The phylogenetic position of the world's smallest passerine, the Pygmy Bushtit *Psaltria exilis*

ULF S. JOHANSSON,[1]* PER G. P. ERICSON,[1] JON FJELDSÅ[2] & MARTIN IRESTEDT[3]

[1]*Department of Zoology, Swedish Museum of Natural History, Box 50007, Stockholm 10405, Sweden*

[2]*Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark*

[3]*Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Box 50007, Stockholm 10405, Sweden*

The Pygmy Bushtit is confined to the montane forests of Java. It is the world's smallest passerine and morphologically resembles a small, drab long-tailed tit or bushtit (Aegithalidae). In its behaviour the Pygmy Bushtit show similarities with the members of the Aegithalidae, but owing to its small size and isolated geographical distribution relative to the other members of the Aegithalidae, it has always been placed in a monotypic genus within the family. The affinities of the Pygmy Bushtit have never been tested in a phylogenetic context and the species has to date not been included in any molecular studies. In this study we use sequence data from four different genetic markers to place it in the passerine phylogenetic tree. Our results confirm the inclusion of the Pygmy Bushtit in the Aegithalidae, but rather than being an isolated lineage, our results strongly suggest that it is nested in the *Aegithalos* clade, and most closely related to the Black-throated Bushtit *Aegithalos concinnus*. The range of the Black-throated Bushtit extends south into subtropical Indochina, with an isolated subspecies occurring in southern Vietnam. The Black-throated Bushtit contains several morphologically and genetically distinct lineages, which could represent distinct species, but the phylogenetic relationships within this complex are poorly resolved and partly in conflict with current taxonomic treatment based on morphology.

Keywords: Aegithalidae, Aves, biogeography, Passeriformes, phylogeny.

The Pygmy Bushtit *Psaltria exilis* is a Javan endemic confined to montane forests in the highlands of western and central Java. It is a nondescript drab grey bird with whitish underparts, whitish irides and yellow legs and feet (Harrap & Quinn 1996, Harrap 2008). In spite of its long tail, it is only 8.5 cm in length, which makes the Pygmy Bushtit the smallest passerine species (Harrap & Quinn 1996). Morphologically it resembles a small, dull bushtit and although very little is known about its biology, it also appears to behave very much as other members of the Aegithalidae. For instance, it lives in small family groups that move actively through the vegetation, builds a 'pouch-like' nest and resembles other long-tailed tits and bushtits in its contact calls and apparent

lack of a territorial song (Harrap & Quinn 1996, Harrap 2008). Based on this resemblance it has long been placed in the Aegithalidae (e.g. Snow 1967, Sibley & Monroe 1990, Dickinson 2003, Dickinson & Christidis 2014). As it is smaller and duller than other aegithalids and is the only tropical member of the clade, the Pygmy Bushtit has been placed in the monotypic genus *Psaltria*.

The genera *Aegithalos*, *Psaltriparus* and *Psaltria* have been grouped together in most classifications since the mid-1800s. Until recently this group was in turn often associated with the tits and chickadees (Paridae). Once designed a separate family, Aegithalidae (Vaurie 1957), the bushtits nevertheless remained linked with the parids due to the lack of evidence suggesting an alternative placement. It was not until molecular data were available, first DNA–DNA hybridization (Sibley & Ahlquist 1990) and later DNA sequencing

*Corresponding author.
Email: ulf.johansson@nrm.se

(Alström *et al.* 2006, Johansson *et al.* 2008, Fregin *et al.* 2012), that the relationship with parids was rejected and it was shown that Aegithalidae are instead most closely related to leaf warblers (Phylloscopidae) and bush warblers (Cettiidae) in the Sylvoidea. These latter studies also revealed that the more warbler-like *Leptopoecile* species are part of the Aegithalidae and sister taxa to *Aegithalos* and *Psaltriparus*. However, the systematic position of the Pygmy Bushtit has been addressed in few systematic studies and to date it has not been included in any molecular phylogeny.

Aegithalos, with between six and nine species, has the largest distribution of the four genera in the family. Members of the genus extend from the Iberian Peninsula and the British Isles across the Palaearctic to Kamchatka and Japan, and south to the Himalayas and Indochina. This large distributional range, however, comprises primarily a single species, the Long-tailed Tit *Aegithalos caudatus*. Another fairly widespread species is the Black-throated Bushtit *Aegithalos concinnus*, which is distributed in the Himalayan foothills and over much of eastern China and along mountain ridges in tropical Indochina. The remaining species in the clade all have rather restricted distributions confined to small regions in the Himalayas or in the mountains of southwestern China. The two *Leptopoecile* species inhabit the mountains north and east of the Tibetan Plateau, primarily above 3000 m, whereas the sole member of the genus *Psaltriparus*, the American Bushtit *Psaltriparus minimus*, inhabits western North America, extending from southwestern Canada to southern Mexico and Guatemala.

