

Intraspecific differentiation of Sino-Himalayan bush-dwelling *Phylloscopus* leaf warblers, with description of two new taxa (*P. fuscatus*, *P. fuligiventer*, *P. affinis*, *P. armandii*, *P. subaffinis*) *

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> Abstract

We investigated external morphology, molecular genetics and bioacoustics of the Chinese leaf warblers *Phylloscopus fuscatus*, *P. fuligiventer*, *P. affinis*, *P. armandii* and *P. subaffinis*, a set of predominantly high-altitude species of open bushy habitats. *Phylloscopus fuscatus* and *P. fuligiventer* were found to be sister taxa; the current subspecies of *P. fuscatus* appeared to be paraphyletic. *P. fuscatus weigoldi* (Qinghai) is excluded from *P. fuscatus* and transferred to *P. fuligiventer* as a subspecies (formerly E Himalayas/SE Tibet only). Several individuals of nominate *fuscatus* carrying private haplotypes were found in China during the breeding season and may locally breed there (Shaanxi, Gansu). Genotyping of type specimens of *P. fuscatus robustus* yielded a composite type series including nominate *fuscatus*. A lectotype of taxon *robustus* is designated since the holotype was lost in World War II. – *Phylloscopus affinis* falls into three distinct population groups which differ by exhibiting marked molecular genetic distance values, in coloration and in vocalisations. Central Asian *Phylloscopus griseolus* is firmly nested in the *P. affinis* clade. The Himalayan (incl. SE Tibet) populations of *P. affinis* on the one hand and the Chinese populations (Yunnan, Sichuan, Qinghai, Gansu) on the other are differentiated on the species level. For the latter populations a new name is introduced (*P. occisinensis* nov. sp.). The Himalayan population group itself is subdivided into a western (*P. affinis perflavus* nov. ssp.) and an eastern group (nominate *affinis*). – *Phylloscopus armandii* generally is subdivided into a larger northern (nominate *armandii*) and a smaller southern Chinese population (ssp. *perplexus*). Their *cytb* and 16S distance values are remarkably high but subspecies status is retained because of only subtle morphological differences and a lack of acoustic divergence. – *Phylloscopus subaffinis* is unstructured with respect to *cytb* and 16S genes throughout China.

> Kurzfassung

Diese Arbeit stellt die äußere Morphologie, zumeist Färbung und Farbmuster, Molekulargenetik und Bioakustik einer Gruppe chinesischer Laubsänger dar, die überwiegend im Hochgebirge in offenen, buschreichen Lebensräumen vorkommen: *Phylloscopus fuscatus*, *P. fuligiventer*, *P. affinis*, *P. armandii* und *P. subaffinis*. *P. fuscatus* und *P. fuligiventer* erwiesen sich als Schwesterarten und die derzeit anerkannten Unterarten von *P. fuscatus* als paraphyletisch. *P. fuscatus weigoldi* (Qinghai) wird von *P. fuscatus* abgetrennt und als Unterart *P. fuligiventer* zugeschlagen (dessen Vorkommen zuvor nur im E Himalaya und SE Tibet). Einige Individuen der Nominatform von *P. fuscatus*, die eigenständige Haplotypen aufwiesen, wurden während der Brutzeit in China gefunden, und sie mögen dort lokal sogar brüten (Shaanxi, Gansu). Die genotypische Charakterisierung der beiden noch erhaltenen Exemplare der Paratypenserie von *P. fuscatus robustus* ergab die Zugehörigkeit zu zwei verschiedenen Gruppen, neben der chinesischen Unterart *robustus* auch die nördliche Nominatform *fuscatus*. Da der Holotypus von *P. fuscatus robustus* im 2. Weltkrieg verloren ging, wird für *robustus* ein Lectotypus bestimmt. – *Phylloscopus affinis* gliedert sich in drei klar umgrenzte Populationsgruppen, die sich durch erhebliche molekulargenetische Distanzwerte, Färbung und Farbmuster unterscheiden. Der zentralasiatische *P. griseolus* ist molekulargenetisch klar dem *affinis*-Ast zugeordnet. Die Himalaya-Populationen von *P. affinis* (mit SE Tibet) einerseits und die chinesischen Populationen aus Yunnan, Sichuan, Qinghai und Gansu andererseits sind auf dem Artniveau differenziert. Für diese Populationen wird ein neuer Name eingeführt, *Phylloscopus occisinensis* nov. sp. Die Himalaya-Population ist zweifach untergliedert, in eine westliche Gruppe (*P. affinis perflavus* nov. ssp.) und in eine östliche Gruppe, die Nominatform *P. affinis affinis*. – *Phylloscopus armandii* ist in eine nördliche Unterart mit größerem Areal (die Nominatform *armandii*) und eine südlichere Unterart (ssp. *perplexus*) mit

kleinerem Areal gegliedert. Ihre Cytochrom-*b*-Distanzwerte sind bemerkenswert hoch; Färbungsunterschiede sind erkennbar. Dennoch wird Unterartstatus für beide beibehalten, da in ihren Gesängen keine Unterschiede gefunden werden konnten. – *Phylloscopus subaffinis* ist über ganz China im Cytochrom-*b*-Gen und 16S-Gen undifferenziert.

> Key words

Old World warblers, *Phylloscopus fuscatus*, *Phylloscopus affinis*, *Phylloscopus occisinensis* nov. sp., *Phylloscopus affinis perflavus* nov. ssp., cytochrome *b*, 16S rRNA, phylogeny, bioacoustics, Sino-Himalaya.

Introduction

On no other Old World group of passerine birds have two new approaches, bioacoustics and molecular genetics, impinged as widely as on *Phylloscopus* and *Seicercus* warblers. Consequently the related developments in evolutionary insight, systematics and taxonomic change were severe and revolutionary (ALSTRÖM, 2006; RHEINDT, 2006). Prior to the invention of molecular sequencing techniques, vocal differences among large *Phylloscopus* populations of current species gave hints of distinct differentiation, but following this insight only a few new species limits were tentatively drawn (HELB *et al.*; 1982, ALSTRÖM & OLSSON, 1990). Only when molecular genetic techniques and their results gained priority in the argumentation did the split of current species into sister species or even species swarms become commonplace. This process coincided with the onset of a vivid discussion of species concepts in ornithology which resulted in a generally distinctly narrower circumscription of “species” than the Mayrian Biological Species Concept admits. However, the rush of “new” species in *Phylloscopus* is not only due to new techniques and a narrowed concept of species. Both aspects enabled a broader understanding, namely that the uniform *Phylloscopus* morphology hides an enormous, historically developed diversity that cannot be recognized otherwise than by analysis of acoustic behaviour and molecular genetics. The 30 species which TICEHURST (1938) accepted became the 56 species that were – still quite restrictively – recognized by DEL HOYO *et al.* (2006).

Most taxonomic changes involved inhabitants of the Sino-Himalayas, a hot spot of diversity in Old World faunas. Up to now only the numerous species of arboreal habitats were investigated, while open-country species were neglected. In the Sino-Himalayan area arboreal passerines, not only in *Phylloscopus*, follow a quite distinct pattern of diversification (JOHANSSON *et al.*, 2007; MARTENS & TIETZE, 2006), but that of open-country species may differ. Open habitat in the Sino-Himalayas is widespread. Large parts of the extended Tibet-Qinghai plateau are covered at best

by bushes, and so are vast areas above timber line in the Himalayas and in Chinese high-altitude mountain chains. These areas are also inhabited by *Phylloscopus* warblers, though by a much smaller species set than that of the adjacent forested mountain regions. Here we focus on four current *Phylloscopus* species hitherto untouched by analysis employing modern techniques. We analyze their molecular genetic differentiation pattern, the voices of representative populations and their differences in morphology. The species in question are the Dusky Warbler *Phylloscopus fuscatus* (BLYTH, 1842) including Smoky Warbler *P. fuligiventer* (HODGSON, 1845), Tickell’s Warbler *P. affinis* (TICKELL, 1833), Sulfur-bellied Warbler *P. griseolus* BLYTH, 1847, Yellow-streaked Warbler *P. armandii* MILNE-EDWARDS, 1865 and Buff-throated Warbler *P. subaffinis* OGILVIE-GRANT, 1900.

Material and Methods

Molecular genetics

For sequence analysis altogether 65 samples from six current Old World warbler species were available: 23 samples of *P. fuscatus*, including *P. fuligiventer*, 18 samples of *P. affinis*, 13 samples of *P. armandii*, four samples of *P. griseolus* and seven samples of *P. subaffinis*. For origin of samples see Table 1 (Appendix) and Figs 1, 2 and 3. For phylogenetic reconstruction several *Phylloscopus* sequences of the target species groups from GenBank were incorporated in the dataset (*P. fuscatus*: DQ17599-DQ17603, Y10729; *P. affinis*: Y10730, L77128, DQ008503; *P. griseolus*: L77137; *P. schwarzi*: AY635061). 22 further *Phylloscopus* taxa (and *Seicercus grammiceps*) were used for sequence analysis and phylogenetic reconstructions based on a concatenated cytochrome-*b* and 16S rRNA data set (Table 1). For hierarchical outgroup rooting we used sequences from warblers and other species belonging

to several genera closely and more distantly related to *Phylloscopus* (*Acrocephalus scirpaceus*, *Abroscopus schisticeps*, *Regulus calendula*).

A 706-bp fragment of the mitochondrial cytochrome-*b* gene was amplified using the primer combination L14841-Cytb (5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3', KOCHER *et al.*, 1989) and H-15547-Cytb (5'-AAT AGG AAG TAT CAT TCG GGT TTG ATG-3', EDWARDS *et al.*, 1991). The PCR protocol was 94° C for 2 min followed by 35 cycles of 92° C for 45 s, 56° C for 1 min and 72° C for 1.5 min with a final extension in 72° C for 5 min. A larger 1079-bp-long fragment was amplified for at least one sample of each taxon investigated with the primer combination L14841 and H15197 (5'-TAG TTG GCC AAT GAT GAT GAA TGG GTG TTC TAC TGG TT-3'). The PCR protocol was 94° C for 10 min followed by 35 cycles of 92° C for 1 min, 53° C for 1 min and 72° C for 2 min with a final extension in 72° C for 10 min. We furthermore amplified a fragment of about 2000 bp of 16S ribosomal RNA for a reduced sample set with each warbler taxon represented by at least one sample. For double-stranded PCR we used the primer combination 12Sa and 16Sbr (PALUMBI *et al.*, 1991). For amplification of the 16Sr RNA fragment we followed the protocol of SPICER & DUNIPACE (2002) with 94° C for 45 s and 30 cycles of 92° C for 45 s, 50° C for 1 min and 72° C for 2 min. From a number of museum specimens DNA was extracted from toe pads which were cut from the toes with sharp scalpel without destroying the toes. DNA extraction from toe pads was carried out with AGOWA@sbeadex Forensic Kit (AGOWA GmbH). Small fragments of about 100–120 bp cytochrome-*b* each were amplified with seven primer combinations, some of them newly designed (see Table 2). In order to prevent contamination, DNA of museum specimens was analysed in a separate lab room for ancient DNA analysis. Sampling, extraction and PCR were carried out under separate sterile benches. Alignment of up to seven fragments yielded short *cytb* sequences of maximally 422 bp, which were used in order to determine the affiliation of name-bearing specimens to distinct mitochondrial lineages.

Sequencing of the PCR products was performed with BigDye™ v. 3.0 and v. 3.1 Dye Terminator Cycle Sequencing Kits (Applied Biosystems) according to the manufacturer's instructions, using L14841-Cytb, L15547-Cytb and 16S500 (SPICER & DUNIPACE, 2002) as sequencing primers, and reactions were electrophoresed with the ABI 377 automatic sequencer. The sequences were aligned by ClustalW using MEGA 3.1 and slightly adjusted by eye. All sequences used for the analysis were deposited at GenBank under the accession numbers FJ155853 – FJ155906 (cytochrome *b*) and EU794911 – EU794952 (16Sr RNA).

An appropriate substitution model for the molecular data set was estimated via Likelihood Ratio Tests with Modeltest 3.04. (POSADA & CRANDALL, 1998). According to Akaike information criterion the best fit model for the cytochrome-*b* dataset was K81uf+G a modification of the K80 model, which in addition includes a transitions/transversions bias and also allows for two different transversion rates (KIMURA, 1980, 1981). Compared to the K80 model, K81uf assumes unequal base frequencies. Likelihood settings were estimated as follows: empirical base frequencies: $\pi A = 0.3039$, $\pi C = 0.3848$, $\pi G = 0.1142$, $\pi T = 0.1971$; proportion of invariable sites $I = 0$; gamma shape parameter $\alpha = 0.1592$; rate matrix: $R(a)[A-C] = 1.0000$, $R(b)[A-G] = 9.2212$, $R(c)[A-T] = 0.4854$, $R(d)[C-G] = 0.4854$, $R(e)[C-T] = 9.2212$, $R(f)[G-T] = 1.0000$. Likewise the best fit model for phylogenetic reconstruction using Bayesian Weights analysis was estimated with MrModeltest. According to the Akaike information criterion, the best fit model for the cytochrome-*b* dataset was HKY+G with the following model settings: empirical base frequencies: $\pi A = 0.3115$, $\pi C = 0.3904$, $\pi G = 0.1088$, $\pi T = 0.1893$; proportion of invariable sites $I = 0$; gamma shape parameter $\alpha = 0.1515$; Ti/tv ratio = 5.4819. Model settings for analysis of the 16Sr RNA data set were estimated as follows: GTR+I+G; empirical base frequencies: $\pi A = 0.3697$, $\pi C = 0.2259$, $\pi G = 0.1822$, $\pi T = 0.2223$; proportion of invariable sites $I = 0.6527$; gamma shape parameter $\alpha = 0.4321$; rate matrix: $R(a)[A-C] = 4.1812$, $R(b)[A-G] = 6.8206$, $R(c)[A-T] = 2.0178$, $R(d)[C-G] = 0.0000$, $R(e)[C-T] = 21.5362$, $R(f)[G-T] = 1.0000$.

Phylogenetic trees were constructed under different approaches: Neighbor Joining and Maximum Parsimony (NJ, MP; SAITOU & NEI, 1987; with MEGA 3.1), Maximum Likelihood (ML, TreePuzzle, SCHMIDT *et al.*, 2000) and Bayesian inference of phylogeny (with MrBayes; HUELSENBECK & RONQUIST, 2001). Robustness of clades was estimated by 1000 bootstrap replicates (FELSENSTEIN, 1985), via quartet puzzling in ML with 1000 puzzling steps (STRIMMER & VON HAESLER, 1996), and via Bayesian posterior probabilities using Markov chain Monte Carlo (MCMC, 1,000,000 generations, burnin = 5000). Tree reconstruction from concatenated *cyt-b* and 16Sr RNA sequences was carried out with MrBayes 3.1.2 under a partitioned model according to the manual of MrBayes 3.1.: the best fit GTR + I + G model and the HKY model were applied to the according sequence partitions and the overall rate was allowed to vary between partitions by setting the priors <ratepr=variable>. Model parameters such as gamma shape, proportion of invariable sites etc. were unlinked across partitions, so that for each partition its own set of parameters was estimated. In order to verify the molecular clock hypothesis, a likelihood ratio test was carried out with Tree Puzzle (SCHMIDT

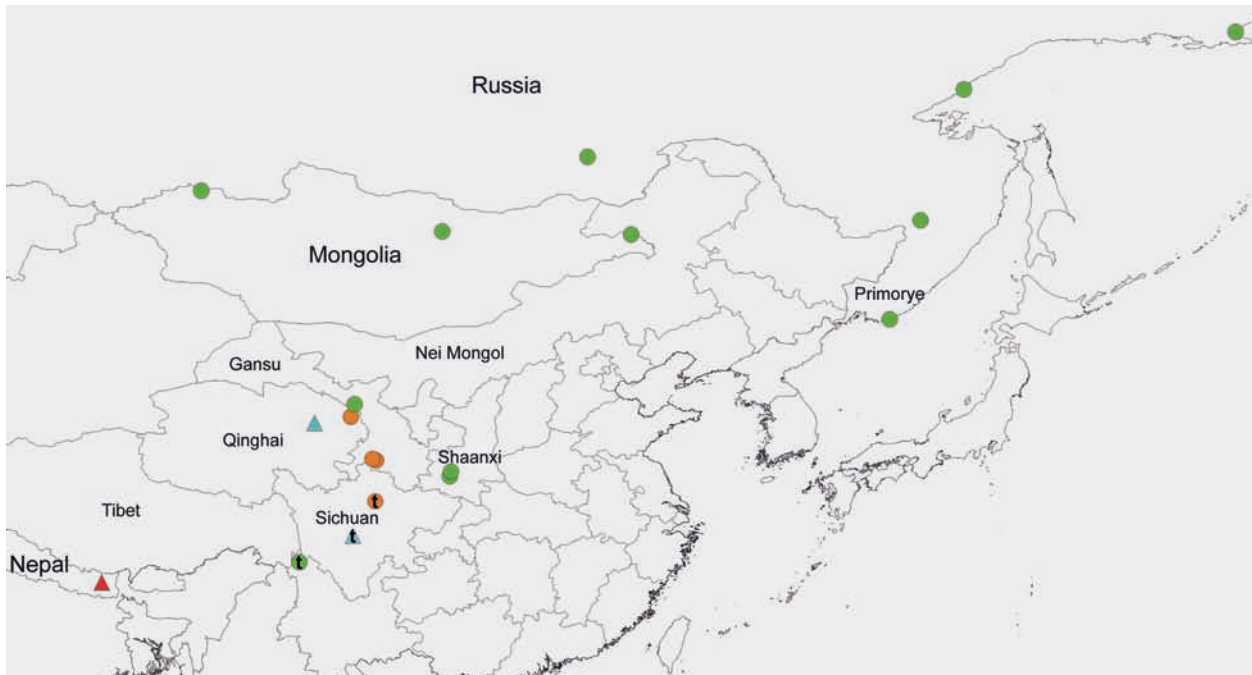


Fig. 1. Origin of samples from the Dusky Warbler complex (*Phylloscopus fuscatus* and *P. fuligiventer*). **Circles, green:** *P. fuscatus fuscatus*; **circles, orange:** *P. fuscatus robustus*; **triangles, blue:** *P. fuligiventer weigoldi*; **triangles, red:** *P. fuligiventer fuligiventer*. Type localities indicated by “t”.

