

# Molecular genetic and bioacoustic differentiation of *Pnoepyga* Wren-babblers

Martin Päckert · Jochen Martens · Wei Liang ·  
Yu-Cheng Hsu · Yue-Hua Sun

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**Abstract** We reconstructed a molecular phylogeny for all four species of the wren-babbler genus *Pnoepyga* and added a comparative analysis of their territorial songs. The genus is divided into two species pairs which can also be distinguished by ecological and bioacoustic features. One species pair, *Pnoepyga albiventer* and *P. formosana*, occupies the higher forested mountain elevations of the

Sino-Himalayas and Taiwan and shares broad-banded songs with a marked element-type variation. The second species pair, *Pnoepyga pusilla* and *P. immaculata*, occupies median and low mountain elevations of the Sino-Himalayas, continental Southeast Asia, and the Sunda region and has characteristic narrow-banded whistled songs. Intraspecific variation of molecular and bioacoustic markers in *P. albiventer* was conspicuous among individuals from Nepal and those from the Chinese provinces of Sichuan and Hubei. A third distinct genetic lineage of *P. albiventer* was found in Myanmar. We suggest that the Chinese form be ranked as a separate species *Pnoepyga mutica* Thayer & Bangs, 1912.

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M. Päckert (✉)  
Senckenberg Naturhistorische Sammlungen, Museum für  
Tierkunde, Königsbrücker Landstraße 159, 01109 Dresden,  
Germany  
e-mail: martin.paeckert@senckenberg.de

J. Martens  
Institut für Zoologie, Johannes Gutenberg-Universität, 55099  
Mainz, Germany

W. Liang  
College of Life Sciences, Hainan Normal University, Haikou  
571158, China

Y.-C. Hsu  
Department of National Resources and Environmental Studies,  
National Dong Hwa University, Shou-Feng, 974 Hualien,  
Taiwan

Y.-H. Sun  
Key Laboratory of Animal Ecology and Conservation, Institute  
of Zoology, Chinese Academy of Sciences, Beijing 100101,  
People's Republic of China

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Cluster analysis

## Zusammenfassung

### Molekulargenetische und bioakustische Differenzierung der *Pnoepyga*-Moostimalien

In einer molekularen Phylogenie und einer Analyse der Territorialgesänge der Moostimalien, Gattung *Pnoepyga*, gliedern sich die vier Arten sowohl nach genetischen als auch nach akustischen und ökologischen Befunden in zwei Gruppen zu je zwei Arten. Das eine Artenpaar mit *P. albiventer* und *P. formosana* lebt in den höheren bewaldeten Gebirgsabschnitten der Sino-Himalayanischen Region und Taiwans. Ihre Gesänge sind breitbandig. Das andere Artenpaar bestehend aus *P. pusilla* und *P. immaculata* besiedelt die mittlere und untere Gebirgszone der Sino-Himalayanischen Region, des südostasiatischen Festlandes und Teilen des Sunda-Archipels, mit Beschränkung von

*P. immaculata* auf den zentralen Himalaya. Beide Arten zeichnen sich durch schmalbandige Pfiffgesänge aus. Individuelle Variabilität der Gesänge des einzelnen Männchens ist äußerst gering. Intraspezifische Variabilität der genetischen und der akustischen Merkmale von *P. albiventer* ist bemerkenswert hoch und betrifft die Populationen Nepals einerseits und jene der chinesischen Provinzen Sichuan und Hubei andererseits. Diese Unterschiede werden als gravierend angesehen und die Populationen Chinas folglich als eigene Spezies gewertet, *Pnoepyga mutica* Thayer & Bangs, 1912.

## Introduction

Wren-babblers of the genus *Pnoepyga* are tiny wren-like passerines of the Sino-Himalayan and Southeast Asian mountain forests with body sizes of only 7.5–10 cm and a body mass of 11–23 g (Martens and Eck 1995; Collar and Robson 2007). They have an almost tailless appearance due to their vestigial rectrices. Because of their secretive behaviour, including foraging and even singing in dense understorey vegetation in forests, they are difficult to observe and have thus not been intensely studied to date. Their extremely similar external morphology makes identification at the species level difficult, even as specimens in collections. This similarity is likely the reason that a third sympatric Himalayan species escaped discovery for a long time. Vocalizations have proven to be the most reliable character to distinguish this species in the field (Martens and Eck 1991).