Here we address the systematic position of the Pygmy Bushtit of Java by including it in a molecular phylogenetic study for the first time. We first verify its position among the bushtits by including it in a large phylogeny of passerine bird species, and then specifically address its relationships to the other species in the Aegithalidae in an analysis with a comprehensive coverage of the taxa currently recognized within this family.

METHODS

DNA extraction, PCR-amplification and sequencing

DNA for this study was obtained from toe-pads of museum study skins. The DNA from *P. exilis* was obtained from a specimen that was collected in western Java on 12 April 1920 (NRM 571647). We also extracted DNA from additional specimens of the Black-throated Bushtit complex including two specimens of the subspecies *A. concinnus annamensis* and representatives of the subspecies *A. c. talifuensis* and *A. c. concinnus* (Table 1).

As the DNA obtained from old museum study skins is generally heavily degraded, the laboratory work was conducted under strict conditions to avoid contamination, following the procedures described in Irestedt *et al.* (2006, in press). We specifically designed primers that PCR-amplified the individual loci in short *c.* 200-bp fragments (Supporting Information Table S1). We sequenced four loci, the mitochondrial cytochrome-*b* gene, and three nuclear intron regions: ornithine decarboxylase (ODC) introns 6 and 7, myoglobin intron 2, and transforming growth factor beta 2 (TGFB2) intron 5.

Phylogenetic analyses

We first aimed to evaluate the phylogenetic position of the Pygmy Bushtit relative to other passerine birds to ascertain its relationships with the Aegithalidae, as suggested by behavioural and morphological data. To cover as broad a range of potential closely related groups as possible, we made use of a previously published dataset of passerine birds (Johansson *et al.* 2008; Supporting Information Table S2). This dataset consists of myoglobin and ODC sequences from a broad representation (90 taxa) of primarily Passerida lineages, with the densest sampling within the Sylvioidea, including all Aegithalidae genera (*Aegithalos*, *Psaltriparus* and *Leptopoecile*). We also included additional *Aegithalos* sequences from GenBank: White-throated Bushtit *Aegithalos niveogularis* (KJ454741, GU434033), Black-browed Bushtit *Aegithalos bonvaloti* (KJ790385, GU434032) and *Aegithalos concinnus iredalei* (DQ008570, GU434027).

While this first analysis confirmed the placement of the Pygmy Bushtit in the Aegithalidae, it also suggested a previously unrecognized affinity for this species within the genus *Aegithalos* near *A. concinnus*. We therefore performed an extended analysis of the relationships within this family based on three markers (ODC, TGFB2 and cytochrome-*b*) and additional sequences primarily obtained from Päckert *et al.* (2010) (Table 1). However, as recent studies of the *A. concinnus* complex (Päckert *et al.* 2010, Dai *et al.* 2011)

Table 1. GenBank accession numbers for the samples used in the three gene analysis of this study.