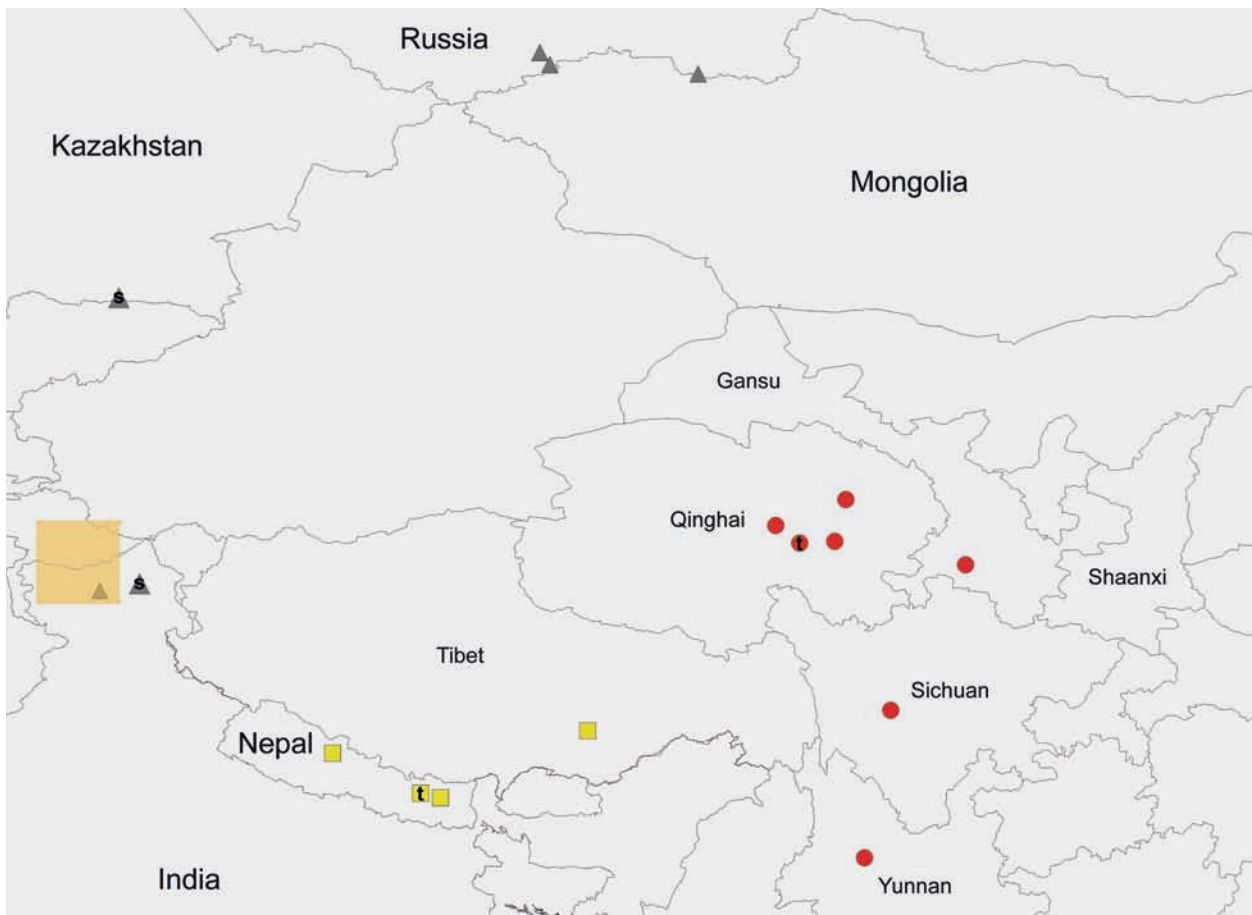


Fig. 2. Origin of samples from the Tickell's Warbler complex (*P. affinis* s.l. and *P. griseolus*). **Squares, yellow:** *P. affinis affinis*; **big square, olive-green shaded:** *P. affinis perflavus* nov. ssp., approximate collecting localities of BMNH specimens and gene sequences deposited at GenBank; **circles, red:** *P. occisnensis* nov. sp.; **triangles, grey:** *P. griseolus*. Type specimens indicated by “t”, specimens identified by song only indicated by “s”.

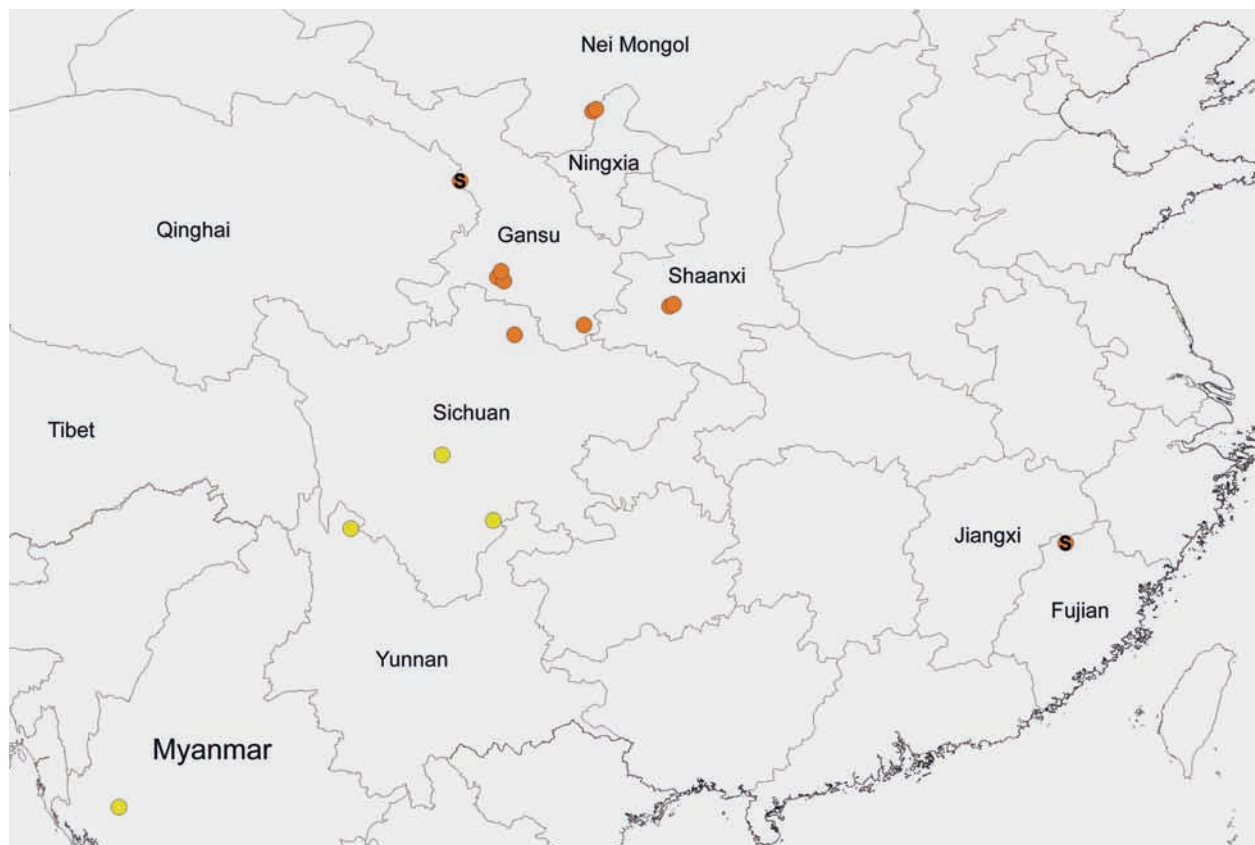


Fig. 3. Origin of samples from the Yellow-streaked Warbler, *P. armandii*. **Circles, orange:** *P. armandii armandii*; **circles, yellow:** *P. armandii perplexus*; specimens identified by song only indicated by “s” (subspecies affiliation not proven).

Tab. 2. Primers used for PCR and sequencing of short cytochrome-*b* fragments from museum specimens; primers without reference were newly designed for this study.

Primer	bp	sequence (5' to 3')	reference
Lcytb-1_new	19	G C Y C A C A T A T G C C G A A A C G	—
Hcytb-1_new	19	T A T G A R C C G T A G T A G A A T C	—
Lcytb0_new	23	T G C A T C T A C T T C C A C A T C G G Y C G	—
Hcytb0_new	21	T A G G A C G T A G C C T A C G A A A G C	—
L15087-Cytb	23	T A C T T A A A C A A A G A A A C C T G A A A	Edwards et al. 1991
H15419-Cytb	34	A A A A C T G C A G C C C T C A G A A T G A T A T T T G T C C T C	Kocher et al. 1989
L15141-Cytb	25	G C C A C T G C T T T C G T A G G C T A C G T C C	—
H454_mod	27	T G T T T G A C C A A T G T A T G G G A T C G C T G A	Ericson et al. 2005
L15120-Cytb	24	A A Y C T A T T C T C A G C G A T C C C A T A C	—
H814_mod	29	A T G A C G A A T G G A A A G A G G A A G T G G A G A G C	Ericson et al. 2005
Lcytb3_new	24	A G T A G A C A A C C C M A C C C T A A C Y C G	—
H598_mod	22	G G R A T G C C T A G K G G G T T G T T T G	Ericson et al. 2005
Lcytb4_new	21	A C C C T A G T C C A Y C T R A C C C T C	—
Hcytb4_new	22	T A G G A T G T C T T T T G T G G A G T A G	—

et al., 2000). The clocklike tree could not be rejected on the $P < 0.05$ level for the entire cytochrome *b* sequence dataset of *Phylloscopus* (log L without clock = -3952.97; log L with clock = -3976.63). The same result was yielded even in a run with all outgroups included (log L without clock = -4056.16; log L with clock = -4078.96).

Genetic distances were calculated using the likelihood settings of the selected model. Nucleotide diversity (π ; Nei 1987: eq. 10.5), haplotype diversity (h ; Nei, 1987: eq. 8.4 and 8.12), $\theta = 2Nf\mu$ (Tajima, 1996: eq. 10) and mismatch distributions with the raggedness index (Harpending, 1994) were estimated with DnaSP v. 4.0 (Rozas & Rozas, 1999).

Acoustics

We compared territorial songs from various populations: *Phylloscopus fuscatus*: nominate *fuscatus*: Siberia 8 recordings (rec.), – ssp. *robustus*: China: Gansu, 2 rec., ssp. *weigoldi*: Qinghai: 6 rec.; – *P. affinis*: E Himalaya (Nepal): 16 rec., Qinghai: 21 rec., Gansu: 1 rec.; – *P. griseolus*: Kazakhstan: 1 rec., India, Ladakh: 1 rec., Russia, Resp. Tyva: 1 rec.; – *P. armandii*: Yunnan, Jiangxi, Sichuan, Shaanxi, Gansu, Qinghai, Ningxia, Nei Mongol: 42 rec.; – *P. subaffinis*: Shaanxi, Sichuan, Yunnan, Jiangxi: 11 rec.

Each song type of a single male was measured once and individual means were calculated for each male for the following song parameters: Maximum and minimum frequency (F_{\max} , F_{\min}), frequency range (Δf), duration (t), total number of elements (z), number of different element types (z_1), speed of repetition ($F_{\text{mod}} = z/t$). Furthermore, for songs of *P. fuscatus* duration of single trill elements (t_1) and the number of frequency maxima (peaks = z_2) per element were measured and frequency modulation of trill elements was accordingly calculated, $F_{\text{mod1}} = (z_2/t_1)$. Basic statistics like parametric and non-parametric tests as well as principal component analysis were carried out with SPSS 11.5 and PAST. Bioacoustic analyses of taxa represented by small sample sizes remained descriptive.

Morphology

Studies of external morphology focused on the classical material in the China collection of Hugo Weigold from Sichuan (1914), including types of *Phylloscopus fuscatus robustus* Stresemann, 1923 and *P. f. weigoldi* Stresemann, 1923 (MTD), of Walter Beick from Qinghai and Gansu (1926–1933; ZMB), of Hugh Whistler from Kashmir (1941, BMNH), of Gerd Diesselhorst from Nepal (1962, ZSM) and on our newly collected specimens in combination with vocal recordings ('song specimens') and tissue samples (skins deposited in ZFMK, MTD, IZAS). In several cases besides the skin, song recordings and a tissue sample were available. This combination allows sound character analysis for populations of questionable status (Table 1). Measurements were taken according to standard method (MARTENS & ECK, 1995), indication of coloration follows MICHEL Farbenführer (2000). In addition to the German colour denomination and the relevant numeral an English colour indication is also given.