To date only four species are accepted within *Pnoepyga* (Collar 2006; Collar and Robson 2007). The Scaly-breasted Wren-babbler, *P. albiventer*, breeds in the Himalayas from West Himachal Pradesh East to the adjacent Southwest Chinese and North Burmese mountain systems. In the past, the insular population from Taiwan has ambiguously been treated as a subspecies of *P. albiventer* (Harrap 1989; Dickinson 2003) as well as affiliated to *P. pusilla* (Cheng 1987; see Harrap 1989)—until it was granted species status (Taiwan Wren-babbler, *P. formosana*; Collar 2006; Collar and Robson 2007). The Pygmy Wren-babbler, *P. pusilla*, is the most widely distributed species and occupies a breeding range from the central and eastern Himalayas in the northwest through large parts of southern China towards populations in continental Southeast Asia, the Malaysian Peninsula and the Sunda Region. The Nepal Wren-babbler, *P. immaculata*, is restricted to the central Himalayas and has only been discovered and described relatively recently. It was determined to be distinctive—based on morphology and song—from the two further sympatrically distributed Nepal species (Martens and Eck 1991; Harrap 2011).

Though *Pnoepyga* Wren-babblers have traditionally been classified under the large family Timaliidae, recent

molecular phylogenetic studies have not confirmed even a close relationship to this family, not even to the morphologically highly similar wren-babblers of the genus *Spelaeornis* (Gelang et al. 2009). As a consequence, Gelang et al. (2009) erected the new monotypic family name of Pnoepygidae for this group, which later received positive comments by Inskipp et al. (2010) and Martens and Bahr (2011).

Previous molecular phylogenies lacked at least *P. formosana* (Päckert et al. 2012) or a second species-level taxon (Gelang et al. 2009 included *P. albiventer* and *P. pusilla* only), so phylogenetic relationships within this species-poor genus remain unresolved to date. In this paper we provide a comparative bioacoustic analysis of the highly distinctive territorial songs flanked by a molecular species-level phylogeny.