| Species | Subspecies | Locality | Specimen no. | GenBank accession no. | | | | | |
				Tgfb2	Ref.	cytb	Ref.	ODC	Ref.
Psaltria exilis		Indonesia, western Java	NRM 571647	KX014777	1	KX014769	1	KX014783	1
Aegithalos concinnus	*concinnus*	Taiwan	NRM 571736	KX014774	1	KX014766	1	Failed	
Aegithalos concinnus	*concinnus*	China, Anhui province	NRM 571732	KX014773	1	KX014765	1	KX014780	1
Aegithalos concinnus	*talifuensis*	Vietnam, Lào Cai province, Sapa	NRM 571740	KX014775	1	KX014767	1	KX014781	1
Aegithalos concinnus	*talifuensis*	Vietnam, Lào Cai province, Sapa	NRM 571743	KX014776	1	KX014768	1	KX014782	1
Aegithalos concinnus	*annamensis*	Vietnam, Lam Dong province, Da Lat	NRM 571725	KX014771	1	KX014763	1	KX014778	1
Aegithalos concinnus	*annamensis*	Vietnam, Dac Lac province, B'sré	NRM 571729	KX014772	1	KX014764	1	KX014779	1
Aegithalos fuliginosus		China, Shaanxi, Taibai Shan, Houzhenzi	MAR809	GU434014	2	GU244444	2	GU434031	2
Aegithalos bonvaloti		China, Yunnan, Jizu Shan	MAR3232	GU434015	2	GU244433	2	GU434032	2
Aegithalos sharpei		Myanmar, Mt. Victoria, Pakokku	MAR50885	GU434020	2	GU244435	2	GU434034	2
Aegithalos leucogenys		Afghanistan, Dar-e-Nur	MAR3955	GU434019	2	GU244439	2	GU434035	2
Aegithalos niveogularis		Nepal, Dolpo District, Ringmo/Lake Phoksumdo	MAR3951	GU434016	2	GU244437	2	GU434033	2
Aegithalos caudatus	*taiti*	France, Pyrénées-Orientales, Serralongue	MAR540	GU434017	2	GU244456	2	GU434030	2
Aegithalos glaucogularis		China, Henan, Dongzhai Nature Reserve	MAR7814	GU434018	2	GU244480	2	GU434029	2
Aegithalos concinnus	*talifuensis*	China, Sichuan, Emei Shan, Hongchun	MAR2068	GU434013	2	AY755556	2	GU434026	2
Aegithalos concinnus	*iredalei*	Nepal, Rasuwa District, W Syabrubesi	MAR4128	GU434011	2	GU244426	2	GU434027	2
Aegithalos concinnus	*manipurensis*	Myanmar, Chin State, Natmataung NP	MAR4722	GU434012	2	GU244428	2	GU434028	2
Psaltriparus minimus	*minimus*	USA, Washington, Pacific C., Grayland	MAR79247	GU434008	2	GU244415	2	GU434022	2

(continued)

Table 1. (continued)

Species	Subspecies	Locality	Specimen no.	GenBank accession no.					
				Tgfb2	Ref.	cytb	Ref.	ODC	Ref.
Psaltriparus minimus	*plumbeus*	USA, Colorado, Fremont C., Camon City	MAR56377	GU434007	2	GU244416	2	GU434023	2
Leptopoecile sophiae		Aviary	MAR2117	GU434010	2	GU244482	2	GU434025	2
Leptopoecile elegans		China, Sichuan, Bang Ba village	MAR5767	GU434009	2	GU244414	2	GU434024	2
Outgroup									
Pnoepyga pusilla			MAR4931	JX518503	2	JX518496	2	JX518521	2

References: 1 = This study; 2 = Päckert *et al.* (2010).

suggest that the current treatment of this taxon as a single polytypic species is questionable, we also included several of the genetically and morphologically distinct subspecies of this species, including two sequences of *A. c. annamensis* from the Da Lat Plateau, southern Vietnam, sequences from two specimens morphologically identified as *A. c. talifuensis* from Sapa, Vietnam, and two sequences from specimens morphologically identified as *A. c. concinnus* from Taiwan and the Anhui Province in China, respectively (Table 1). The taxa *A. c. manipurensis* and *A. c. iredalei* included in the study by Päckert *et al.* (2010) were also included.

The study by Dai *et al.* (2011) furthermore suggests that the delineation of species boundaries within *concinnus–talifuensis* is more complex than indicated by current taxonomy, with at least three distinct lineages being present. Dai *et al.* (2011) refer to these clades as A, B and C, and concluded that '[i]n terms of the current taxonomy, subspecies *A. c. talifuensis* includes lineages A and B, whereas lineages C, … exhibits strong concordance between the geographical mtDNA lineage and the currently described nominate subspecies, *A. c. concinnus*'. Furthermore, the two '*talifuenisis*' clades A and B do not form a monophyletic group, as clade B appears as the sister-group of '*concinnus*' (clade C) with high support, and there is little genetic divergence between the two clades. In contrast, the two '*talifuensis*' clades are separated by a genetic distance of 4.8% (cytochrome-*b*, uncorrected p-distance). As no morphological characters are yet known to distinguish between the two '*talifuensis*' clades and their respective geographical distributions have not been circumscribed, these lineages can at present only be identified using molecular data. Therefore, we performed a third analysis in which we included cytochrome-*b* sequences from 112 *Aegithalos* individuals, primarily sourced from GenBank (Supporting Information Table S3), in order to fully investigate the relationship of the Pygmy Bushtit to the *A. concinnus* complex. This analysis includes all currently recognized species in Aegithalidae (including the Rufous-fronted Bushtit *Aegithalos iouschistos*) and of the subspecies in the Black-throated Bushtit complex we lacked sequence data only from *A. c. pulchellus* from eastern Myanmar.