The following acronyms are used:

BMNH	Bird Group, Natural History Museum, formerly British Museum Natural History, Tring, UK
IZAS	Institute of Zoology, Academy of Sciences, Beijing, PR China
MAR	tissue collection J. Martens
MNE	Naturkundemuseum Erfurt, Germany
MTD	Staatliche Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Dresden, Germany
NHMW	Naturhistorisches Museum Wien, Austria
UWBM	Burke Museum of Natural History and Culture, Washington, USA
ZFMK	Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany
ZIH	Zoologisches Institut der Universität, Halle, Germany
ZMB	Museum für Naturkunde, Humboldt-Universität, Berlin, Germany
ZSM	Zoologische Staatssammlung, München, Germany

Results

Molecular genetics

79 cytochrome-*b* sequences of Old World *Phylloscopus* warblers produced a 666-bp-long alignment. There were 195 variable sites (29.3 %) in the alignment, of which 172 (25.8 %) were parsimony informative (outgroups *Acrocephalus*, *Abroscopus* and *Regulus* excluded). Though the relationships between the different deeply split *Phylloscopus* clades were not well resolved in the various phylogenies, all molecular trees resulting from different reconstructions based on cytochrome-*b* sequences showed a largely congruent interior sub-grouping of Dusky Leaf Warbler (*Phylloscopus fuscatus*) and Tickell's Leaf Warbler (*P. affinis*) with strong bootstrap support for most nodes. The Bayesian haplotype tree for the cytochrome-*b* data set is shown in Fig. 4. Each of the two groups

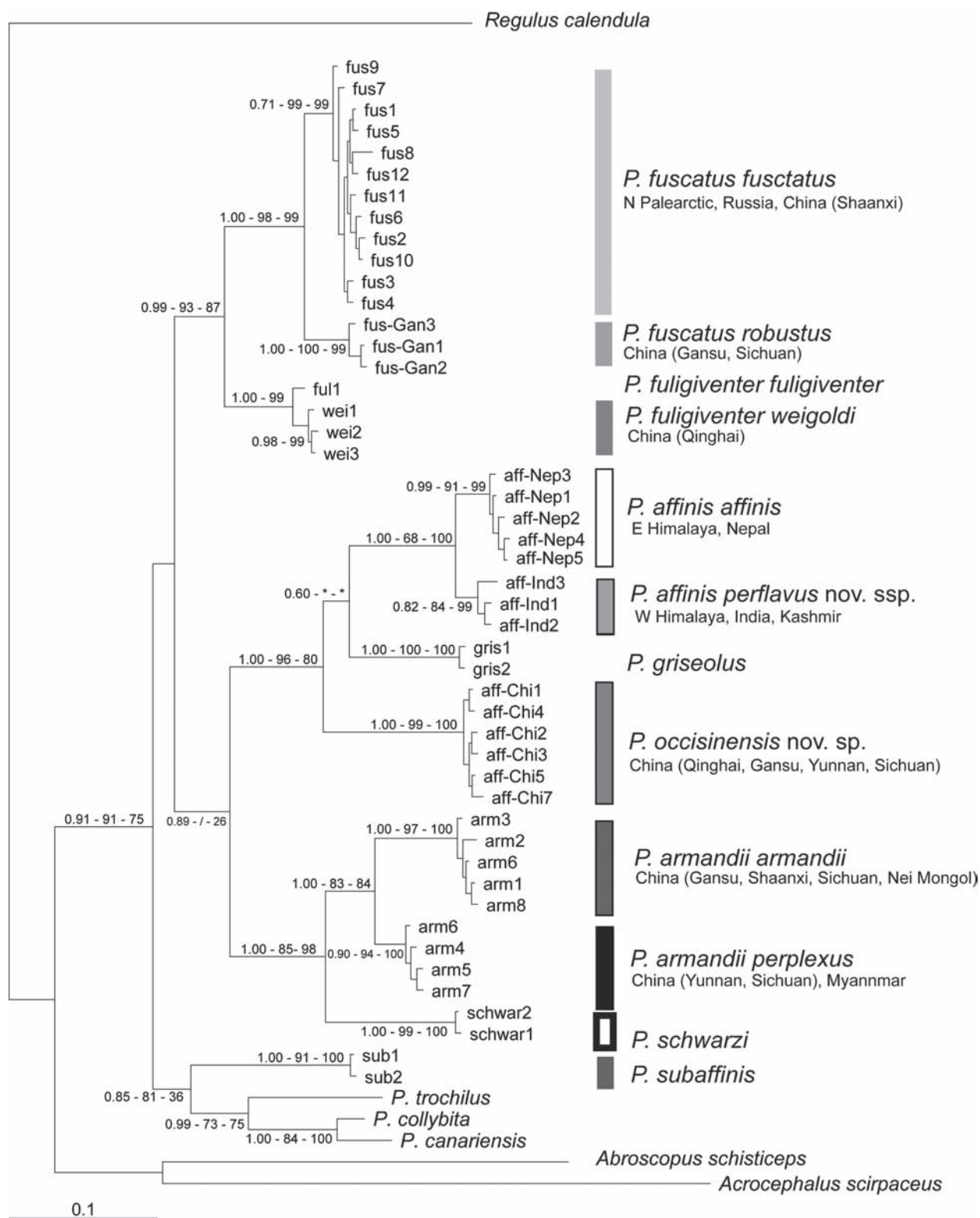


Fig. 4. Interspecific phylogenetic relationships of Dusky Warbler (*Phylloscopus fuscatus* complex), Tickell's Leaf Warbler (*P. affinis* complex) and Yellow-streaked Warbler (*P. armandii*). Haplotype tree based on 666 bp cytochrome *b*, Bayesian Weights, 1,000,000 generations, clade credibility values and bootstrap values indicated at nodes (Bayes – ML – NJ).

comprises three different mitochondrial lineages and a fourth lineage representing a different currently valid species, *P. fuligiventer* and *P. griseolus*, respectively. Sequencing with the forward primer 16S500 yielded a

fragment of approximately 800 bp that covered almost the complete t-RNA-val gene and parts of the 16S rRNA region. Thirty-nine sequences of Old World warblers produced a 679-bp-long alignment. There were

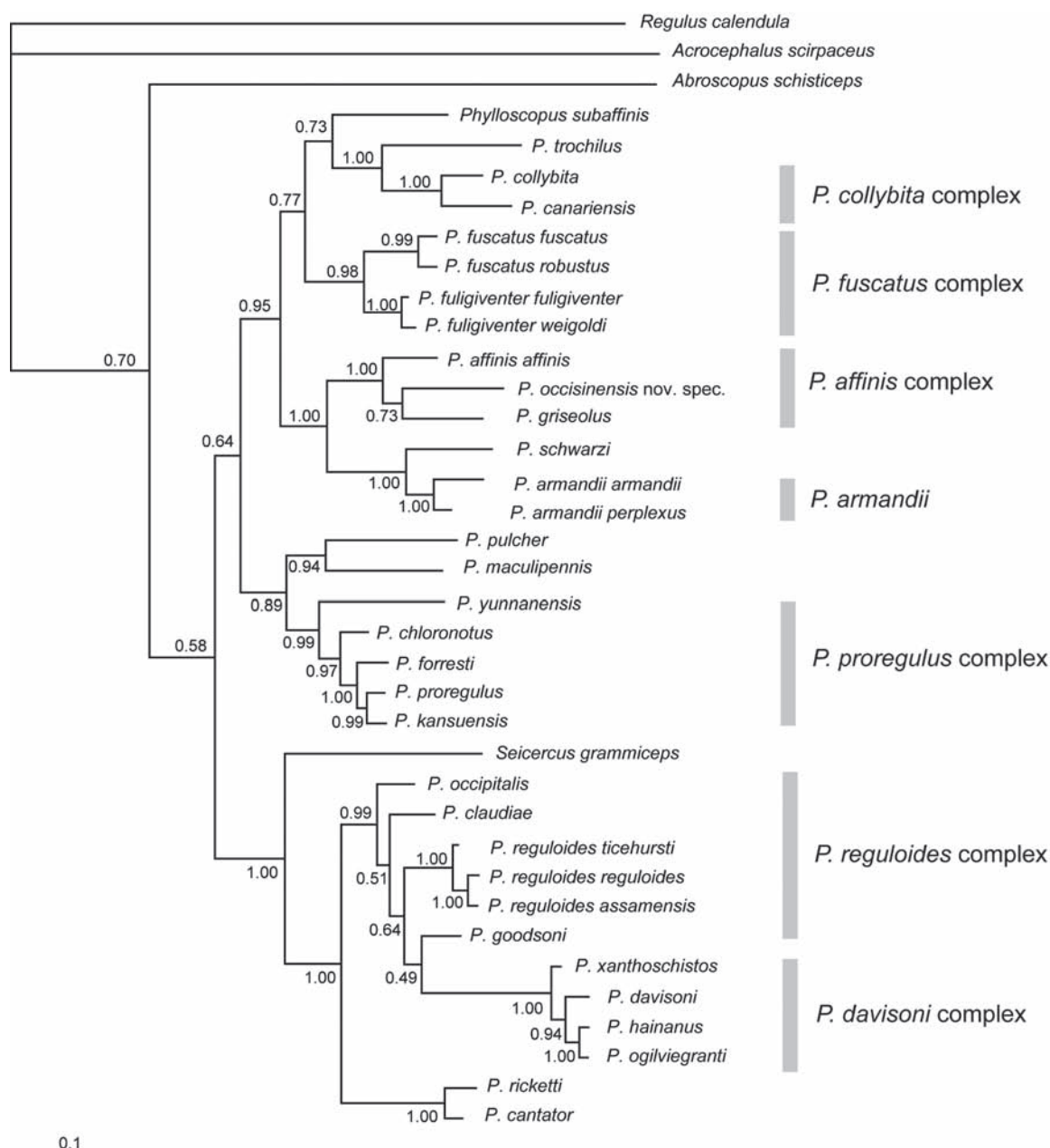


Fig. 5. Interspecific phylogenetic relationships for 34 Old World warbler taxa (*Phylloscopus* incl. *Seiurus grammiceps*); Bayesian tree based on 1563 bp of partial sequences of tRNA (Val), 16S rRNA and cytochrome *b*; 1,000,000 generations, clade credibility values indicated at nodes.

84 variable sites (12.4%) in the alignment of which 67 (9.9%) were parsimony informative (outgroups excluded).

Phylloscopus fuscatus

Molecular genetics

In the Dusky Warbler complex (*P. fuscatus* incl. *P. fuligiventer*) we found a total of 18 different cytochrome-

b haplotypes (out of 30 sequences, including those taken from GenBank) of which the most common and most widespread was restricted to the North Palearctic range of nominate *fuscatus* (nine specimens including a stray from northeastern Germany, Table 1). All other haplotypes were unique ones found in single specimens.

Northern haplotypes of *P. fuscatus* from Russia and Mongolia form a well-supported cluster including three haplotypes from the Qinling chain, Shaanxi, China (Fig. 4). Unexpectedly, we found three nominate *fuscatus* specimens during the breeding season in Shaanxi (MAR 4932 [MTD C 63097], MAR

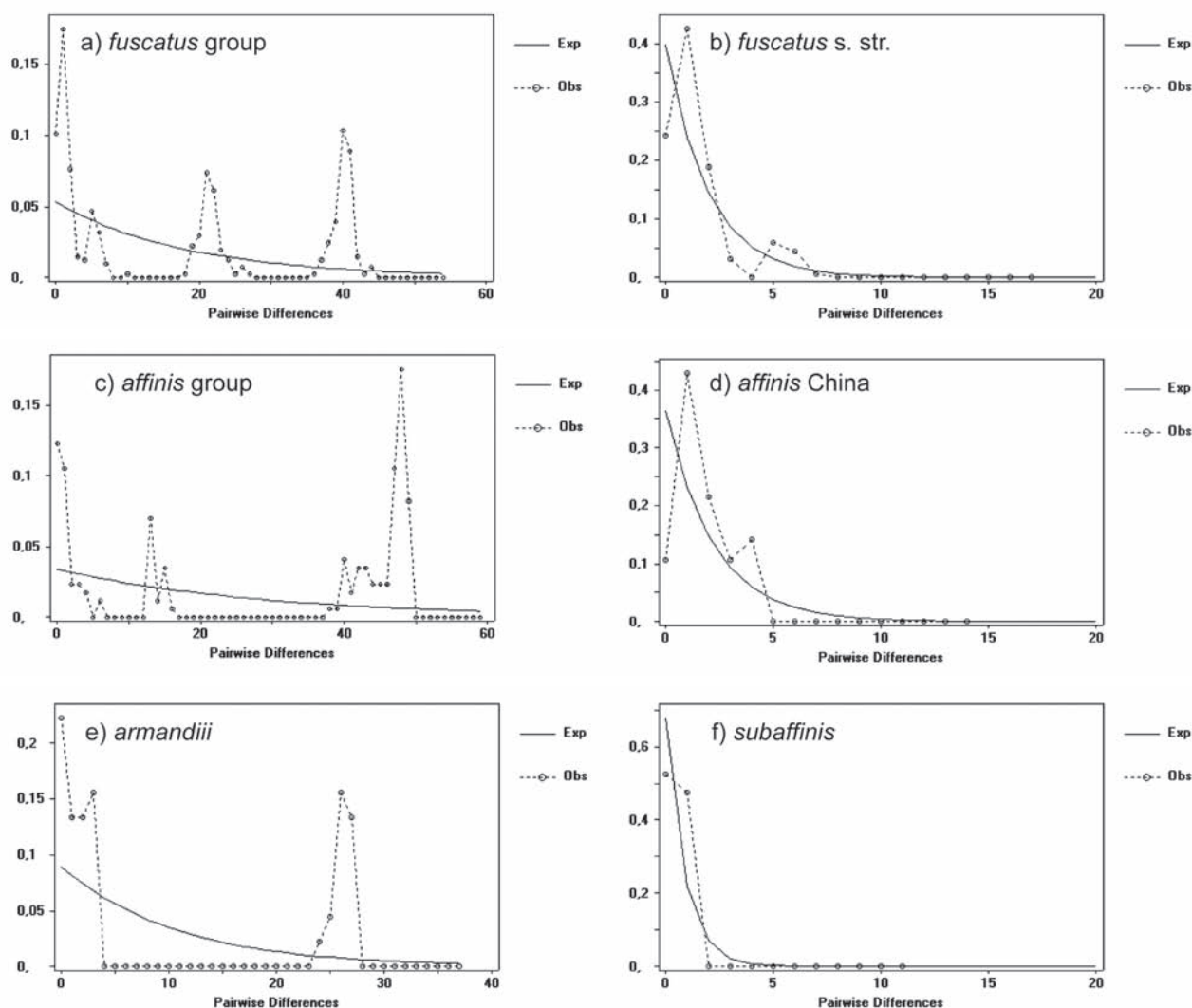


Fig. 6. Mismatch distributions for entire clades or subclades of the *Phylloscopus fuscatus* complex, the *P. affinis* complex, for *P. armandii* and *P. subaffinis*.

5747 [MTD C 63099], MAR 5748 [MTD C 63098], Table 1). Their haplotypes are unique ones and were not found in Siberia and Mongolia (fus6, fus7, fus8, Fig. 4, Table 1). In the starlike North Palearctic haplotype network (not shown) two of the latter Chinese haplotypes (fus6, fus7) are derived from the central most common haplotype (fus1) by only one and two substitutions, respectively, while haplotype fus8 from Shaanxi differs from fus1 by seven substitutions. The entire North Palearctic *fuscatus* clade represents the sister group to a Chinese haplotype cluster from South Gansu (Figs 4, 5). The second major clade of the Dusky Warbler complex comprises the remaining Chinese haplotypes from Qinghai (taxon *weigoldi*) and one haplotype of *P. fuligiventer* from Nepal. The latter is sister clade to the Chinese *weigoldi* cluster. The interior substructure of the *P. fuscatus* clade in the 16S rRNA tree (not shown) and the phylogeny reconstructed from concatenated sequences (Fig. 5) are the same as in the *cytb* tree. Monophyly of the entire Dusky

Warbler clade (incl. *fuligiventer*) is strongly supported in all reconstructions. The two available paratypes of *P. fuscatus robustus* belong to different mitochondrial lineages: A short *cytb* sequence of one male from Sungpan, Sichuan (MTD C 23280, MAR 6499; 26.VI. 1914) matched the most common haplotype of newly collected material from Gansu (Table 1). A sequence obtained from another paratype specimen from Atentsze, Sichuan (MTD C 40817, MAR 6500; 26. IX. 1915) matched the most common haplotype from the northern nominate *fuscatus* cluster. Another specimen from Gansu collected during the breeding season also carried a northern *fuscatus* haplotype (north of Xining, Sung-schi-dschuang, ZMB 35.513, MAR 6639; Beick collection).

Pairwise cytochrome-*b* distances among the clades of the *P. fuscatus* group are remarkably different: They are highest between the two main clades, i.e. pooled nominate *fuscatus/robustus* Siberian and Gansu populations vs. pooled *P. fuligiventer/weigoldi* Himalayan



Fig. 7. Vocalisations of the Dusky Warbler complex (*P. fuscatus* incl. *P. fuligiventer*). *P. fuscatus fuscatus*: Russia: a-m) Slavianska, Amur, 14. VI. 1990, JM; n-u) Amur, 1996, MP; *P. fuscatus robustus*: China, Gansu: a₁-d₁) Malu, 20. VI. 2007; e₁-m₁) Zhuoni, 20. VI. 2007; all JM. *P. fuligiventer weigoldi*: a₂-m₂) China, Qinghai, Koko Nor (Qinghai lake), Heimahe, 10. VI. 1996, JM; data-base numbers indicated for single males, sound archive J.M., University of Mainz, Germany.