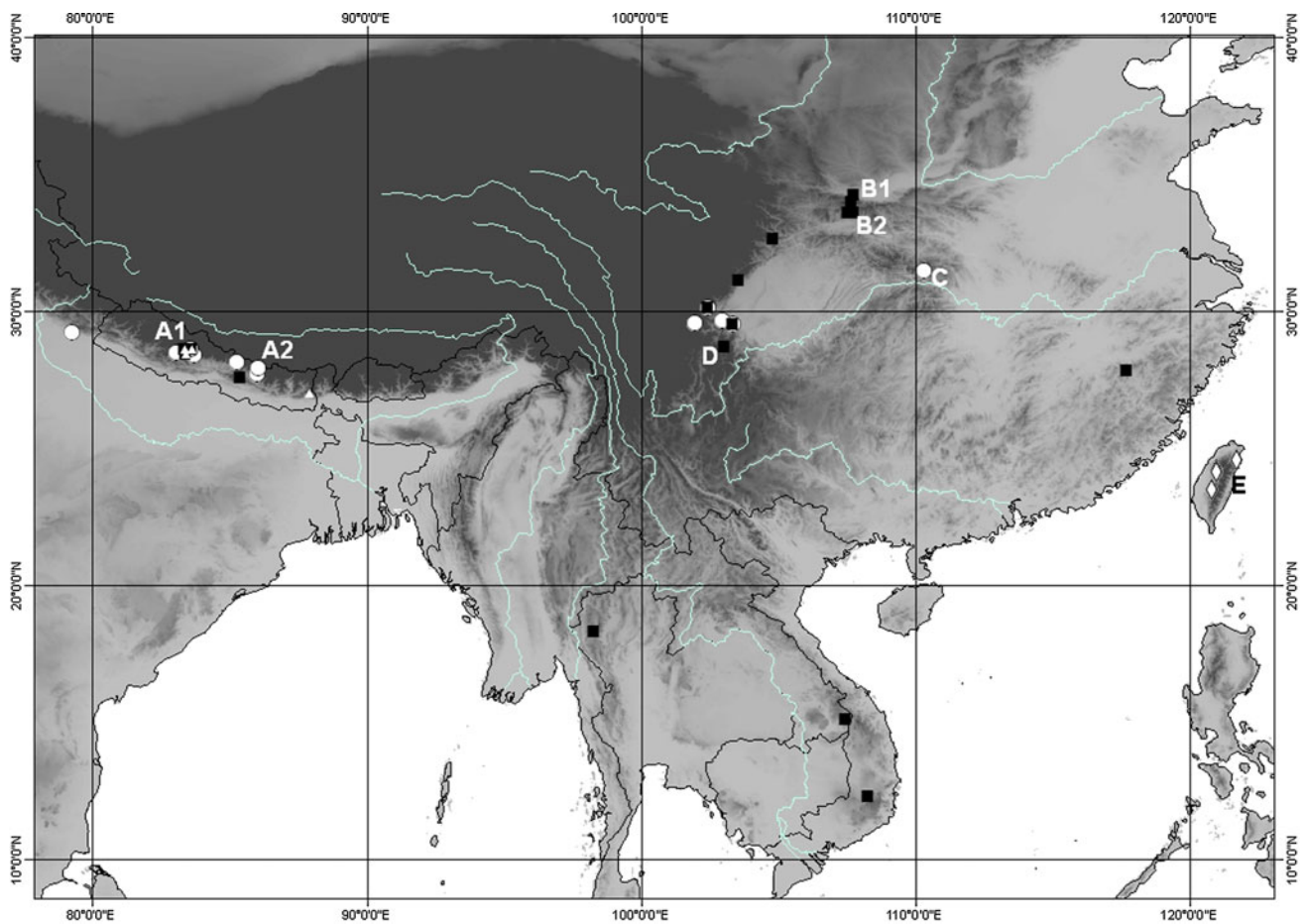
## Materials and methods

### DNA extraction, PCR

DNA was extracted from blood and tissue samples in a chloroform–isoamyl isolation procedure. From fresh samples we amplified and sequenced two mitochondrial genes [cytochrome b (cytb), NADH dehydrogenase-2 (ND2)] and three nuclear introns [glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ornithine decarboxylase (ODC), transforming growth factor beta (TGFB)], obtaining a total of 11 samples. Table 1 presents data on the origin of the samples and the GenBank accession nos; the sampling sites are indicated on Fig. 1. The primer combinations used for amplification and sequencing are provided in Electronic Supplementary Material (ESM) Table 2. The PCR protocols were as described by Päckert et al. (2010). Sequencing of the PCR products was performed using the BigDye™ v. 3.0 and v. 3.1 Dye Terminator Cycle Sequencing kits (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Reactions were electrophoresed with the ABI 377 automatic sequencer (Applied Biosystems), and the sequences were aligned by ClustalW using MEGA ver. 4 (Tamura et al. 2007) and slightly adjusted by eye.

### Sequence analysis

Phylogenetic reconstructions were based on a concatenated alignment of 3,457 bp (cytb: 1,043 bp; ND2: 961 bp; GAPDH: 280 bp; TGFB: 518 bp; ODC: 655 bp). For hierarchical outgroup rooting we used two long-tailed tit species (*Aegithalos caudatus*, *Ae. concinnus*) and one further sequence of *Spelaeornis chocolatinus*. Three further wren-babbler sequence data sets from Moyle et al. (2012) were inferred from GenBank and included in the analysis.



**Fig. 1** Origin of sound recordings (symbols) and genetic samples (uppercase letters) from *Pnoepyga* Wren-babblers. Recordings: white circles *P. albiventer*, white triangles *P. immaculata*, white

diamonds *P. formosana*, black squares *P. pusilla*. Extralimital recording sites in Malaysia and on Timor are not shown

Phylogenetic trees were reconstructed according to maximum likelihood using RAxML7.2.8 (Stamatakis 2006; using the GUI python application by Silvestro and Michalak 2011) and Bayesian inference of phylogeny with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001). The concatenated data set was partitioned by gene and codon (codon partition applied to the mitochondrial markers cytb and ND2). The appropriate substitution model for each of the five sequence data sets was estimated for each of the four genes analysed using MrModeltest ver. 2.3 (Nylander 2004), and model settings were applied accordingly to each of the partitions: cytb = GTR + I, ND2 = HKY + I +  $\Gamma$ , GAPDH = HKY + I, TGFB = HKY, ODC = GTR + I. Due to the limitations of the software in RAxML analysis, the GTR + I +  $\Gamma$  model was applied to all partitions, and model parameters were unlinked across partitions. Bayesian inference of phylogeny was carried out using the Metropolis-coupled Markov Chain Monte Carlo algorithm with two parallel runs, each with one cold and three heated chains. Convergence of the two runs was confirmed by average standard deviations of split frequencies approaching zero. The

chains ran for  $10^6$  generations with every 100th generation sampled (burn-in: 3,000). The remaining trees were used for generating a 50 % majority rule consensus tree. Maximum likelihood (ML) bootstrap support was obtained by 1,000 thorough bootstrap replicates with raxML.