We used the same basic approach for all three datasets. The sequences were aligned by eye using

BioEdit 7.2.5 (Hall 1999). For the analysis of the phylogenetic position of the Pygmy Bushtit within Passeriformes we used the same alignment as Johansson *et al.* (2008), excluding the 625-bp-long insertion observed in ODC for *Motacilla* and *Amaurocichla* (Johansson *et al.* 2008). Phylogenetic analyses were conducted with Bayesian inference (BI) using MrBayes 3.2.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) using RAxML v8.1.21 (Stamatakis 2014), as implemented in RAxML-GUI v1.5b1 (Silvestro & Michalak 2012). The model of sequence evolution was selected with the Bayesian information criterion (BIC) using PartitionFinder (Lanfear *et al.* 2012). In the higher-level study ('Passeriformes') the $GTR+\Gamma_4+I$ model was selected for ODC and a $GTR+\Gamma_4$ substitution model was selected for myoglobin intron 2. For the Aegithalidae ODC/TGFB2/cytochrome-*b* analysis the HKY model was selected for ODC and TGFB2, and $GTR+\Gamma_4$ was selected for cytochrome-*b*. For this latter three-gene dataset we ran four separate analyses, one on the concatenated data, and one on each of the individual gene trees using the same models suggested by PartitionFinder. The $GTR+\Gamma_4$ model was also selected for the more inclusive cytochrome-*b* analysis. In all MrBayes analyses we used default priors. For each of the different datasets, two independent runs, each with four Metropolis-coupled MCMC chains, were run for 20 million generations with trees sampled every 500 generations. Trees sampled before the chain reached apparent stationarity (burn-in) were discarded and the posterior probabilities for the topology were estimated from the remaining generations. To ascertain whether the chains had reached stationarity we: (1) plotted the log-likelihood values for each run and compared the posterior probabilities among runs, (2) checked that the average standard deviation of split frequencies had converged towards zero, (3) determined that the potential scale reduction factor approached 1.0 for all parameters and (4) used Tracer v1.6 (Rambaut & Drummond 2007) to determine whether the sampling of the posterior distribution had reached an adequate effective sample size. The ML analyses were performed with rapid bootstrap (1000 replicates) and a thorough ML search for one run and 1000 replicates under a GTR+G model. The dataset was partitioned by gene and branch-lengths were calculated independently for each partition.

RESULTS

Phylogenetic position within the Passeriformes

Our analysis of the higher-level relationships of the Pygmy Bushtit based on myoglobin intron 2 and ODC sequences confirms that it belongs in the Aegithalidae. The monophyly of the Aegithalidae (*Aegithalos*, *Psaltriparus*, *Leptopoecile* and *Psaltria*) was recovered with strong support (posterior probability (PP) = 1.00), and within this clade *Leptopoecile* is placed basal relative to *Aegithalos* and *Psaltriparus* (Fig. 1). However, the Pygmy Bushtit is not placed as an isolated lineage within the Aegithalidae as assumed by its current taxonomic treatment by recognition of a monotypic genus, but is nested within *Aegithalos* and sister to *A. concinnus*, represented here by the subspecies *A. c. iredalei* (Supporting Information Fig. S1). This group in turn is the sister-group to a clade containing *A. caudatus*, *A. niveogularis* and *A. bonvaloti*.

Phylogenetic position within the Aegithalidae

In the analysis addressing phylogenetic relationships within Aegithalidae based on ODC, TGFB2 and cytochrome-*b* sequences, the Pygmy Bushtit also falls inside the *Aegithalos* clade (Fig. 1) and within this clade there is strong support (PP = 1.00, ML bootstrap = 98%) for placing it in a clade together with the taxa in the Black-throated Bushtit complex. Indeed, it is nested within this complex together with *A. c. manipurensis*, *A. c annamensis* and *A. c. iredalei* as the sister-group of *A. c. concinnus* and *A. c. talifuensis*. However, the support for this placement is weak (PP = 0.89, ML bootstrap = 57%) and in fact none of the relationships within Black-throated Bushtit complex is strongly supported.