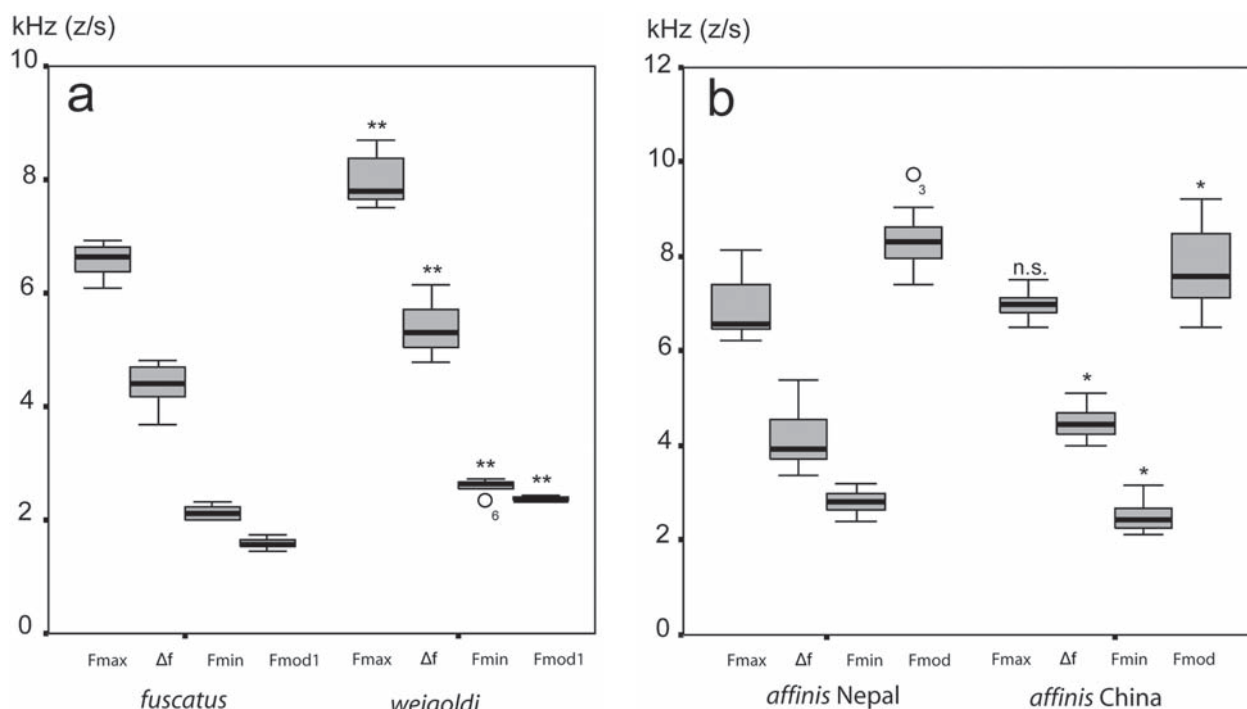


Fig. 8. Differentiation of spectral song parameters in a) the Dusky Warbler complex (*P. fuscatus*) and in b) the Tickell's Warbler complex (*P. affinis*); boxplot of sonographic measurements of maximum and minimum frequencies (F_{\max} , F_{\min} / in kHz), frequency range (Δf / in kHz), speed of trills (F_{mod} / in elements per second = z/s); two outliers (males 3 and 6) are indicated by open circles; outliers: any observation outside an interval of three times the length of a box (3 x the interquartile range).

and Qinghai populations (10.1–11.5%). Nominat *fuscatus* and *robustus* differ by 4.8%, and the Himalayan *fuligiventer* differs from the Chinese *weigoldi* by 1.3% (Table 3). The strong phylogeographic structure of this group is reflected by a multimodal mismatch distribution showing three peaks at different levels of molecular differentiation (Fig. 6a). Even within the North Palearctic nominate *fuscatus* clade, observed frequencies of pairwise differences deviate from the expected distribution due to marked sequence divergence between Shaanxi and Russian haplotypes (low second peak at five substitutions, Fig. 6b). The significantly negative Tajima's-D value indicates recent population expansion of the northernmost nominate *fuscatus* group (Siberia, Mongolia; Table 4).

Acoustics

Sonographic measurements were taken from 260 songs of sixteen males. Territorial songs in the *P. fuscatus* group are rather simply structured and include almost exclusively trills, i.e. repetitions of single element types (Fig. 7a-h, l, n, s, t). The individual male possesses a rich note repertoire. Three nominate *fuscatus* from Siberia displayed six, eight and fifteen verse types each, two *robustus* from Gansu nine verse types each. The single element can be steeply descending (Fig. 7b, f, o), ascending (Fig. 7g) or highly modulated

(Fig. 7d, e, h, q). The first song element is often a short one or is otherwise modified. Among eight males of the North Palearctic nominate *fuscatus* only two songs with repeated two-note syllables ('note groups') were found (Fig. 7m, u). Double trills are common in repertoires of both nominate *fuscatus* and Chinese *robustus* (Fig. 7i, k, k₁-m₁). Likewise, differences in spectral parameters are subtle: songs from the Chinese *robustus* population are somewhat higher-pitched and frequency range is broader (from 2.1 up to 5.8 kHz) compared to Siberian songs (2.2–5.1 kHz). In addition, elements in *robustus* are more widely spaced within the verse, giving the verse a slow and somewhat 'tired' auditory impression. Its verses comprise 4.5-7 (mostly 5) notes/s (2 males), while nominate *fuscatus* verses comprise 6 to 12 (mostly 8-12) notes/s (8 males).

At first sight, songs of ssp. *weigoldi* (Qinghai; Fig. 7a₂-m₂) seem to match the overall song pattern of the North Palearctic *fuscatus* group, but songs of both groups distinctly differ with respect to syntax and note repertoire. Several notes include harmonics – a rare acoustic feature in *Phylloscopus* songs. Trill units of Qinghai *weigoldi* birds are much more complex than those of Siberian *fuscatus*: True trills are common (Fig. 7a₂-b₂), but several song types include trill units composed of several short elements in rapid succession, i.e. temporally highly compressed syllables. Song syntax in *weigoldi* is considerably more complex than in *fuscatus/robustus*. Apart from simple trill songs, many

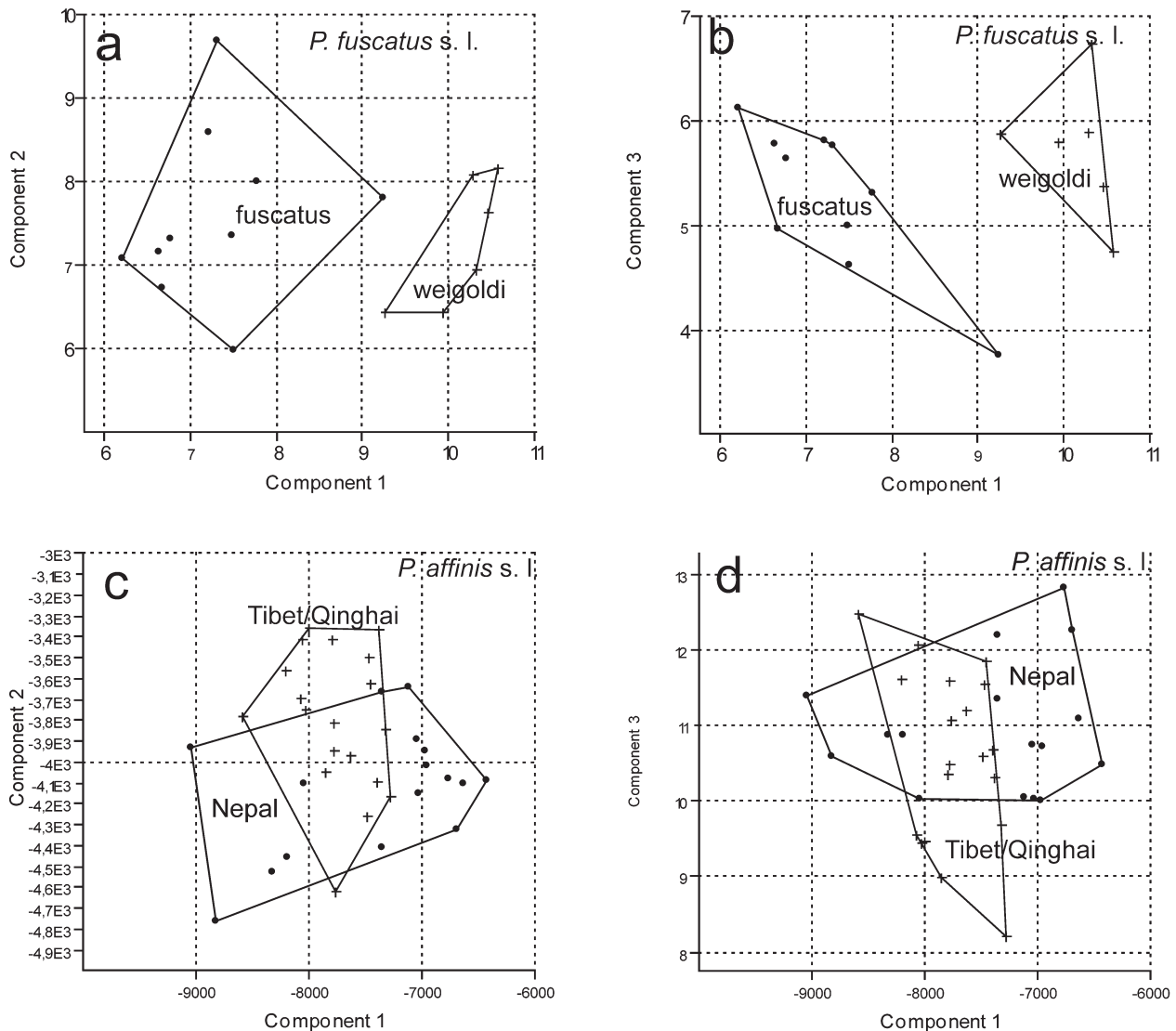


Fig. 9. Acoustic differentiation in a, b) the Dusky Warbler complex (*P. fuscatus* s.l.) and c, d) Tickell's Warbler complex (*P. affinis* s.l.); scatterplots of principal components PC1, PC2 and PC3 extracted from ten and six song parameters, respectively.

song types comprise from two to four successive phrases. A phrase comprises identical notes or syllables, while the note or syllable types of subsequent phrases differ in frequency range, modulation and/or length (Fig. 7d₂-m₂). Apart from structural features, songs of *weigoldi* differ significantly from those of *fuscatus/robustus* in several spectral parameters: they are higher pitched and more broad-banded, and single elements are more strongly modulated (Fig. 8a; Mann-Whitney-U-test, $p < 0.01$). A principal component analysis was carried out with individual means of ten song parameters. Three components with Eigenvalues > 1 were extracted, of which PC1 (Eigenvalue = 2.54) explained 61.6% and both PC1 and PC2 (Eigenvalue = 0.86) explained a cumulative 82.5% of the acoustic variation. The scatterplots of PC1 vs. PC2 and PC3, respectively, illustrate the marked differentiation of *weigoldi* songs from those of nominate *fuscatus* and *robustus* (Fig. 9a, b).

Morphology

Five 'morpho-types' can be distinguished in the *P. fuscatus/P. fuligiventer* clade, which differ quite remarkably in coloration and correspond to four molecular sub-branches (Figs 4, 5).

***P. fuscatus fuscatus*:** Material from the Weigold collection (from Jehol, Northeast China) generally has a quite gloomy appearance. Breast band only weakly developed or nearly absent; belly with a broad dirty whitish-tinged centre, consequently extension of the brown of the flanks is much reduced. Except for the lower tail coverts, this form is the least warmly tinged one.

***P. fuscatus robustus*:** It was described on the basis of "dirty yellowish" belly (instead of a shiny white one in nominate *fuscatus*) (Stresemann 1923). The five newly

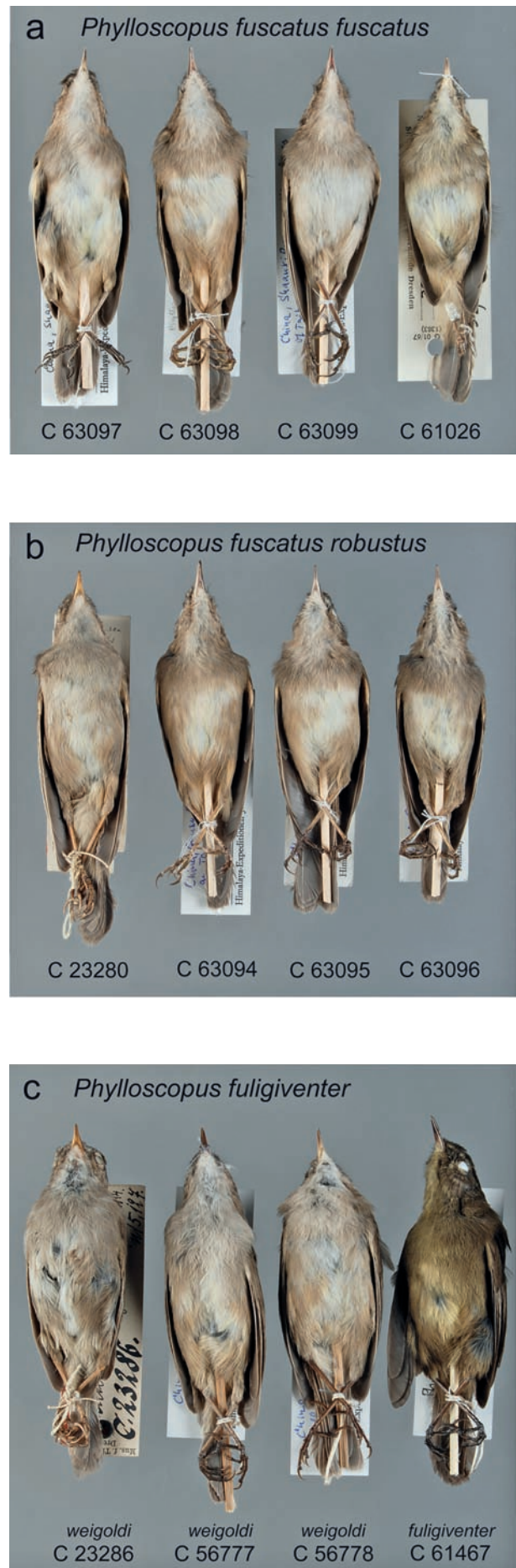
collected skins from Gansu (May, June) have a rather broad gloomy but warm brownish breast band, which is continued by the brownish flanks; the latter with a slightly more yellowish touch (Fig. 10b). Only the narrow centre of the belly is dirty whitish rather than yellowish, otherwise constricted by the extended brownish flanks. These characters match fairly well one of the two paratypes of *robustus* (MTD C 23280, MAR 6499, Fig. 10b). This specimen carries a haplotype of the Chinese Gansu-clade. The 2nd paratype (MTD C 40870, MAR 6500) with more yellowish belly is closer in coloration to the northern nominate *fuscatus*. Coincidentally, this specimen, a September bird, carries a northern nominate *fuscatus* haplotype. In the Beick collection from the Gansu/Qinghai border (north of Xining) one specimen from May 16 (ZMB 29545, MAR 6640) coincides with this coloration type, pattern and proven haplotype as well.

***P. fuscatus* cf. *fuscatus*:** The three specimens from Shaanxi (MAR 4932 [MTD C 63097], MAR 5747 [MTD C 63099], MAR 5748 [MTD C 63098], all from May; Fig. 10a) fall in the nominate *fuscatus* clade. They are remarkably lighter on breast and belly and the whitish belly centre is more extended than in *robustus*. The brownish coloration on the flanks tends to a touch of reddish. General coloration of the underside including throat is conspicuously lighter than in *robustus* and the brown has a warm yellowish tinge (Fig. 10a, three on the right side).

P. 'fuscatus' weigoldi (Fig. 10c, three on the left side): The two paratypes and four additional newly collected skins lack a clear breast band and a warm brownish tinge on the underside is missing. The difference in coloration from nominate *P. fuligiventer*, to which it is attached as sister taxon by the *cytb* analysis, is remarkable (Fig. 10c, right).

***P. fuligiventer fuligiventer*:** A conspicuously dark-coloured form with blackish olive upper and underside; quite unlike *P. fuscatus weigoldi* and the various forms of *P. fuscatus* (Fig. 10c, right).