Sonographic analysis

We analysed 315 songs of 73 males from all four *Pnoepyga* Wren-babbler species with Avisoft-SASLab-Pro (Fig. 1). Information on the origin and deposition of sound recordings is provided in ESM Table 3. Sonographic measurements were taken for the following 14 parameters: maximum frequency of whole song and of the first two elements ( $F_{max}$ ,  $F_{max1}$ ,  $F_{max2}$ ), minimum frequency and frequency span of song ( $F_{min}$ ,  $\Delta f = F_{max} - F_{min}$ ), frequency course of song from the first to the last element ( $\Delta F_{max} = F_{maxfinal} - F_{max1}$ ), total duration of song ( $t$ ) plus maximum and minimum duration of longest and shortest element ( $t_{max}$ ,  $t_{min}$ ), maximum and minimum frequency bandwidth of broadest and narrowest element ( $\Delta f_{max}$ ,

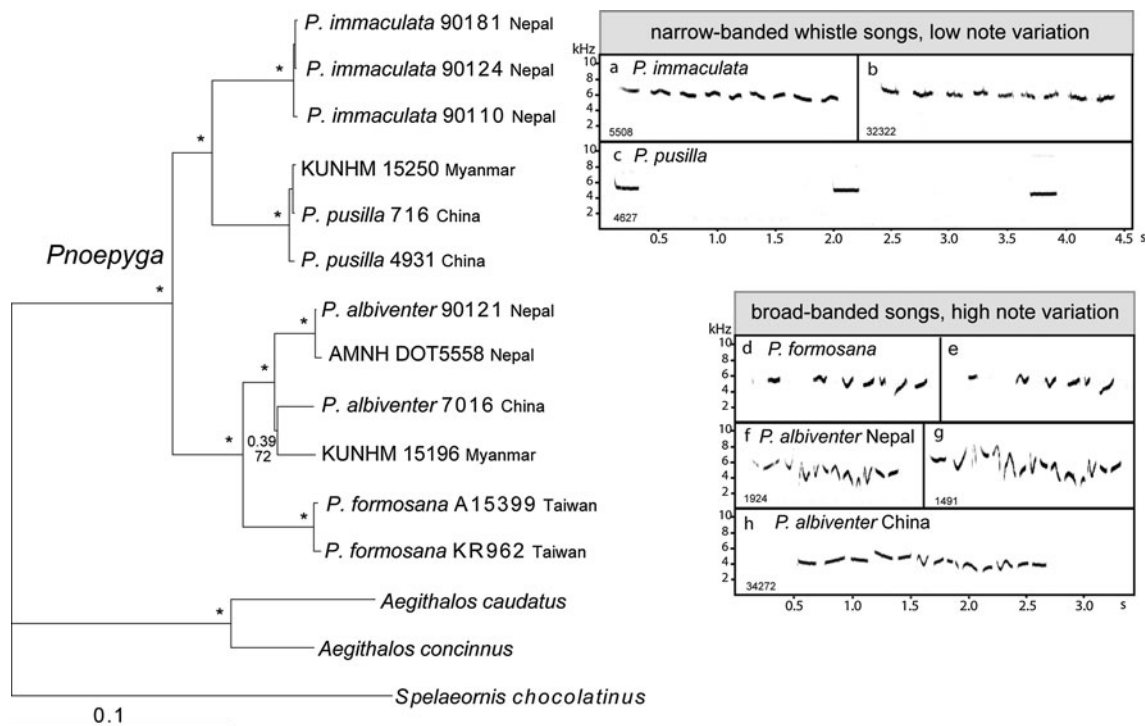
**Table 1** Origin and GenBank accession numbers of *Pnoepyga* Wren-babbler samples used for the molecular analyses

Origin and GenBank accession numbers	Sample	Specimen no. <sup>a</sup>	Species	Country	Province	Locality	Mitochondrial genes		Nuclear introns		
							Cytb	ND2	TGFB	GAPDH	ODC
	MAR 07016	MTD C64175	<i>Pnoepyga albiventer</i>	China	Hubei	Shennongjia	JF804114	JX518512	JX518505	–	JX518520
	MAR 90042	–	<i>Pnoepyga albiventer</i>	Nepal	Sindhu Palchok Distr	Dadar Danda, Kalinchok	JX518495	JX518507	–	–	–
	MAR 90121	ZFMK 2000.047	<i>Pnoepyga albiventer</i>	Nepal	Mustang Distr	Lethe Khola	JF804116	JX518508	JX518504	JX518531	JX518516
	KR962	–	<i>Pnoepyga formosana</i>	Taiwan			JX518493	JX518513	JX518501	JX518525	JX518522
	A15399	–	<i>Pnoepyga formosana</i>	Taiwan			JX518494	JX518514	JX518500	JX518524	JX518523
	MAR 90110	ZFMK 2000.044	<i>Pnoepyga immaculata</i>	Nepal	Mustang Distr	Lethe Khola	JX518489	JX518506	JX518498	JX518528	JX518519
	MAR 90124	MTD C52955	<i>Pnoepyga immaculata</i>	Nepal	Mustang Distr	Lethe Khola	JX518490	JX518509	JX518499	JX518529	JX518518
	MAR 90181	ZFMK 2000.045	<i>Pnoepyga immaculata</i>	Nepal	Myagdi Distr	Upper Myagdi Khola	JX518491	JX518510	JX518497	JX518530	JX518517
	MAR 00716	MTD C56725	<i>Pnoepyga pusilla</i>	China	Shaanxi	Taibai Shan	JX518492	JX518511	JX518503	JX518526	JX518515
	MAR 04931	MTD C62977	<i>Pnoepyga pusilla</i>	China	Shaanxi	Foping	JX518496	–	JX518502	JX518527	JX518521
	MAR 06972	MTD C63823	<i>Pnoepyga pusilla</i>	China	Sichuan	60 km NNW Meigou	JF804115	–	–	–	–
	GenBank <sup>b</sup>										
		KUNHM15196	<i>Pnoepyga albiventer</i>	Myanmar			JN827149	JN826623	JN826368	–	–
		KUNHM15250	<i>Pnoepyga pusilla</i>	Myanmar			JN827150	JN826624	JN826369	–	–
		AMNH_DOT5558	<i>Pnoepyga albiventer</i>	Nepal			JN827148	JN826622	JN826367	–	–