The close affinity of the Pygmy Bushtit with the Black-throated Bushtit complex is supported in all gene trees (Fig. S2a–c). However, the exact placement differs among the different analyses. The ODC gene tree is mostly unresolved, but one of the few supported nodes in this tree places the Pygmy Bushtit with some of the taxa in the Black-throated Bushtit complex (PP = 0.60, ML bootstrap = 52%; Fig. S2a). The TGFB2 gene tree is much more resolved although many relationships
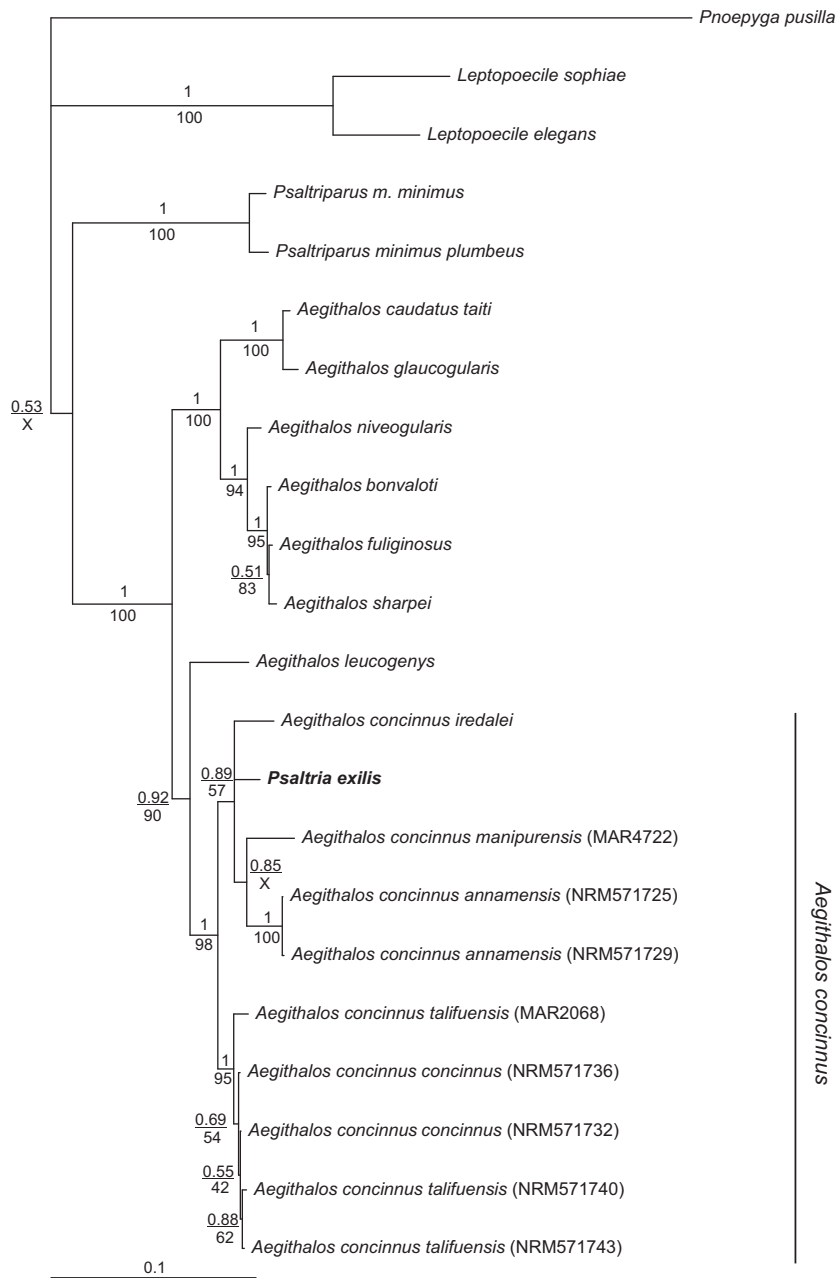
Figure 1. Molecular phylogeny of the Aegithalidae. The tree is a Bayesian consensus tree based on three genes, nuclear ODC and TGFB2 and mitochondrial cytochrome-*b* (total 1586 bp). Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis.

have comparably low posterior probabilities or bootstrap support values (Fig. S2b). Of the three gene trees, the cytochrome-*b* gene tree is the most resolved and is also the tree in which most nodes have comparably high support (Fig. S2c). Overall, the TGFB2 and the cytochrome-*b* gene trees recover a similar topology but differ at a few poorly supported nodes. With respect to the placement of the Pygmy Bushtit, the TGFB2 gene tree places it basal relative to a monophyletic Black-throated Bushtit complex, whereas in the cytochrome-*b* gene tree the species is placed within this clade as the sister-group of *A. e. iredalei*. In both gene trees the support for the Pygmy Bushtit in a

clade with the taxa in the Black-throated Bushtit complex has high support (TGFB2: PP = 1.00, ML bootstrap = 89%; cytochrome-*b*: PP = 0.99, ML bootstrap = 84%) whereas the support for its relationship to the taxa within this complex is lower, but at least in the TGFB2 gene tree its position as basal relative to a monophyletic Black-throated Bushtit has a posterior probability 0.96 and a ML bootstrap support value of 77%.