Fig. 10. Coloration differentiation in the Dusky Warbler complex (*Phylloscopus fuscatus* s.l.). – **a**) *P. fuscatus fuscatus*: C63097: China, Shaanxi, Qin Ling range, Foping (May); C 63098, C 63099: China, Shaanxi, Qin Ling range, Houzhengzi (May); C 61026: Germany, Mecklenburg-Vorpommern, Greifswalder Oie (October); **b**) *P. fuscatus robustus*: Lectotype C 23280: China, Sichuan, Sungpan (May); C 63094, C 63095, C 63096: China, Gansu, Taohe valley, south of Malu (May); **c**) *P. fuligiventer weigoldi*: Paratype C 23286: China, Sichuan, Sue Shan; C 56777, C 56778: China, Qinghai, Koko Nor (Qinghai lake), Heimahe (June); *P. fuligiventer fuligiventer*: C 61467, Nepal, Bhojpur Distr., Arun valley below Tumlingtar (April).



Tab. 3. Model-corrected genetic distances (605 bp, cytochrome *b*).

	fus	rob	wei	ful	sub	aff	perf	occi	gris	arm	perp
<i>fuscatus</i>											
<i>robustus</i>	0.048										
<i>weigoldi</i>	0.115	0.113									
<i>fuligiventer</i>	0.101	0.109	0.013								
<i>subaffinis</i>	0.137	0.149	0.125	0.124							
<i>affinis</i>	0.172	0.172	0.153	0.143	0.154						
<i>perflavus</i> nov. ssp.	0.205	0.199	0.161	0.146	0.163	0.034					
<i>occisinensis</i> nov. sp.	0.181	0.168	0.192	0.199	0.202	0.160	0.158				
<i>griseolus</i>	0.179	0.224	0.197	0.195	0.152	0.128	0.129	0.138			
<i>armandii</i>	0.166	0.185	0.152	0.148	0.150	0.149	0.160	0.182	0.177		
<i>perplexus</i>	0.203	0.201	0.158	0.153	0.187	0.190	0.204	0.190	0.183	0.052	
<i>schwarzi</i>	0.229	0.235	0.217	0.217	0.183	0.179	0.200	0.200	0.208	0.114	0.101

Tab. 4. DNA polymorphism within several subclades of four Old World warbler clades; n= number of sequences, n_{hap} = number of haplotypes, \hat{h} = haplotype diversity, π = nucleotide diversity, $\theta = 2N\mu$, Tajima's D: Fu & Li's D-Test, * = $p < 0.05$, ** = $p < 0.01$.

	n	n_{hap}	\hat{h}	π	θ	Tajima's D
<i>P. fuscatus</i> complex	30	16	0.871	0.0266	0.0251	0.22693
<i>P. f. fuscatus</i>	20	10	0.758	0.0024	0.0061	-2.1738**
<i>P. affinis</i> complex	20	13	0.879	0.0485	0.0314	2.2182*
<i>P. occisinensis</i> nov. sp.	9	5	0.806	0.0030	0.0037	-0.7397
<i>P. a. affinis</i>	8	4	0.429	0.0007	0.0007	0.3335
<i>P. armandii</i>	10	6	0.778	0.0168	0.0176	-0.203
<i>P. subaffinis</i>	7	2	0.476	0.0008	0.0007	0.559

Phylloscopus affinis

Molecular genetics

Among 26 cytochrome-*b* sequences of Tickell's Leaf Warbler (*P. affinis* complex, including *P. griseolus*) 17 different haplotypes were found, most of which were private ones – only two common haplotypes were found in only three males from China (Qinghai) and in three males from Nepal and Tibet, respectively (Table 1). In the two molecular phylogenies the *P. affinis* group is divided in two deeply split mitochondrial lineages: One bifurcated Himalayan clade and a second Chinese clade (Figs 4, 5). These two lineages form a well supported monophyletic group with the Sulfbellied Leaf-Warbler *P. griseolus* from Northwest India and Russia, however, phylogenetic relationships between the respective clades are poorly resolved and conflicting among different reconstructions (Figs 4, 5). The Himalayan haplotype group is subdivided into an East Himalayan (Nepal)/Southeast Tibetan clade and a West Himalayan (Northwest Indian) clade; monophyly of both genetic clusters is well supported. Pairwise cytochrome-*b* distances among the branches of the

affinis clades (including *P. griseolus*) are remarkably differentiated (Table 3): They are highest between the Qinghai/Gansu cluster and the Himalayan cluster (incl. *P. griseolus*), amounting to 15.8-16.0%. *P. griseolus* differs from both Himalayan sub-clades by 12.8-12.9%. Even the latter sub-clades (western and eastern Himalayas) differ from one another by 3.4%. As in the *P. fuscatus* group, the phylogeographic structure of the entire *P. affinis* clade is corroborated by a strongly multimodal mismatch distribution (Fig. 6c). Even within the Qinghai/Gansu population, observed and expected frequencies of pairwise differences slightly differ (Fig. 6d). Haplotype and nucleotide diversities are comparatively low for the nominate population from the East Himalayas (Table 4).

Acoustics

Sonographic measurements were taken from 170 songs of 34 males. All members of the *P. affinis* complex (including *P. griseolus*) share the same song pattern – simple trill songs – as found in the *P. fuscatus* group (Fig. 11). Songs are generally introduced by one or two unmodified call notes (Fig. 11a-c). Gener-

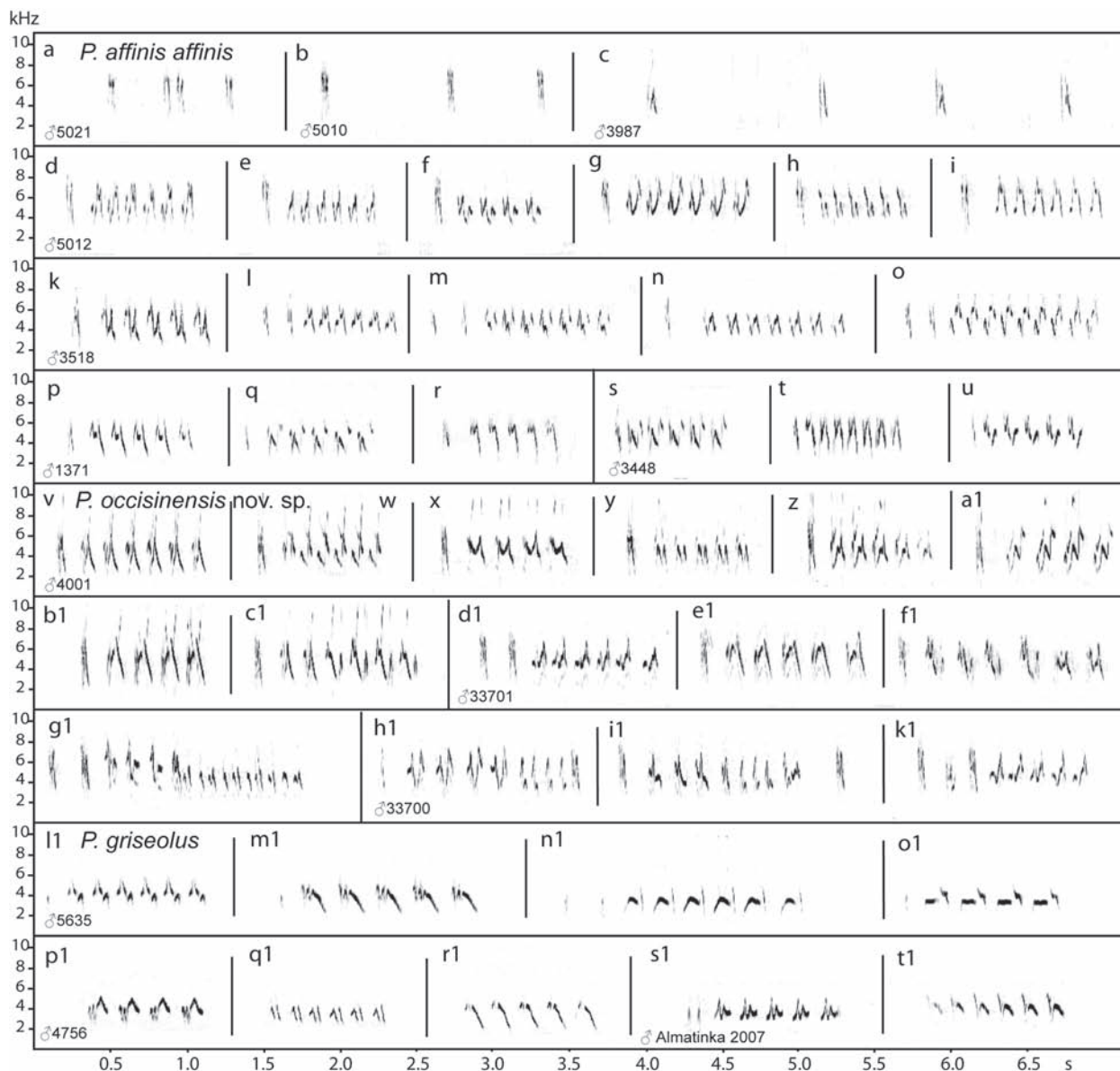


Fig. 11. Vocalisations of the Tickell's Warbler complex (*Phylloscopus affinis* incl. *P. griseolus*). *Phylloscopus affinis affinis*, Nepal: **a–b**) call series from two different males, Mustang Distr., Purano Marpha, 12. and 9. V. 1995; **d–i**) territorial song: **d–i**) Purano Marpha, Mustang Distr., 12. V. 1995; **k–o**) Phoksumdo Lake, Dolpo Distr. 7. VI. 1973; **p–r**) Purano Marpha, 7. VII. 1973; **s–u**) Phoksumdo Lake, 31. V. 1970. *Phylloscopus occisinensis* nov. sp., China, Qinghai: **c, v–c₁**) Koko Nor (Qinghai Lake), Heimahe, 11. and 12. VI. 1996; **d₁–g₁** and **h₁–k₁**) China, Qinghai, Donggi Cona Lake, both 2. IX. 2001. *Phylloscopus griseolus*: **l₁–o₁**) Russia, Resp. Tyva; **p₁–r₁**) India, Jammu and Kashmir, Shey Gompa, 03. VI. 1976; **s₁, t₁**) Kazakhstan, Almatinka jezero, south of Almaty, 03. VI. 2007 – All recordings JM (except **d₁–g₁** and **h₁–k₁**: A. Gebauer, **l₁–o₁**: B. Veprintsev, **s₁–t₁**: H.H. Bergmann); database numbers indicated for single males, sound archive J.M., University of Mainz, Germany.

ally, this uniform song pattern is distributed all over the breeding range from the Himalayas to adjacent Tibet, Qinghai and Gansu, and only slight taxon-bound differences in spectral and temporal parameters were found (Fig. 8b, 9c, d): The voice of Chinese birds (Qinghai/Gansu) covers a lower and slightly broader frequency range than that of Nepal birds (F_{\min} , Δf , $p < 0.05$, Mann-Whitney U-test), and trills are more rapid in the Nepal population (F_{mod} , $p < 0.05$, Mann-Whitney U-test). Only in the Qinghai/Gansu population were song types of an aberrant pattern

found: double or even triple trills, some of these with a markedly descending frequency course (Fig. 11g₁–i₁). These song types were found in repertoires of four out of 19 Qinghai/Gansu males. A single male possesses up to eleven different song types.

Compared to all populations of the *P. affinis* group, the song of *P. griseolus* is lower-pitched and conspicuously more narrow-banded (Fig. 11l₁–o₁). Element types are less steep and less modulated, and besides homogeneous trill songs repertoires of *P. griseolus* also encompass phrases of repeated two-note syllable

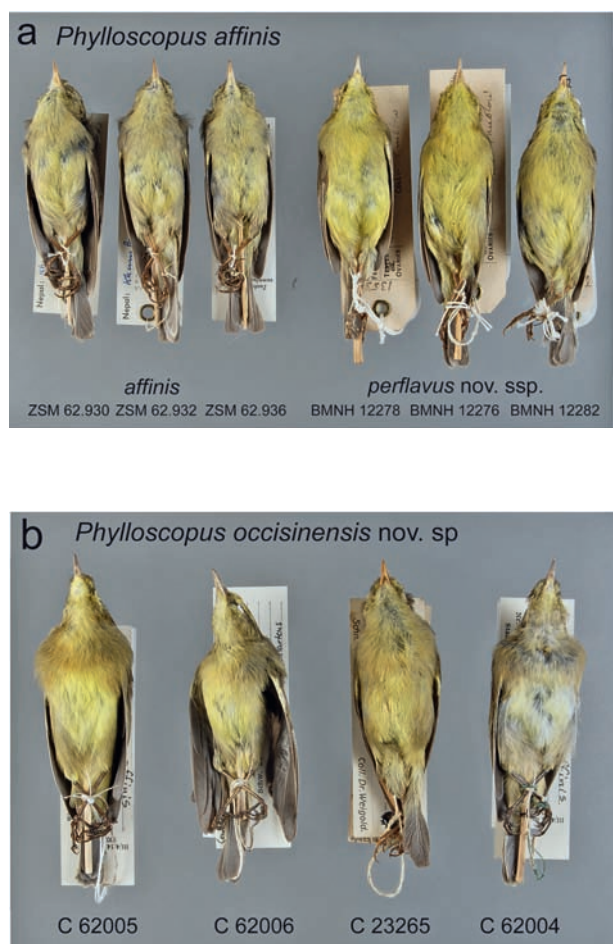


Fig. 12. Coloration differentiation in the Tickell's Warbler complex. **a)** *Phylloscopus affinis affinis*: ZSM 62.932, ZSM 62.930, ZSM 62.936 (neotype): Nepal, Solukhumbu Distr., Kundzhang village (= Khumjung; June, July); *P. affinis perflavus* nov. ssp.: BMNH 12276 (holotype), BMNH 12278 (paratype), BMNH 12282: India, Kashmir and Pakistan (see Table 1); **b)** *P. occisinensis* nov. sp.: C 23265: China, Sichuan, near Sungpan; C 62005 (holotype): China, Qinghai, Donggi Cona Lake; C 62004: China, Qinghai, Balong; C 62006: China, Yunnan, Jizu Shan.

bles (Fig. 11n–o). Songs of this species are also introduced by short call-like click-notes but these are faint and not as accentuated as in *P. affinis* songs.

Morphology

Individuals of *P. affinis* fall into three distinct colour morphs (Fig. 12) which apparently are allopatrically distributed and which are to be attributed to three taxa. Characters are outlined here only in brief; for details of taxonomy see Discussion and Appendix.

P. affinis affinis, eastern Himalaya (Fig. 12a): Birds from Nepal, Mt. Everest area (June, July), are dull greenish yellow on the underside with marked pattern, sides of breast and upper belly with a greyish tinge; supercilium light greenish, marked, but not very con-

trasting. This population represents nominate *affinis* (see Appendix).

P. affinis perflavus nov. ssp., western Himalaya (Fig. 12a): Birds from Kashmir (May to July) are bright citron yellow on the whole underside, including throat and breast, no breast band, flanks unmarked; contrasting bright-yellow supercilium; upperside dark greyish with conspicuously greenish tinge. This population represents a separate subspecies-level taxon which is introduced here as new (see Appendix).

P. occisinensis nov. sp. western China (Fig. 12b): Birds from Yunnan, Gansu and Qinghai (April to July) are bright yellow on the underside with a slight orange touch in single breast and belly feathers, breast band and extended flanks yellow-brownish to greenish; upperside similar to nominate *affinis*. A newly moulted September bird (Fig. 12b, C 62005) and a May bird (C 63177) show a broad breast band of beige with an orange touch in the centre. This population represents a separate species-level taxon which is introduced here as new (see Appendix).

Phylloscopus armandii

Molecular genetics

Among 13 cytochrome-*b* sequences of Yellow-streaked Warbler (*P. armandii*) eight different haplotypes were found, the most common one in four specimens from Shaanxi and Gansu. A monophyletic clade of all north-western Chinese (haplotypes from Shaanxi, Sichuan and Nei Mongol, nominate *armandii*) is deeply separated from a southern clade comprising haplotypes from Sichuan, Yunnan and Myanmar (ssp. *perplexus*, Figs 4, 5). Sister-group relationship between *P. armandii* and *P. schwarzi* is well supported in all phylogenetic reconstructions (Figs 4, 5). Sequence divergence between the two subspecies of *P. armandii* amounts to 5.2%. These differ from the sister species *P. schwarzi* by 10.1–11.4%. A multimodal mismatch distribution confirms the phylogeographic structuring of the complex due to the deep lineage split between nominate *armandii* and ssp. *perplexus* (Fig. 6e).