Cytb, cytochrome B; ND2, NADH dehydrogenase-2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ODC, ornithine decarboxylase; TGFB transforming growth factor beta

<sup>a</sup> Collection acronyms of vouchers: MTD, Senckenberg Natural History Collections, Dresden, Germany; ZFMK, Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; KUNHM, Kansas University Natural History Museum, USA; AMNH, American Museum of Natural History, New York, USA

<sup>b</sup> Three sequence data sets from Moyle et al. (2012) were inferred from GenBank and added to the analysis



**Fig. 2** *Left* Bayesian multigene phylogeny for *Pnoepyga* Wren-babblers based on 3,457 bp of mitochondrial cytochrome b (1,043 bp) and NADH dehydrogenase-2 (961 bp) and nuclear introns of glyceraldehyde-3-phosphate dehydrogenase (280 bp), transforming growth factor beta (518 bp) and ornithine decarboxylase (655 bp).

*Markov Chain 1,000,000 generations, burning = 3,000, partitioned by gene and codon. Asterisk indicates full nodal support from Bayesian and likelihood analysis (lower posterior probabilities and bootstrap values shown at one weakly supported node). Right* Sonograms of territorial songs

$\Delta_{fmin}$ ), total number of elements ( $z$ ) and relative number of whistle elements as percentage of  $z$  ( $z_{rel\_whistle} = z_{whistle}/z$ ) and duration of interval between first and second element ( $\Delta t$ ). Among- and within-species differentiation of song was analysed using principal component analysis (PCA) and discriminant analysis with SPSS ver. 11.5 (SPSS, Chicago, IL). Cluster analysis dendrograms were built with PAST.