Phylogenetic position with dense sampling of *Aegithalos*

The tree based on the more inclusive cytochrome-*b* analysis (Fig. 2) is congruent with that of the multi-locus concatenated analysis (Fig. 1), with the exception that the previously identified *concinnus/talifuensis* clade is not monophyletic. Similar to the concatenated analysis a group with *A. c. concinnus* and *A. c. talifuensis* forms the sister-group of all other Black-throated Bushtit taxa (including the Pygmy Bushtit), but separated from this is a clade of '*talifuensis*' specimens from Yunnan that emerge as the sister-group to *A. c. manipurensis* (Fig. 2). This clade corresponds to lineage A of Dai *et al.* (2011). All our samples from the concatenated analysis of *concinnus* and *talifuensis* fall into the former clade, and thus this '*talifuensis*' lineage A is at present unsampled by the nuclear data.

DISCUSSION

The Pygmy Bushtit of Java is reminiscent of a small long-tailed tit (Aegithalidae), both in morphology and in behaviour (Harrap 2008) and our molecular phylogenetic analyses confirm this relationship. More surprisingly, our analyses suggest that the Pygmy Bushtit is not an isolated lineage within the Aegithalidae, but rather is nested in the genus *Aegithalos* and is particularly closely related to the Black-throated Bushtit.

The Black-throated Bushtit, although significantly larger than the Pygmy Bushtit, is also among the smaller members of the Aegithalidae, being about 10.5 cm in length. It inhabits mixed forest and lightly wooded and bushy habitats, including *Rhododendron* and bamboo at mid-elevations. It has a relatively large distribution that extends further south than any other member of the genus and includes the Himalayan foothills from northern Pakistan to eastern China and Taiwan, and reaches south to 20°N in the mountains of Myanmar and northern Laos. There is also an isolated population at 11–15°N on the Da Lat Plateau of southern Vietnam. Within this range it is divided into six subspecies (*concinnus, talifuensis, manipurensis, iredalei, pulchellus* and *annamenis* (Harrap 2008)), which differ primarily in the colour of the crown and the amount and intensity of rufous on the body underparts. Previous molecular studies have shown that these taxa are separated by large genetic distances, but that the systematics of this group is not entirely congruent with the recognized subspecies boundaries. The subspecies *A. c. annamensis* and *A. c. iredalei*, which occupy the extreme southeast and west of the species' range, form at least two, possibly three genetically distinct lineages well differentiated from the other populations in this complex (Päckert *et al.* 2010).