Acoustics

Songs of 42 males were sonographically analysed with respect to song pattern, and measurements were taken from 197 songs of fourteen males. Song pattern of *P. armandii* is much more variable and individual song

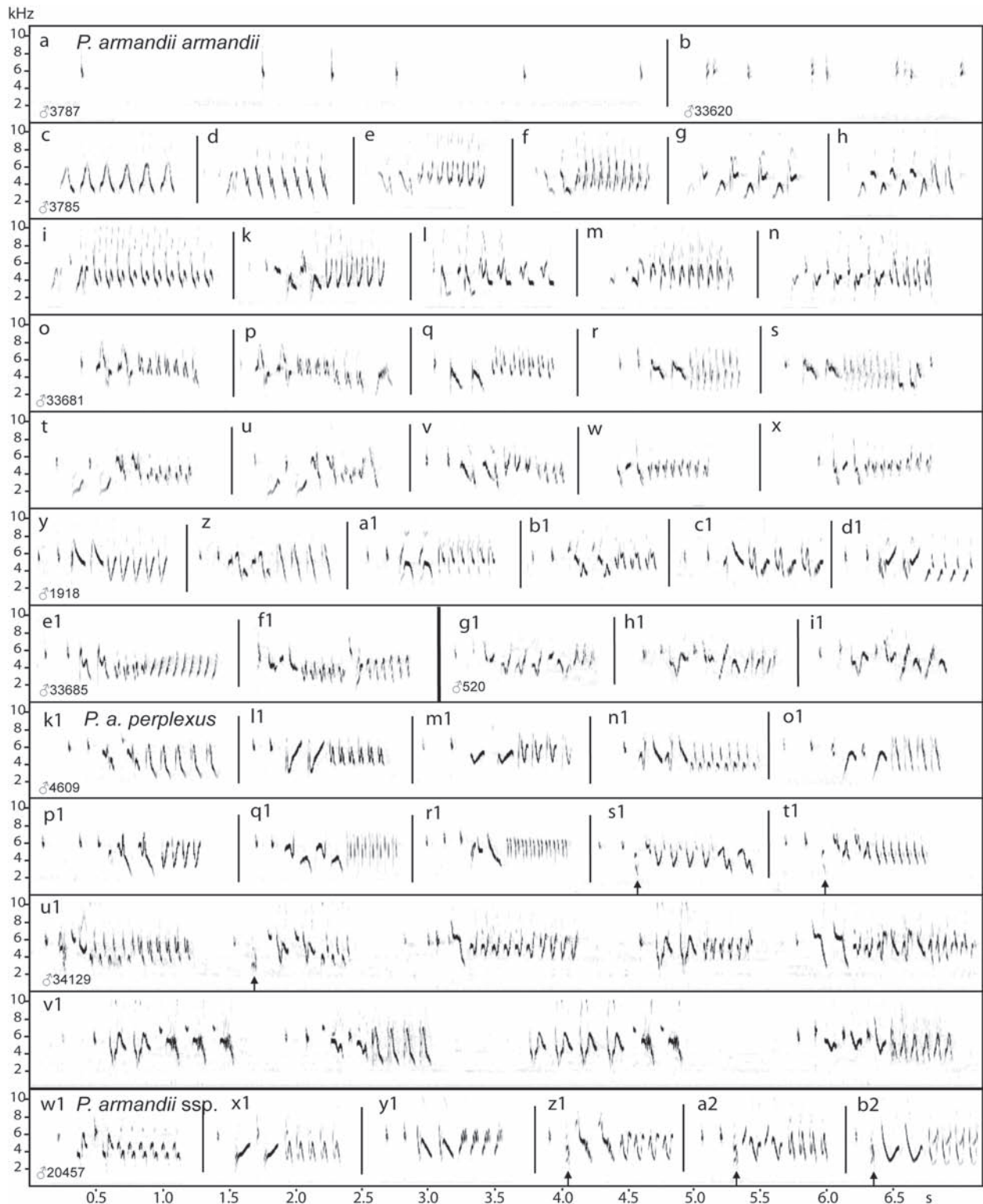


Fig. 13. Vocalisations of the Yellow-streaked Warbler complex (*Phylloscopus armandii*; all from China). – *P. armandii armandii*: **a–b**) calls series; **c–i₁**): territorial song – **a**) Qinghai, Bei Shan, 23. V. 1996; **b**) Gansu, Lianhua Shan, 26. VII. 2007. **c–n**) Qinghai, Bei Shan, 23. V. 1996; **o–x**) Ningxia, Helan Shan, Suyukou, 5. VIII. 2007; **y–d₁**) Shaanxi, Laoxiancheng, 4. V. 2005; **e₁–f₁**) Nei Mongol, Ala Shan South Temple, 6. VIII. 2007; **g₁–i₁**) Shaanxi, Taibai Shan, Houzhenzi, 9. VI. 1997; *P. armandii perplexus*: **k₁–t₁**) Yunnan, Geza N Zhongdian, 6. V. 2002; **u₁, v₁**) Sichuan, Mamize Nature Reserve: two continuous series of songs in highly aggressive display (one male); *P. armandii* ssp.: **w₁–b₂**) Jiangxi, Wuji Shan, 03. VI. 2006 – All recordings J.M.; database numbers indicated for single males, sound archive J.M., University of Mainz, Germany.

repertoires of males are even larger than in those of the *P. fuscatus* and the *P. affinis* group. As in the latter two

taxa, the large intraspecific variation of song pattern in *P. armandii* comprises simple trills and double trills

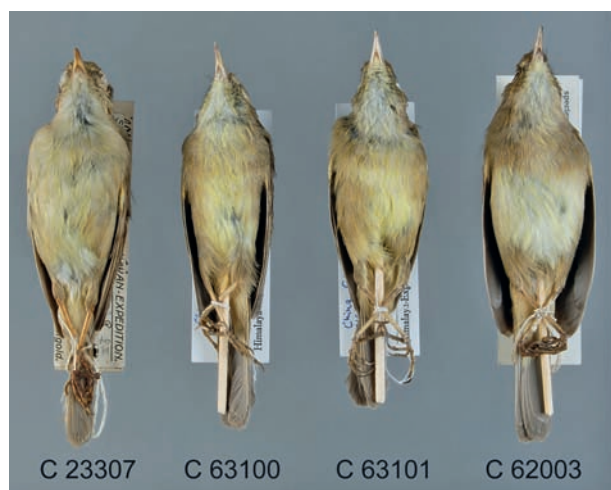


Fig. 14. Coloration differentiation in *Phylloscopus armandii*. *P. a. armandii*: C 23307: China, Sichuan, Sungpan (Weigold collection, 1914); C 63100: China, Gansu, Kangxian; C 63101: China, Shaanxi, Laoxiancheng; *P. a. perplexus*: C 62003: China, Yunnan, Geza N Zhongdian (Martens collection, 2002–2006).

(AAAAA, AABBBBB, Fig. 13c-f), but also clearly bipartite songs composed of a syllable part (i.e. note group part) and a trill part (ABAB CCCC, Fig. 13h–n). In most bipartite songs (including double-trill verses) the second trill part is lower-pitched than the first part. Rare song types start with a trill or a double note but terminate in a phrase of repeated syllables (AABCB-CBCBC, Fig. 13d₁) or entirely lack a trill part (Fig. 13g). Some males also perform triple trills (AABBBB-CCCC, Fig. 13p, v, e₁) or combinations of syllables and trills (Fig. 13f₁-h₁). Two singing males were recorded far east of the known breeding range of the species at Wuji Shan range, Huanggang Shan, Jiangxi Province, on 1. and 3. VI. 2006 (Figs 3, 13w₁-b₂).

Males have large repertoires: a maximum number of 38 different song types were found in a single male (out of 34). Despite the large intra- and inter-individual song variation, single song types are widely distributed. The most common song types were found in four to twelve out of 39 different males (Fig. 13q, w-a₁, u₁, t₁; 10–31%). Only two song types were found in all but one of the breeding populations (Fig. 13z, not present in Yunnan; Fig. 13a₁, not found in Jiangxi). Even in a single male, song types can be varied by adding different trills or syllables to the song (Fig. 13o/p, r/s) or by switching different trill parts into the same introduction (Fig. 13q/r). All songs are preceded by one up to three short calls (Fig. 13a, b: call series). In several individual song types from all southern populations (ssp. *perplexus* and the two individuals from Jiangxi) the introducing call notes are followed by an additional lower-pitched note with harmonics (Fig. 13s₁, t₁, u₁, z₁-b₂: arrows). This pattern was not found in northern song types of ssp. *armandii*. Apart from this slight ge-

ographical variation, no striking differences between songs of nominate *armandii* and ssp. *perplexus* are detectable with respect to frequency range or temporal parameters. Maximum and minimum frequencies of song cover a broad range between 2.1 and 7.4 kHz in both subspecies.

Morphology

The four recently collected specimens of *perplexus* (all from May, one of them Fig. 14, North Yunnan, MTD C 62003, MAR 3252) are less intensively yellowish below than nominate *armandii*. In both subspecies the yellow parts may have a more or less streaked appearance, which is sometimes completely lacking. Subspecies *perplexus* has a more distinctly developed broad breast band which is marked brownish beige. Eight fresh nominate *armandii* from the same season (May to early August) are bright yellowish most of them indistinctly streaked on breast and belly, breast band only indistinctly marked (Fig. 14, three at left).

Phylloscopus subaffinis

Molecular genetics

Only two haplotypes out of seven samples were found in the Buff-throated Warbler (*P. subaffinis*); the most common one was present in five specimens covering large parts of the Chinese breeding range (Sichuan, Shaanxi, Jiangxi), the remaining one originates from North Yunnan. There is no evidence of any geographical substructure either from *cytb* and 16S rRNA phylogenies or from mismatch distributions (Figs 4, 5, 6f). Haplotype and nucleotide diversity as well as theta value reached by far the lowest values of all Old World Warbler taxa investigated here (Table 4).

Discussion

Species structure, taxa and monophyly

The bush-dwelling and in several cases high-altitude *Phylloscopus* species of the Himalayas and China investigated here exhibit – except for one case, *P. subaffinis* – a pronounced molecular-genetic substructure.

This is all the more surprising as for one of these species, *P. affinis*, TICEHURST (1938: 74) stated: "Over the huge breeding area of *affinis* I can see not the slightest geographical variation; this, I think, is what might be expected in a bird whose haunts are at very high elevations in the same kind of terrain and climate wherever it is found." Furthermore, in the *P. fuscatus* complex (incl. *P. fuligiventer*) and in the *P. affinis* complex (incl. *P. griseolus*), the currently accepted species are paraphyletic according to molecular data and the arrangement of least-inclusive taxa does not reflect monophyletic entities on the species level. Furthermore, high p-distances of cytochrome-*b* sequences and partly acoustic differences influence the picture of species taxa to be accepted. Here we mainly consider these characters in deciding on the taxonomic steps to be taken. We assign species level to populations in which – so far as available – morphology, molecular genetic markers and acoustic characters are differentiated. The search for contact zones of closely related allopatric species taxa remains a constant challenge. Only through the study of population interactions will we gain detailed insight into evolutionary processes in Southeast Asian mountain systems.

Phylloscopus fuscatus complex

The Dusky Warbler is distributed in two disjunct areas: nominate *fuscatus* in Siberia/Mongolia and ssp. *robustus* and ssp. *weigoldi* in parts of West China (*robustus*: parts of Sichuan, Gansu, Shaanxi, *weigoldi*: Qinghai, parts of Sichuan; CHENG, 1987; CLEMENT, 2006; TICEHURST, 1938; VAURIE, 1951, 1959). However, current distributional maps differ: CLEMENT (2006) draws a continuous area across the deserts of Inner Mongolia, in CHENG (1987), CHRABRYJ *et al.* (1989) and GLUTZ v. BLOTZHEIM & BAUER (1991) two area parts, a northern one and a southern one, are separated by the Mongolian deserts.

Carriers of haplotypes of one well supported northern clade occur in Siberia/Outer Mongolia and disjunctly in China (Sichuan, Gansu and Shaanxi). The haplotypes of the three newly collected specimens from Shaanxi were found only in these Chinese individuals. Though collected in the breeding season (11. V. 2005 and 18. V. 2006) their breeding status is not proven and they may belong to late migrants of northern nominate *fuscatus* (but see Morphology). One male had undeveloped testes, one female may have produced eggs and the developmental state of the third bird is unknown. Also in the Sichuan collection of Hugo Weigold we discovered one bird of the northern subclade

of *fuscatus* (a September paratype of *robustus*, MTD C 23280; STRESEMANN, 1923), as well as in the Gansu collection of Walter Beick (STRESEMANN *et al.*, 1937) a May bird (ZMB 35.513). Coloration of the Gansu bird differs only slightly from northern *P. fuscatus* (see above). Because haplotypes of these four birds are firmly nested within the northern *fuscatus* clade, these birds do not merit any nomenclatorial consideration.

P.f. robustus STRESEMANN, 1923 is regarded as a weakly differentiated subspecies and its validity had been rejected (TICEHURST, 1938; VAURIE, 1954, 1959) though accepted by DICKINSON (2003). Five newly collected Gansu specimens with proven *cytb* haplotypes clearly differ from nominate *fuscatus* from China (May birds; Martens collection) as well as from Siberian/North Chinese specimens. Cytochrome-*b* sequences of parts of the classical collection of Weigold and Beick coincide with the new material. However, for comparison of skins fresh material is needed because lipochromes in old specimens may change. We regard *robustus* as a valid subspecies which is diagnosable by coloration, colour pattern and molecular genetic markers, but only slightly by territorial song.

The East Himalayan /SE Tibetan *P. fuligiventer* was placed close to or even treated as conspecific with *P. fuscatus* to date (VAURIE, 1959; CHENG, 1987; GLUTZ v. BLOTZHEIM & BAUER, 1991). A sister species relationship of these two taxa was recently suggested by molecular data (JOHANSSON *et al.*, 2007). However, our molecular data show that *P. fuligiventer* is firmly rooted in the *P. fuscatus* complex and turns out as sister to the North Chinese high-altitude subspecies *weigoldi* of the latter. According to these findings, the *P. fuscatus* complex needs taxonomic re-arrangement: *P. "fuscatus" weigoldi* is part of *P. fuligiventer* not of *P. fuscatus*. RASMUSSEN & ANDERTON (2005) already suggested close relationships between *P. fuligiventer* and *P. fuscatus weigoldi* and affiliated *P. fuligiventer tibetanus* to *P. fuscatus*. This would make *P. fuligiventer* monotypic and restrict it to a small area of the eastern Himalayas. Like the Qinghai *weigoldi* population, *P. fuligiventer* is a high-altitude species restricted to habitats above timber-line in Southeast Tibet (ssp. *tibetanus*) and in the eastern Himalayas (nominate *fuligiventer*) (LUDLOW & KINNEAR, 1944; MARTENS & ECK, 1995; DICKINSON, 2003).

Taxonomic recommendations

All taxa of the *P. fuscatus* complex turned out to be monophyletic; they are allopatrically distributed and

are treated as a superspecies here (see AMADON 1966). For distribution of the *P. fuscatus* complex (incl. *P. fuligiventer*) see CHRABRYJ *et al.* (1989).

It should be noted that neither tissue samples nor voice recordings of ssp. *tibetanus* were available to us. Our proposal for the current re-arrangement of the *P. fuscatus* complex is as follows:

Phylloscopus [*fuscatus*]

Phylloscopus fuscatus

Phylloscopus fuscatus fuscatus (BLYTH, 1842) – Type locality: Calcutta [India] – (general distribution: Siberia, Mongolia; as a breeding bird possibly Shaanxi, Sichuan and Gansu, China).

Phylloscopus fuscatus robustus STRESEMANN, 1923 – Type locality: Sungpan [China, Sichuan] – (general distribution: W and NW China: Sichuan, Qinghai, Gansu).

Phylloscopus fuligiventer

Phylloscopus fuligiventer fuligiventer (HODGSON, 1845) – Type locality: Nepal – (general distribution: eastern Himalayas).

Phylloscopus fuligiventer tibetanus TICEHURST, 1937 – Type locality: Tsari, S Tibet – (general distribution: SE Tibet).

Phylloscopus fuligiventer weigoldi STRESEMANN, 1923 – Type locality: Dschiesongea, near Tatsienlu [Sichuan, China] – (general distribution: China: Qinghai, parts of high-altitude Sichuan).