**Results**

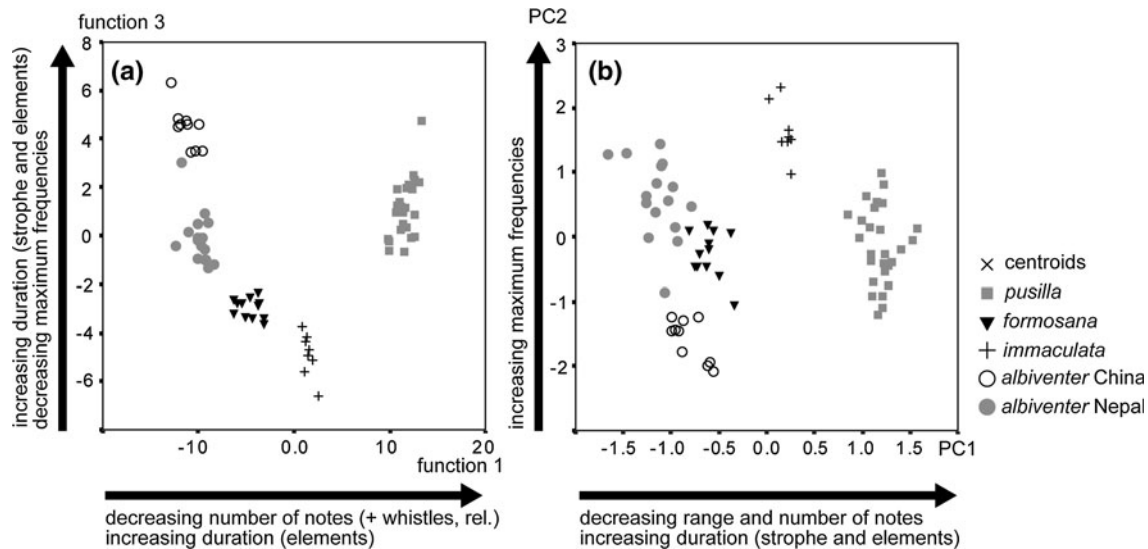
**Molecular phylogeny**

All phylogenetic reconstructions separated two sister species pairs, *P. albiventer* and *P. formosana*, on one clade in opposition to *P. immaculata* and *P. pusilla* on a second clade. The Bayesian tree reconstruction is shown in Fig. 2, and its topology did not differ from that inferred from the ML reconstructions. Almost all nodes of the *Pnoepyga* clade received strong support from both reconstructions (Fig. 2), and even when phylogeny was inferred from nuclear sequence data alone (1,454 bp) the same nodes received full Bayesian support (except for internal nodes of *P. albiventer* because the two GenBank sequences with

only one of the three nuclear markers available were excluded from analysis). Considerable intraspecific differentiation was found among Chinese and Himalayan samples of *P. albiventer*, which were separated by a rather deep split from each other and from a third lineage from Myanmar for which sequence data were inferred from GenBank (Fig. 2). However, the interior topology of the *P. albiventer* clade was not resolved among the three equally diverged lineages. The three *albiventer* lineages differed from each other by genetic distances of mitochondrial DNA (cytb, uncorrected  $p$  distances) of 4.0 % and from *P. formosana* by values of 7.3–8.1 %. Genetic distances among *P. immaculata* and *P. pusilla* ranged at 5.9 %. The phylogeny inferred from the nuclear intron data alone was identical to the total evidence tree in Fig. 2 and received full support at all nodes (however, the three GenBank specimens were excluded from the analysis because only TGFB was available for these; tree not shown).

**Song differentiation**

Sonograms of territorial songs are indicated at the associated clades of the *Pnoepyga* tree in Fig. 2. The pure-whistle songs of *P. pusilla* represent the most simple song



**Fig. 3** Differentiation of territorial songs among *Pnoepyga* species based on the measurements of 14 song parameters (whistles, rel. = relative number of whistles). **a** Discriminant analysis, **b** principal component analysis of individual song parameter means

structure of the genus. In these pure-whistle songs, usually three—but in the Himalayas and in parts of continental Southeast Asia there are only two—long whistle notes were found (often starting with a steep downstroke) with rather long intervals in between, the first note slightly longer than the second and higher in pitch. In other parts of the range, three-note songs are given in a series of whistles slightly descending in frequency from note to note (Fig. 2c). Whistle series with more than three elements are rare and were only occasionally found beyond the Nepal breeding range of the species, such as in Indonesia. Songs of Himalayan *P. immaculata* have the highest pitch compared to the other three species, consisting of a more rapid series of moderately modulated short whistles slightly descending in frequency (Fig. 2a, b). Songs of the other two species differ strongly from the typical whistled songs of the latter two by a greater variation in different element types, covering a much larger frequency range. Generally, songs of *P. albiventer* and *P. formosana* start with two or three whistled notes (that might be slightly ascending or descending in frequency) which are then followed by a variety of highly modulated hook-shaped elements (either opened upwards or downwards). Intraspecific variation is insignificant. Apparently, the individual male produces only one verse type, which is repeated with utmost accuracy. Thus, every male can be recognized by a single verse. Also, inter-individual verses within Nepal are quite similar and even identical in parts of the verse; for example, eight males from various areas in West and East Nepal sang identical sequences of the last four elements in all verses (sonagrams in Martens and Eck 1991).

On a larger geographic scale, intraspecific song variation in *P. albiventer* is apparent. Differences among the two regions (Nepal and China) were significant for all frequency and time parameters of song except for minimum frequency  $F_{\min}$  (Mann–Whitney  $U$  test  $p < 0.05$ ). Compared to the songs of the Nepal populations, songs from China (Sichuan and Hubei Provinces) are longer, with a mean duration of 1.98 s (compared to 1.48 s in Nepal, Mann–Whitney  $U$  test  $p < 0.01$ ) and have a considerably smaller mean bandwidth of 3.5 kHz (compared to 4.7 kHz in Nepal; Mann–Whitney  $U$  test,  $p < 0.01$ ; Fig. 1f–h). Songs from Sichuan and Hubei populations were considerably homogeneous among localities. Two males from Omei Shan from different localities sang the last seven elements in an identical manner in each verse (sonagrams in Martens 1998), and two additional males from different sites shared the last five elements. Each male performed a single song type only.