The situation with the non-monophyletic *talifuensis*, first identified by Dai *et al.* (2011), appears to be more intricate than the phenotypic variation suggests. At present, no distinguishing characters have been identified that separate the two *talifuensis* groups and there is no obvious geographical barrier to prevent interaction between the two clades (Dai *et al.* 2011). Dai *et al.* (2011) suggested that the two clades evolved in isolation in the early Pleistocene (c. 2.4 million years ago, 95% CI = 4.29–0.88 Ma), but later came into secondary contact. The two lineages may reflect the presence of two cryptic species in this region, but it seems more plausible to interpret the apparent sympatry of the two clades in Yunnan as a case of introgression of mtDNA following an expansion of the widespread subspecies *A. c. concinnus* of southeastern China into the range of *talifuensis* in the Hengduan Mountains in the southwest. Several similar cases have been described in recent years, where distinct mitochondrial haplotype groups have been mixed over sometimes very large geographical areas (e.g. Zink *et al.* 2008, Webb *et al.* 2011, Pentzold *et al.* 2013). In some cases highly divergent lineages are found to coexist in a panmictic population, such as in the Common Redstart *Phoenicurus phoenicurus* over most of Europe (Hogner *et al.* 2012). For a definitive interpretation it would be necessary to obtain a broader geographical sampling and to include sequence data from nuclear genes, including Z-linked markers. A detailed analysis of the Black-throated Bushtit complex is beyond the scope of this paper, but in view of these findings we agree with Päckert *et al.*
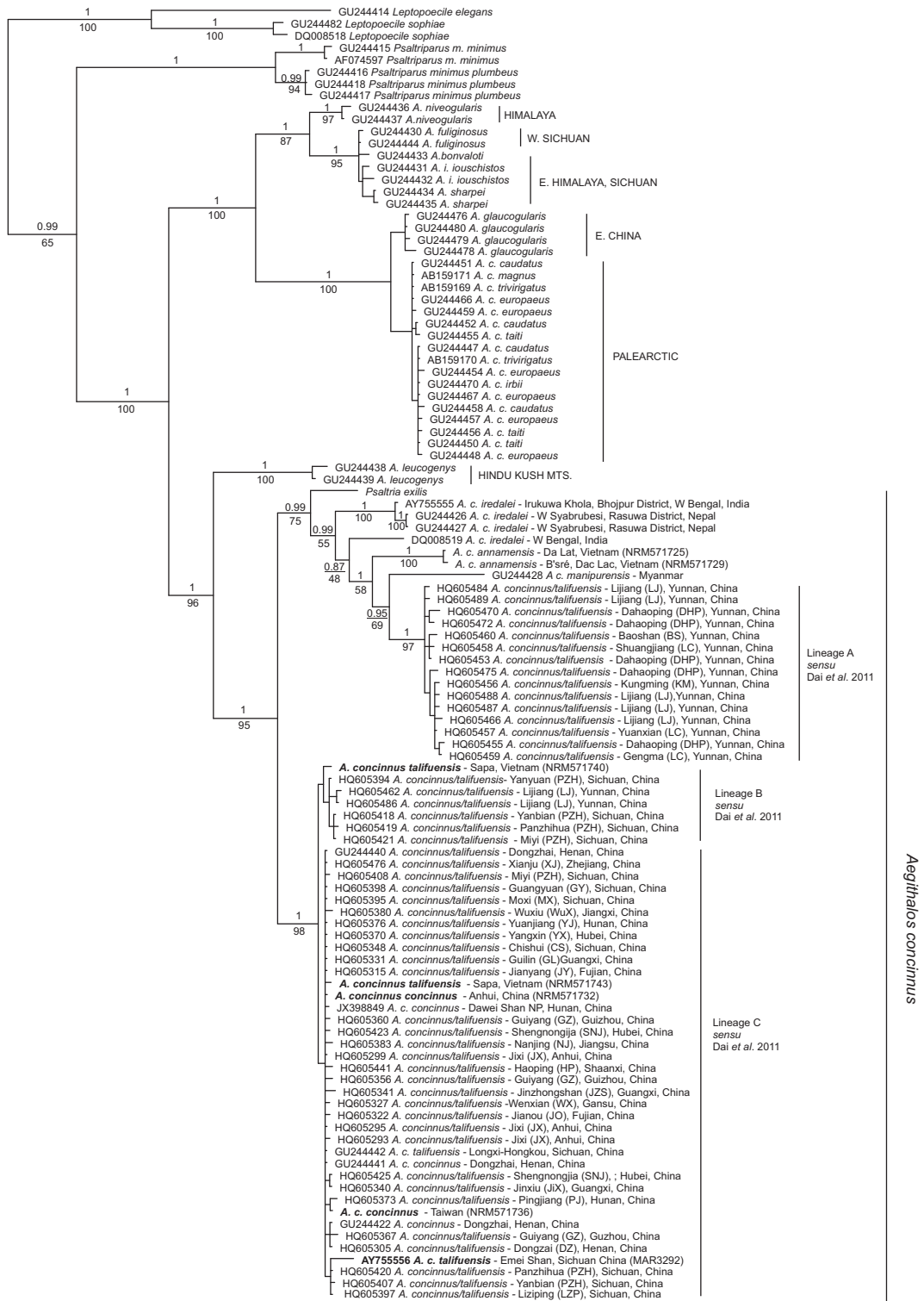
Figure 2. Bayesian consensus tree of the analysis of 112 cytochrome-*b* sequences. Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis. Sequences used in the three gene analysis are indicated in bold.

(2010) that it is premature to make any taxonomic recommendations for the group at this point.