Phylloscopus affinis complex

The genetic differentiation of this group is complicated and, accordingly, its present taxonomic treatment as a monotypic species is inappropriate. First of all, Tickell's Warbler appears to be paraphyletic, too, though the position of *P. griseolus* in the *P. affinis* clade is conflicting. Furthermore, the Himalayan sub-clade is deeply split from the Qinghai/Gansu populations. Even the populations of the Himalayan chain are bipartite, though on a markedly lower level of differentiation. Their contact zone has not yet been located, but may be situated considerably west of the western border of Nepal. A winter bird from Nichlanl, Uttar Pradesh, India (W. Koelz leg., 31. I. 1947, ZSM 58.4), probably just south of the Himalayan breeding area, belongs to nominate *affinis*. No other characters ever indicated such a heavy sub-structuring except for

the fact that *P. griseolus* since its original description was always treated as a species of its own. At least the molecular tree, with underlying genetic distances up to 16% of the *cytb* gene, requires a thorough re-arrangement of taxa within this complex. Coloration and colour pattern are distinct between the three molecular *affinis* clades. Acoustic differentiation between (East) Himalayan (MARTENS, 1980) and Qinghai/Gansu populations is slight and mainly concerns subtle but significant differences in frequency range and syntax pattern. Songs of the West Himalayan population are unknown.

Taxonomic recommendations

All taxa of this complex are monophyletic and are - so far as is known - allopatrically distributed; the group is treated as a superspecies here (see AMADON, 1966). Our proposal for the current re-arrangement of the *affinis* complex is as follows below (for description of new taxa see Appendix). Primarily on grounds of molecular genetics, morphology and bioacoustics *Phylloscopus affinis* is to be split into two species. The vast *affinis* area part from the eastern Tibet-Qinghai Plateau, Yunnan, Sichuan and Gansu is excluded from *P. affinis* s. str. and attributed to a species of its own which is introduced below (see Appendix). For a distributional map of the traditional *affinis* species see VIETINGHOFF-SCHEEL (1986). The distributional areas and possible contact zones of the resulting two species are not yet known; they are likely to be found in the easternmost Himalayas or in southern Yunnan.

Subdivision of *Phylloscopus affinis* s. str. into two subspecies is justified by their genetic differentiation: Nominate *affinis* is the eastern Himalayan representative, *P. a. perflavus* n. ssp. is the western one; they are separated by a *cytb* distance value of 3.4 %. *P. griseolus* invariably stands as an independent species; its area is apparently vicariant to that of *P. affinis* (LOSKOT & VIETINGHOFF-SCHEEL, 1991).

Phylloscopus [*affinis*]

Phylloscopus affinis sensu stricto

Phylloscopus affinis affinis (TICKELL, 1833) – Type locality: Bengal: Barabhum and Dholbhum [India] – (general distribution: eastern Himalayas incl. all Nepal, SE Tibet, probably [this taxon] east to the Tsangpo bend; western border west of Nepal, details unknown).

Phylloscopus affinis perflavus n. ssp. – Type locality: India, vale of Kashmir – (general dis-

tribution: western Himalayas west of Nepal; eastern border unknown).

Phylloscopus griseolus (BLYTH, 1840) – Type locality: Calcutta – (general distribution: Tian Shan, Altai, western part of Qinghai-Tibet Plateau) – monotypic.

Phylloscopus occisinensis nov. sp. – Type locality: China, Qinghai, Lake Donggi Cona near Huashixia – (general distribution: eastern Qinghai-Tibet Plateau, Yunnan, Gansu, Sichuan) – monotypic.

Phylloscopus armandii

This widespread species (China from Yunnan across the country to north of Beijing; VIETINGHOFF-SCHEEL, 1984) was monotypic until TICEHURST (1934) described *P. a. perplexus* from the southern part of its range, Southwest Sichuan and North Yunnan, which is a quite restricted range. Suspected breeding areas in the mountains of Myanmar remained unproven (CLEMENT, 2006). The five samples available (North Yunnan, S Sichuan; Myanmar: the latter from early spring; March 29) yielded strong genetic divergence compared to all northern samples (Shaanxi, N Sichuan, Gansu, Ningxia, Nei Mongol; 5.2% in *cytb*) and a taxonomic high-level rank for both subspecies is indicated. Based on the vocal recordings available at present geographic differentiation of territorial songs of *P. armandii* is not diagnosable. A possible contact zone of both subspecies has not yet been traced but is expected to be located in Sichuan: According to *cytb* sequences a specimen from North Sichuan (near Jiuzhaigou; MAR 4946) belongs to *armandii*, three other specimens from C Sichuan to *perplexus* (Mamize Nature Reserve: MAR 6964, 6965, Kangding: MAR 6985; Fig. 3, Table 1). According to CHENG (1987) distribution points and text affiliation for the two taxa are somewhat contradictory, southern *perplexus* being mentioned for the northern province of Ningxia but correctly not so shown on the map. In the southeastern part of the area, mapped points for both taxa somewhat “interdigitate”; confirmation is needed. TICEHURST (1934, 1938) gives as breeding range for *perplexus* Southwest Sichuan, Southeast Chuanben (i.e. eastern part of Sichuan), North Yunnan. CLEMENT (2006) largely adheres to Cheng’s indication. There is a marked difference in coloration between the four fresh *perplexus* specimens available and a fresh series of nominate *armandii*, *perplexus* displaying a more distinct breast band and lighter belly with less conspicuous yellow.

Taxonomic recommendations

Both taxa are monophyletic; so far known they are allopatrically distributed and despite the high level of genetic differentiation (5.2% *cytb* distance) are tentatively distinguished at subspecies level. Morphological differences in the fresh material are marked, but acoustic differentiation is nearly absent according to present knowledge.

Phylloscopus armandii

Phylloscopus armandii armandii (MILNE-EDWARDS, 1865) – Type locality: originally not indicated, types from mountains west and northwest of Beijing – (general distribution: W central China to NE China).

Phylloscopus armandii perplexus TICEHURST, 1934 – Type locality: Chien-Chuan Valley, northwest Yunnan, 26°40’N (general distribution: SW central China [Sichuan, Yunnan]; NW Myanmar in winter only).

Phylloscopus subaffinis

The Buff-throated Warbler was treated a monotypic species ever since it was identified. Song recordings and mitochondrial DNA sequences (*cytb*, 16Sr RNA) from major parts of the breeding range (Yunnan, Sichuan, Shaanxi, Jiangxi; Table 1) yielded no internal genetic structure. Consequently, we do not propose any taxonomic changes.

Biogeographic evidence for species richness in *Phylloscopus* in East Asia

In Siberia and the Sino-Himalayan region bird distribution often follows a distinct pattern. A Siberian area part is separated from a West Chinese/Himalayan one (maps for various species in HARRISON, 1982). Traditionally, both area parts are attributed to a single polytypic species. Acoustic and molecular genetic analysis, however, proved this view to be too simple. There are not only considerable differences between populations of northern (Siberian) and southern (Sino-Himalayan) area parts regarding voice and molecular markers, but also the Sino-Himalayan area part is subdivided into

several, mostly three sub-regions, as indicated by a marked population differentiation (JOHANSSON *et al.*, 2007). Such a configuration is well established (with modifications) for the Pallas's Warbler, *Phylloscopus proregulus* complex (MARTENS *et al.*, 2004), the Greenish Warbler, *Phylloscopus trochiloides* complex (IRWIN, 2000; IRWIN *et al.*, 2001, 2005), the Eurasian Treecreeper, *Certhia familiaris* complex (TIETZE *et al.*, 2006), the Coal Tit, *Parus ater* complex (MARTENS *et al.*, 2006) and the Goldcrest, *Regulus regulus* (MARTENS & PÄCKERT, 2003; PÄCKERT *et al.*, 2003). Among the species treated here only the *P. fuscatus* complex

follows this differentiation pattern, although it is not an arboreal species. Even in *P. affinis* this pattern is recognizable though a Siberian area part is missing. The same phylogeographic scenario was found in the complex comprising Blyth's and White-tailed Leaf Warblers, *P. reguloides* and *P. davisoni* (OLSSON *et al.*, 2005). Most of the populations restricted to either of the North Palearctic and Sino-Himalayan subregions have reached species level, and the underlying differentiation processes do not only apply to arboreal species but bush facies and high-altitude steppe inhabitants as well, although with slight modifications.

Appendix

Designation of types, description of taxa

Lectotype of *Phylloscopus fuscatus robustus* STRESEMANN, 1923

The type was destroyed in WW II (ECK & QUAISER, 2004) and of the originally six paratypes (STRESEMANN, 1923) only two are in the Dresden Museum für Tierkunde (MTD C23280, from Sungpan; MTD C40817 from Atentsze). The deposition of the other paratypes is unknown (ECK & Quaiser, 2004). These remaining paratypes form a composite series. MTD C 23280 matches the *cytb* haplotypes known from newly collected specimens from Gansu (Table 1, haplotypes fus-Gan1, fus-Gan2) and represents the taxon *robustus*. MTD C 40817 is congruent with the northern nominate *fuscatus* haplotype cluster (Table 1). This bird, collected on Sept. 26th, apparently is a northern migrant. In order to correctly identify the taxon *robustus* we herewith declare according to the CODE (article 73.2.2) specimen MTD C23280 a lectotype for this taxon.

Description of lectotype: Male, China, Sichuan, Sungpan, H. Weigold leg. 26 June 1914 (MTD C 23280, tissue sample MAR 6499, Fig. 10b). Wing 64 mm (tip slightly damaged), tail 56 mm, tarsus 23 mm, bill 14.5 mm. This specimen agrees well with five recently collected *robustus* specimens from Gansu though its breast band is less marked (Table 1; Fig. 10b, left bird vs. 3 birds on the right side).

Neotype of *Motacilla offinis* TICKELL, 1833

Apparently, the type specimens of *Motacilla offinis* [sic] TICKELL, 1833 are lost; TICEHURST (1938) was unable to locate them and a neotype is to be designated. The type locality of *offinis* is in low-altitude Bihar, India, localities Barabhum and Dholbhum, and probably refers to birds of the East Himalayan clade population. But there is no proof for that assumption and even migratory birds from the Qinghai/Gansu-clade population may have been involved. Nevertheless, in accordance with the close proximity of the type locality to the East Himalayan population we chose as neotype a specimen from the East Nepal breeding birds. From this area we had 46 relatively recently collected specimens at our disposal (27 sp. ZSM, Diesselhorst collection [1962], 19 sp. ZFMK, Martens collection [1969 to 1973]). The respective specimen was genetically identified as a member of the East Himalayan sub-clade by short fragments of *cytb*.

Neotype: Male with active testis; Nepal, Solukhumbu District, Khumbu, Khumdzung, 3950 m (27°50' N 86°42'E), G. Diesselhorst leg., 01. VII. 1962 (ZSM 62.936, tissue sample MAR 3937; Fig. 12a, Table 1).

Diagnosis of taxon: A medium-sized *Phylloscopus* leaf warbler with a marked dirty-yellowish supercilium, dark brownish upperside and light, dull green-

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR); deposition of vouchers: **BMNH** = Natural History Museum, Tring, UK; **IZAS** = Institute of Zoology, Chinese Academy of Sciences, Beijing, PR China; **MNE** = Naturkundemuseum Erfurt, Germany; **MTD** = Staatliches Museum für Tierkunde Dresden, Germany (collection numbers provided: C 59791 etc.); **NHMW** = Naturhistorisches Museum Wien, Austria; **UWBM** = Burke Museum of Natural History and Culture, Washington, USA; **ZIH** = Zoologisches Institut Halle, Germany; **ZMB** = Museum für Naturkunde, Berlin, Germany; **ZSM** = Zoologische Staatssammlung München, Germany; Song specimen (song spec.): **yes** = Song recording and voucher available, song record = only song recording available; **Acc. No** = GenBank accession number.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/ origin	voucher	song	Acc.No cytb/16SrRNA
NEP 133	<i>Phylloscopus affinis</i>	<i>affinis</i>	Nepal	Mustang Distr.	Purano Marpha	aff-Nep1	J. Martens, 10.5.1995	no	no	EU851090 EU794937
MAR 2681	<i>Phylloscopus affinis</i>	<i>affinis</i>	Nepal	Bhojpur Distr.	Irkawa Khola	aff-Nep1	J. Martens, 9.4.2001	no	no	FJ155877
MAR 6602	<i>Phylloscopus affinis</i>	<i>affinis</i>	China	Tibet	Xiongse valley, near Lhasa	aff-Nep1	Lu Xin, 19.6.2007	no	no	FJ155878
MAR 2705	<i>Phylloscopus affinis</i>	<i>affinis</i>	Nepal	Bhojpur District	Irkawa Khola	aff-Nep2	J. Martens, 10.4.2001	no	no	FJ155879
U64	<i>Phylloscopus affinis</i>	<i>affinis</i>	Nepal			aff-Nep3	T. Price fide B. Harr	no	no	FJ155880
MAR 6603	<i>Phylloscopus affinis</i>	<i>affinis</i>	China	Tibet	Xiongse valley, near Lhasa	aff-Nep5	Lu Xin, 19.6.2007	no	no	FJ155881
MAR 6606	<i>Phylloscopus affinis</i>	<i>affinis</i>	China	Tibet	Xiongse valley, near Lhasa	aff-Nep4	Lu Xin, 19.6.2007	no	no	FJ155883
MAR 6605	<i>Phylloscopus affinis</i>	<i>affinis</i>	China	Tibet	Xiongse valley, near Lhasa	aff-Nep4	Lu Xin, 19.6.2007	no	no	FJ155882
GenBank	<i>Phylloscopus affinis</i>	<i>perflavus</i> nov. ssp.	Pakistan	NW Frontier Province	-	aff-Ind1	ALSTRÖM et al. 2006	no	no	DQ008503
GenBank	<i>Phylloscopus affinis</i>	<i>perflavus</i> nov. ssp.	India	Kashmir	-	aff-Ind2	PRICE et al. 1997	no	no	Y10730
GenBank	<i>Phylloscopus affinis</i>	<i>perflavus</i> nov. ssp.	India	Kashmir	-	aff-Ind3	RICHMAN 1996	no	no	L77128
MAR 4904	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Shaanxi	Laoxiancheng	arm1	J. Martens, 4.5.2005	MTD: C 63170	yes	EU851092 EU794939
MAR 5753	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Gansu	Kangxian	arm1	J. Martens, 21.5.2006	MTD: C 63101	yes	FJ155895
MAR 6411	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Gansu	SW of Zhuni town, research station	arm1	J. Martens, 20.7.2007	MTD: C 63100	yes	FJ155896
MAR 6413	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Gansu	20 km E of Zhuni	arm1	J. Martens, 21.7.2007	MTD: C 63172	yes	FJ155897
MAR 6415	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Gansu	Lianhua Shan Nature Reserve	arm8	J. Martens, 22.7.2007	no	yes	FJ155901
MAR 2003	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Shaanxi	Houzhenzi, Taibai Shan	arm2	J. Martens, 6.5.2000	no	no	FJ155898

