleri	Himachal Pradesh, NW India (ca. 32N, 77E)	b	Cyt*b*	NRM 20037017
			12S	
			Myo	

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