The situation within the Black-throated Bushtit complex aside, the close affinity of the Pygmy Bushtit with this group seems to be strongly supported. Both the higher-level phylogeny of the Passeriformes based on myoglobin intron 2 and ODC, as well as the more specific analysis of the relationships within the Aegithalidae based on ODC, TGFB2 and cytochrome-*b*, place the Pygmy Bushtit with the Black-throated Bushtit complex with high posterior probabilities. Whether the two species are sister-groups or the Pygmy Bushtit is nested within the latter species remains unresolved.

The Pygmy Bushtit is found in montane forests in western and central Java. Of the subspecies in the Black-throated Bushtit complex, *A. c. manipurensis* occurs along the border between western Myanmar and India, *A. c. pulchelles* in eastern Myanmar and northwestern Thailand, *A. c. annamensis* in the highlands of southern Laos/Vietnam and *A. c. iredalei* in the Himalayas. To the north and to the east, *A. c. concinnus* is widely distributed in southeastern China, whereas the two *talifuensis* lineages are distributed primarily in southwestern China. The Pygmy Bushtit is thus found 3000 km and 26 degrees of latitude south of the range of the nearest population of *A. c. pulchellus* in eastern Myanmar. Our data do not confidently establish the relationship of the Pygmy Bushtit with the taxa within the Black-throated Bushtit complex, but they do, with weak support, indicate that it may have originated from an ancestral population of the *iredalei–manipurensis–annamensis* clade (Fig. 1).

Several other Javan taxa show a similar disjunct distribution with its closest relative on the Asian mainland. The most prominent example concerns the mammalian megafauna, with the occurrence of subfossil and extant oxen, rhinos and elephants in Java (Morley 2012), but there are also several examples of mainly dry-habitat birds on Java with similar distributions (Sheldon *et al.* 2015).

Over the last 2.5 Ma, Sundaland has repeatedly been connected with and disconnected from mainland Asia due to cyclical sea level fluctuations when development of arctic glaciers tied up oceanic water (Hall 2011). Thus, for much of the Pleistocene, the Gulf of Thailand and the area between the Malay Peninsula, Sumatra, Borneo and Java/Bali was dry land, which resulted in a continental climate with drier conditions in the interior of this land area. Pollen studies have given rise to a theory that central Sundaland was the site of a savanna corridor extending along the east coast of the Malay Peninsula to Java during the peaks of the glacial periods (Heaney 1991, Bird *et al.* 2005), which explains how the Asian megafauna may have reached Java in the late Pliocene through the mid-Pleistocene (Cranbrook 2000) and how birds could adapt to drier environments. However, the savanna corridor may not explain the dispersal of birds associated with humid montane forests, such as the long-tailed tits. Such habitats in the Sinohimalayan Mountains have long been isolated by deciduous forests from the evergreen montane forests of Java (Morley 2012). In this context it may be more relevant to consider vegetation formations associated with special soils. Coarse-textured and often poorly drained soils in eastern Sumatra and western Borneo generally underlie extremely nutrient-poor peat swamps and heath forests, and this sediment type extends across the sea south of the Malay Peninsula, between eastern Sumatra and western Borneo (Slik *et al.* 2011). Heath-forests, as found in many nutrient-poor parts of Sundaland today, differ from tropical rainforests in terms of structure and productivity and are more similar to the forest of nutrient-poor mountain ridges. We therefore suggest that the dispersal of montane forest birds may have been possible along the ridge tops between the Myanmar–Thailand border and Malay Peninsula, and then onward to the highlands of Java during periods of cold climate and low sea levels.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Molecular phylogeny based on the data set from Johansson et al. (2008) indicating the position of the Pygmy Tit Psaltria exilis relative to the Aegithalidae and other passerine lineages. The tree is a Bayesian consensus tree of the combined, mixed-model analysis of two nuclear data partitions: myoglobin intron 2 and ODC introns 6 and 7, along with intervening exon 7 (total 1597 bp). Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis.

Figure S2a. Bayesian consensus tree based on introns 6 and 7, along with intervening exon 7, of the ornithine decarboxylase (ODC) gene (ODC). Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis.

Figure S2b. Bayesian consensus tree based on the fifth intron of the transforming growth factor β2 (TGF β2) gene. Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis.

Figure S2c. Bayesian consensus tree based on mitochondrial cytochrome b gene. Numbers above the branches indicate Bayesian posterior probabilities and numbers below bootstrap support values from the maximum likelihood analysis.

Table S1. Primers used in this study.

Table S2. GenBank accession numbers for the samples used in the 'higher level' phylogeny.

Table S3. GenBank numbers for the sequences used in the extended cytochrome *b* analysis.