Discriminant analysis largely distinguished five clusters and achieved 100 % correct assignment of males to the corresponding species. The two *P. albiventer* subspecies from Nepal and China were separated into distinct groups (with 100 % correct assignment, although one male from Nepal closely approached the Chinese cluster in the scatterplot, Fig. 3a). The first four functions were used in the analysis (all had Eigenvalues  $>1$ ); function 1 and 2 explained a cumulative 92 % of the total variance. Only one variable,  $\Delta f$ , failed the tolerance test and was subsequently excluded from the analysis. The highest correlations occurred among function 1 and  $t_{\min}$ ,  $z_{el}$  and  $z_{rel\_whis}$ , and the highest correlation coefficients of function 2 were likewise associated with the latter two element variables.

However, none of the parameters showed the highest correlations with function 2; thus, the discriminant scatterplot (Fig. 3a) was created by plotting functions 1 and 3 against each other. The highest correlations with function 3 occurred with the following parameters:  $F_{\min}$ ,  $F_{\max 1}$ ,  $F_{\max 2}$ ,  $\Delta t$ ,  $t$ ,  $t_{\max}$ .

In the PCA, the first three principal components had Eigenvalues of  $>1$  and explained a cumulative 83 % of the total variation of the song parameters. The first two principal components were loaded by roughly the same variables that correlated most strongly with discriminant functions 1 and 2 (temporal parameters and number of elements for component 1 and maximum frequencies for component 2). The scatterplot of the first two principal components separated the same groups as found in the discriminant analysis, however, with a less clear distinction among the two *P. albiventer* populations and *P. formosana* (Fig. 3b).

In the hierarchical cluster analysis, nearly all individuals of the same genetic lineage fell into the same cluster (dendrograms not shown). Only one *albiventer* male from Nepal was nested in the Chinese *albiventer* clade in both clustering methods (male 31731, outlier from the grey *P. albiventer* cluster in Fig. 3a, b). This male performed extremely low-pitched and long songs with 15 elements per strophe ( $z < 14$  in all other Nepal males). In the paired-group clustering, the *P. albiventer* cluster was opposed to the three remaining clusters, while in single-linkage clustering the *P. pusilla* cluster was opposed to the remaining three groups. The single-linkage dendrogram showed a second *P. albiventer* male from Nepal as an outlier basal to all *P. albiventer*, *P. formosana* and *P. immaculata*. Songs of this male (male 1491; Fig. 2g) differed from those of other Nepal *albiventer* birds by having a higher frequency pitch, broader bandwidth and higher number of elements ( $z = 15$ , as for male 31731).

## Discussion

### Phylogeny and distribution

The two *Pnoepyga* species pairs represent a quite common phylogeographic pattern in Southeast Asian passerines: a subtropical foothill clade opposed to a temperate/subalpine clade of the high-elevation forests (Päckert et al. 2012). Elevational extent of breeding ranges in the following comparisons refers to Martens and Eck (1995) and to Collar and Robson (2007). In the Himalayas, the breeding range of *P. albiventer* covers mid- and high-elevation forests between 2,370 up to 4,000 m a.s.l., and *P. formosana* reaches up to 2,780 m a.s.l. on Taiwan. In contrast, *P. immaculata* is found breeding in low- to mid-elevation Himalayan forests between 1,730 and 3,100 m a.s.l., and

*P. pusilla* is found at even lower elevations between 1,500 and 2,640 m a.s.l. (however, with local records up to 3,050 m a.s.l.). Except for the crucial case of the allopatric Taiwan form *formosana* (see below), the biological species status of continental *Pnoepyga* species is undoubted because in the Nepal Himalayas the three mainland species display territorial song in close sympatry and even syntopy (Martens and Eck 1995).

Due to the limited number of species in the genus, it is hard to judge whether shared song patterns of sister species originated from a common ancestry or from common habitat preferences. In fact, low-altitude breeders sing simple whistled songs of a narrow bandwidth—however, the voice of *P. pusilla* is considerably lower and even more narrow-banded than that of *P. immaculata*. In Nepal, *P. pusilla* is restricted to low-bush and herb vegetation along fast-running and noisy mountain streams and is hardly to be heard in habitats away from running water (Martens and Eck 1991). In view of the exclusively whistled song, the relatively high frequencies used and the known habitat requirements, Martens and Geduldig (1990) regarded *P. pusilla* as a member of the mountain passerine torrent-accompanying guild. Their songs are characterized by pure whistles and a high frequency to overcome the noise of running water. Among those species range some ten additional ones, such as *Phylloscopus magnirostris* and *Stachyris nigriceps* (see Martens and Geduldig 1990). Also, *P. immaculata*, with similar vocal characteristics, can be regarded as a torrent-accompanying species occupying habitats close to fast-flowing streams and rivers (Collar and Robson 2007); it is most often encountered in deeply recessed narrow valleys densely covered by bush and herb vegetation. In contrast, *P. albiventer* in the Sino-Himalayas partly prefers forest types of the upper rather Palearctic climate belt with less densely overgrown and lighter bush and herb layers (Martens and Eck 1995). Thus, vocal differences among the two species pairs might be further enhanced by vocal adaptation to habitat structure because in dense vegetation birds tend to sing at lower frequencies and at a narrower bandwidth compared to males of their own or closely related species adapted to open habitats (Hunter and Krebs 1979; Boncoraglio and Saino 2007). Badyaev and Leaf (1997) found that in closed habitats, leaf warbler songs comprise rather un-modulated notes separated by greater intervals—a strongly distinctive song characteristic of *P. pusilla*, which compared to its congeners occupies the lowest elevation belt and nearly exclusively forages in dense understorey.

### Taxonomy

A considerable number of specific and subspecific names have been proposed for *P. albiventer* over the years. To the

Himalayas belong *pallidior* Kinnear, 1924 (West Himalayas), *albiventer* Hodgson, 1837 (central and East Himalayas), *squamata* Gould, 1837 (Himalayas), *unicolor* Hodgson, 1845 (central Himalayas) and *vegeta* Koelz, 1954 (Northeast India, Naga Hills). From China originate the names *mutica* Thayer and Bangs, 1912 (from Wa Shan, Sichuan; as a species of its own) and *magnirostris* Rothschild, 1925 [Cheng (1987) gives the type locality as Longchuan River, Yunnan]. Except for *pallidior*, Mayr and Paynter (1964) already synonymized all of these taxa with the nominate form *albiventer*. Central Himalayan *unicolor* had already been recognized as a juvenile form of *albiventer* by Sharpe (1881) and has not been in use since that time (see Martens and Eck 1991). Wolters (1980) largely followed this classification but apparently overlooked *vegeta* and *magnirostris*. Thus, to date, subspecific classification within *P. albiventer* (Dickinson 2003) is confined to *P. a. albiventer*, *P. a. pallidior* and *P. a. formosana*. The latter term is confined to Taiwan (for status, see following text), and all China presently is covered by nominate *albiventer*. However, Chinese and Himalayan populations distinctly differ by molecular genetics and in territorial song. Thus, a separate name for Chinese birds is appropriate, and taxon *mutica* Thayer and Bangs (type locality Wa Shan, Sichuan) is available for these. Additionally, differences in both marker systems (molecular and bioacoustic) are so pronounced that we affiliate biospecies rank to the Chinese populations and herewith revalidate the appropriate name: *Pnoepyga mutica* Thayer and Bangs, 1912. Objections to this taxonomic decision are likely because molecular analyses of the *mutica* type specimen have not been performed as yet. However, one of our song recordings originates from the type locality (no. 30822; archive J. Martens), and we counterchecked the correct affiliation of our field recording site Wawu Shan (102°56'E 29°39'N, located East of former Kiating, now Leshan) to the historical name Wa Shan. This name is correctly indicated at the place now called Wawu Shan in the original expedition diary map sketches of the botanical expedition of E. H. Wilson on which W. R. Zappety worked as the bird collector (Thayer and Bangs 1912). Diaries are deposited at Harvard University and are freely available via the internet (Wilson 1896–1952). Furthermore, all other China *albiventer* songs analysed exhibit the characters described above (six localities from Sichuan and Hubei Provinces, ten males recorded). We therefore assume that the name *mutica* is correctly affiliated to all of them. The type locality Wa Shan, now Wawu Shan, received some publicity through the rediscovery of the Sichuan treecreeper *Certhia tianquanensis* (Martens et al. 2002). It would be important to learn where the Himalayan *albiventer* and Chinese *mutica* actually meet—if at all—and how they may behave in local contact.

Whether the separate genetic *P. albiventer* lineage from Myanmar represents a taxon of its own remains to be verified on a larger basis of specimens and voice recordings. Until then we refrain from providing explicit taxonomic recommendations.

The species status of *P. formosana* is well confirmed by our data due to its distinctiveness in both molecular markers and territorial song. After the systematic position of this taxon had changed several times as a subspecies of *P. albiventer* and *P. pusilla* (see Cheng 1987; Harrap 1989), it was finally affiliated to *P. albiventer* (Dickinson 2003). However, Harrap (1989) and Inskipp et al. (1996) had already suggested species status of *formosana* with respect to differences in morphology and acoustics, and Collar (2006) finally took action and elevated *formosana* to species rank. P.I. Holt (in Collar 2006) found that territorial *formosana* males did not react to the playback of Chinese *pusilla* and Chinese *albiventer* songs—an additional important hint to the biospecies status of *formosana*.

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