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/ origin	voucher	song	Acc.No cytb/16SrRNA
MAR 4946	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Sichuan	close to Jiuzhaigou	arm3	J. Martens, 21.5.2005	MTD: C 63171	yes	FJ155899
MAR 6496	<i>Phylloscopus armandii</i>	<i>armandii</i>	China	Nei Mongol	Helan Shan, South Temple Forest Park	arm6	J. Martens, 6.7.2007	MTD: C 63174	yes	FJ155900
MAR 6964	<i>Phylloscopus armandii</i>	<i>perplexus</i>	China	Sichuan	Manize Nature Reserve	arm5	J. Martens, 17.5.2008	MTD: C 63173	no	FJ155903
MAR 6965	<i>Phylloscopus armandii</i>	<i>perplexus</i>	China	Sichuan	Manize Nature Reserve	arm6	J. Martens, 17.5.2008	MTD: C 63175	no	FJ155904
MAR 6985	<i>Phylloscopus armandii</i>	<i>perplexus</i>	China	Sichuan	Kangding	arm7	J. Martens, 23.5.2008	MTD: C 63176	no	FJ155905
MAR 3252	<i>Phylloscopus armandii</i>	<i>perplexus</i>	China	Yunnan	Geza, 60 km N of Zhongdian	arm4	J. Martens & D.T. Tietze, 6.5.2002	MTD: C 62003	yes	EU851087 EU794945
MAR 4557	<i>Phylloscopus armandii</i>	<i>perplexus</i>	Myanmar	Chin State	Natmataung National Park	arm5	D.T. Tietze, 29.3.2005	no	no	FJ155902
MAR 2633	<i>Phylloscopus fuliginenter</i>	<i>fuliginenter</i>	Nepal	Bhojpur Distr.	Arun bank below Tumlingtar	ful1	J. Martens, 3.4.2001	MTD: C 61467	no	EU851097 EU794923
MAR 1333	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	East Middle Amur	Malishevo; 70 km E Khabarovsk	fus1	A.A. Nazarenko, 15.6.1992	no	no	FJ155853
MAR 1424	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	East Middle Amur	Malishevo, 70 km E Khabarovsk	fus1	O. Valchuk	no	no	FJ155854
MAR 5907	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Primorye	near Vladivostok	fus1	J.O. Kriegs, 15.9.2006	no	no	FJ155855
MTD 92	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Germany	Mecklenburg-Vorpommern	Greifswalder Oie	fus1	R. Dittich, 22.10.201	MTD: C 61026	no	EU851094 EU794933
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Russian Far East	near Magadan	fus1	BENSCH et al. 2006	no	no	DQ174603
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Russian Far East	near Magadan	fus1	BENSCH et al. 2006	no	no	DQ174600
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Russian Far East	near Magadan	fus1	BENSCH et al. 2006	no	no	DQ174601
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Russian Far East	near Magadan	fus1	BENSCH et al. 2006	no	no	DQ174599
UWBM 59905	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Mongolia	Dornod Aymag	Burkhan, Halin Gol	fus1	C.S. Wood	UWBM 59905	no	FJ155856
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Russian Far East	near Magadan	fus9	BENSCH et al. 2006	no	no	DQ174602

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/origin	voucher	song	Acc.No cytb/16SrRNA
UWBM 66011	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Mongolia	Töv Aymag	Ulaanbataar	fus12	C.S. Wood	UWBM 66011	no	EU851093
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>				fus10	Ma et al. (unpublished)	no	no	DQ119527
MAR 1425	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	East Middle Amur	Malishevo, 70 km E Khabarovsk	fus2	O. Valchuk	no	no	FJ155857
MAR 4022	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Mongolia		Tes Gol	fus3	M Stubbe	ZIH	no	FJ155858
MAR 4480	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Ayano-Maiski District	Ayan	fus4	A. Gamauf, 2.7.2001	NHMM	no	FJ155859
MAR 4481	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	Russia	Chitinskaya Oblast	Jenda	fus5	A. Gamauf, 27.8.2004	NHMM	no	FJ155860
MAR 4932	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	China	Shaanxi	Foping	fus6	J. Martens, 11.5.2205	MTD: C 63097	no	FJ155861
MAR 5747	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	China	Shaanxi	Houzhenzi, Taibai Shan	fus7	J. Martens, 18.5.2006	MTD: C 63099	no	FJ155862 EU794941
MAR 5748	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	China	Shaanxi	Houzhenzi, Taibai Shan	fus8	J. Martens, 18.5.2006	MTD: C 63098	no	FJ155863
GenBank	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>				fus11	Price et al. 1997	no	no	Y10729
MAR 5778	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	Taohe river, S Malu	fus-Gan1	J. Martens, 29.5.2006	MTD: C 63094	no	EU851096 EU794943
MAR 6408	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	Taohe river, S Malu	fus-Gan1	J. Martens, 20.7.2007	IZAS	yes	FJ155874
MAR 6410	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	SW Zhuoni	fus-Gan1	J. Martens, 20.7.2007	IZAS	yes	FJ155873
MAR 5779	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	Taohe river, S Malu	fus-Gan1	J. Martens, 29.5.2006	MTD: C 63096	no	FJ155875 EU794944
MAR 5780	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	Taohe river, S Malu	fus-Gan2	J. Martens, 29.5.2006	MTD: C 63095	no	FJ155876
MAR 446	<i>Phylloscopus fuscatus</i>	<i>weigoldi</i>	China	Qinghai	Koko Nor, village Heihame	wei1	J. Martens, 16.6.1996	MTD: C 56778	yes	FJ155870
MAR 1796	<i>Phylloscopus fuscatus</i>	<i>weigoldi</i>	China	Qinghai	Koko Nor, village Heihame	wei1	M. Kaiser & J. Fiebig, 27.6.2001	ZMB	no	FJ155871
MAR 414	<i>Phylloscopus fuscatus</i>	<i>weigoldi</i>	China	Qinghai	Koko Nor, village Heihame	wei2	J. Martens, 10.6.1996	MTD: C 56776	yes	EU851095 EU794942

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/ origin	voucher	song	Acc.No cytb/16SrRNA
MAR 441	<i>Phylloscopus fuscatus</i>	<i>weigoldi</i>	China	Qinghai	Koko Nor, village Heihame	wei3	J. Martens, 15.6.1996	MTD: C 56777	yes	FJ155872
GenBank	<i>Phylloscopus griseolus</i>	<i>griseolus</i>	India	Kashmir	Zanskar Valley	gris1	RICHMAN 1996	no	no	L77137
UWBM 66478	<i>Phylloscopus griseolus</i>	<i>griseolus</i>	Russia	Respublika Tyva	Mongun-Taiginskiy Kozhuun	gris1	R.C. Faucett	UWBM 66478	no	EU851091 EU794938
UWBM 66479	<i>Phylloscopus griseolus</i>	<i>griseolus</i>	Russia	Respublika Tyva	Mongun-Taiginskiy Kozhuun	gris1	R.C. Faucett	UWBM 66479	no	FJ155892
UWBM 66404	<i>Phylloscopus griseolus</i>	<i>griseolus</i>	Russia	Respublika Tyva	Erzynskiy-Taiginski Kozhuun	gris1	R.C. Faucett	UWBM 66404	no	FJ155893
UWBM 66570	<i>Phylloscopus griseolus</i>	<i>griseolus</i>	Russia	Respublika Tyva	Bai-Taiginskiy Kozhuun	gris2	R.C. Faucett	UWBM 66570	no	FJ155894
MAR 3647*	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Donggi Cona Lake [holotype]	aff-Chi5	J. Martens, 1.9.2002	MTD: C 62005	yes	EU851089
MAR 2088	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Koko Nor, village Heihame	aff-Chi1	A. Gebauer & M. Kaiser, 10.07.1994	no	no	FJ155884
MAR 3635	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Wenquan	aff-Chi1	J. Martens, 31.8.2002	no	no	FJ155885
MAR 439	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Koko Nor, village Heihame	aff-Chi1	J. Martens, 15.6.1996	no	no	FJ155886
MAR 3204	<i>Phylloscopus occisinenensis</i> n. sp.		China	Yunnan	Jizu Shan [paratype]	aff-Chi2	J. Martens & D.T. Tietze, 26.4.2002	MTD: C 62006	no	FJ155887
MAR 3627	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Balong [paratype]	aff-Chi3	J. Martens, 27.8.2002	MTD: C 62004	no	EU851088 EU794936
MAR 1797	<i>Phylloscopus occisinenensis</i> n. sp.		China	Qinghai	Koko Nor, village Heihame	aff-Chi4	M. Kaiser & J. Fiebig, 27.6.2001	ZMB 2001/3036	no	FJ155889
MAR 5800	<i>Phylloscopus occisinenensis</i> n. sp.		China	Gansu	Lianhua Shan, upper temples	aff-Chi5	J. Martens, 2.6.2006	no	no	FJ155888
MAR 6469	<i>Phylloscopus occisinenensis</i> n. sp.		China	Gansu	Lianhua Shan, above Shahtan [paratype]	aff-Chi7	J. Martens, 30.7.2007	IZAS	no	FJ155890
MAR 6986	<i>Phylloscopus occisinenensis</i> n. sp.		China	Sichuan	Pamuling Monastery [paratype]	aff-Chi5	J. Martens, 24.5.2008	MTD: C 63177	Yes	FJ155891
MAR 1379	<i>Phylloscopus schwarzi</i>		Russia	Primorye	Birakan	schwar1	A.A. Nazarenko, 1.6.1993	no	no	AY616187 EU794946
MAR 1377	<i>Phylloscopus schwarzi</i>		Russia	Primorye	Birakan	schwar1	A.A. Nazarenko, 1.6.1993	no	no	FJ155906

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/ origin	voucher	song	Acc.No cytb/16SrRNA
GenBank	<i>Phylloscopus schwarzi</i>		China	Hebei		schwar2	OLSSON et al. 2004	no	no	AY636051
MAR 920	<i>Phylloscopus subaffinis</i>		China	Sichuan	Omei Shan	sub1	J. Martens, 2.6.1998	MTD: C 59792	yes	FJ155864
MAR 4906	<i>Phylloscopus subaffinis</i>		China	Shaanxi	Laoxiancheng	sub1	J. Martens, 4.5.2005	no	yes	FJ155865
MAR 4910	<i>Phylloscopus subaffinis</i>		China	Shaanxi	Laoxiancheng	sub1	J. Martens, 5.5.2005	no	yes	FJ155866
MAR 5718	<i>Phylloscopus subaffinis</i>	<i>subaffinis</i>	China	Jiangxi	Huanggang Shan in the Wuyi Shan	sub1	J. Martens, 10.5.2006	MTD: C 63178	yes	FJ155867
MAR 5723	<i>Phylloscopus subaffinis</i>	<i>subaffinis</i>	China	Jiangxi	Huanggang Shan in the Wuyi Shan	sub1	J. Martens, 10.5.2006	MTD: C 63179	yes	FJ155868
MAR 5746	<i>Phylloscopus subaffinis</i>	<i>subaffinis</i>	China	Shaanxi	Houzhenzi, Taibai Shan	sub1	J. Martens, 18.5.2006	MTD: C 63180	yes	EU851086
MAR 3247	<i>Phylloscopus subaffinis</i>	<i>subaffinis</i>	China	Yunnan	Geza, 60 km N of Zhongdian	sub2	J. Martens & D.T. Tietze, 3.5.2002	MTD: C 63181	yes	FJ155869
Related Old World warbler species										
03320	<i>Phylloscopus occipitalis</i>		Nepal	Humla District	near Simikot		M. Fischer, 22.6.2002	MNE	no	EU851080
03092	<i>Phylloscopus trochilus</i>		Germany	Lake Constance			D.T. Tietze, 29.8.2002	no	no	AY616191
04138	<i>Phylloscopus reguloides</i>	<i>reguloides</i>	Nepal	Rasuwa District	W Syabrubesi, 3. camp		J. Martens, 8.4.2004	no	no	EU851077
04751	<i>Phylloscopus reguloides</i>	<i>assa-mensis</i>	Myanmar	Chin State	Naumataung National Park		D.T. Tietze, 2.4.2005	no	no	EU851078
03246	<i>Phylloscopus reguloides</i>	<i>ticehursti</i>	China	Yunnan	60 km N Zhongdian		J. Martens, 2.5.2002	no	no	EU851079
05755	<i>Phylloscopus claudiae</i>		China	Gansu	Kangxian		J. Martens, 22.5.2006	MTD: C 63121	yes	EU851083
05710	<i>Phylloscopus goodsoni</i>	<i>fokiensis</i>	China	Jiangxi	Huanggang Shan, Wuyi Shan		J. Martens, 08.5.2006	MTD: C 63125	yes	EU851081
00854	<i>Phylloscopus davisoni</i>	<i>davisoni</i>	Thailand	Chiang Mai	Doi Inthanon		J. Martens, 25.2.1998	MTD: C 59788	yes	EU851084
05702	<i>Phylloscopus hainanus</i>		China	Hainan	Diao Luo Shan		J. Martens, 2.5.2006	MTD: C 63142	yes	EU851082
03290	<i>Phylloscopus ogilviegrantii</i>	<i>disturbans</i>	China	Sichuan	Longxi-Hongkou reserve		J. Martens, 21.5.2002	MTD: C63138	yes	EU851085

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/ origin	voucher	song	Acc.No cytb/16SrRNA
90076	<i>Phylloscopus xanthoschistos</i>	<i>xanthoschistos</i>	Nepal	Kaski District	Potana		J. Martens, 28.4.1995	no	no	EU771072 EU794919
02542	<i>Phylloscopus collybita</i>		Germany	Rheinland-Pfalz	Mainz		J. Martens, 23.3.2001	MTD: C 61452	no	EU851075 EU794930
03508	<i>Phylloscopus canariensis</i>		Spain	Canary Islands	El Hierro		M. Päckert, 1.4.2003	no	no	EU851076 EU794931
00733	<i>Phylloscopus yunnanensis</i>		China	Shaanxi	Taibai Shan		J. Martens, 15.6.1997	MTD: C56748	no	AY583264 EU794927
02947	<i>Phylloscopus chloronotus</i>		Nepal	Humla District	near Chala		M. Fischer, 26.6.2001	MNE	no	AY583255 EU794925
00353	<i>Phylloscopus kansuensis</i>		China	Qinghai	Bei Shan		J. Martens, 24.5.1996	MTD: C65773	yes	AY583245 EU794928
01396	<i>Phylloscopus proregulus</i>		Russia	S Ussuriland	Ussuri river		A.A. Nazarenko, 4.7.1993	no	no	AY583240 EU794947
00941	<i>Phylloscopus forresti</i>		China	Sichuan	Omei Shan		J. Martens, 12.6.1998	no	no	AY583251 EU794948
00918	<i>Phylloscopus pulcher</i>		China	Sichuan	Omei Shan		J. Martens, 12.6.1998	MTD: C59791	no	AY583269 EU794926
03215	<i>Phylloscopus maculipennis</i>		China	Yunnan	Jizu Shan		J. Martens, 27.4.2002	no	no	AY583271 EU794929
02781	<i>Phylloscopus cantator</i>		Nepal	Nepal	Bhojpur District, near Tumlingtar		J. Martens, 24.4.2001	MTD: C 61465	yes	AY606157 EU794922
00903	<i>Phylloscopus ricketti</i>		China	Sichuan	Omei Shan		J. Martens, 29.5.1998	MTD: C 59793	yes	AY606172 EU794921
01944	<i>Seicercus grammiceps</i>				from aviary		<i>fide</i> R. Pfeifer, 15.9.1999	MTD: C 59823	no	AY606139 EU794940
03088	<i>Abroscopus schisticeps</i>				from aviary		<i>fide</i> R. Pfeifer, 10.102002	no	no	EU861032 EU794952
MTD16	<i>Acrocephalus scirpaceus</i>		Germany	Baden-Württemberg	Egenhausen		H. Löhrl	MTD: C 61991	no	EU861030 EU794950
MTD290	<i>Acrocephalus palustris</i>		Germany	Sachsen	Dresden-Reick		S. Eck	MTD: C 59838	no	EU861031 EU794951
Tissue samples from museum specimens										
03937	<i>Phylloscopus affinis</i>	<i>affinis</i>	Nepal	Solukhumbu District	Khumdzung (= Khumjung) [neotype]		G. Diesselhorst, 1.7.1962	ZSM 62.936		FJ384557
06724	<i>Phylloscopus affinis</i>	<i>affinis</i>	China	Tibet	Tangu		E. Schäfer, 1938	ZMB 2001.772		FJ384556

Tab. 1. Origin of samples used for molecular analysis (Martens collection, MAR) – continuation.

sample No	taxon	subspecies	state	region	locality	haplotype	collector/origin	voucher	song	Acc.No cytb/16SrRNA
06726	<i>Phylloscopus affinis</i>	<i>affinis</i>	India	Sikkim	Tangu		E. Schäfer, 1938	ZMB 2001.773		FJ384555
03940	<i>Phylloscopus affinis</i>	<i>perflavus</i> nov. ssp.	India	Kashmir	Lopara [holotype]		H. Whistler, 29.5.1931	BMNH 1949.Whi.1.12276		FJ384558
03941	<i>Phylloscopus affinis</i>	<i>perflavus</i> nov. ssp.	India	Kashmir	Warwan Valley [paratype]		H. Whistler, 7.6.1931	BMNH 1949.Whi.1.12278		FJ384559
06499	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Sichuan	Sungpan [lectotype]		H. Weigold, 26.6.1914	C 23280		FJ384554
06500	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	China	Sichuan	Atentsze		H. Weigold, 26.9.1915	C 40817		FJ384552
06639	<i>Phylloscopus fuscatus</i>	<i>fuscatus</i>	China	Gansu	Sung-shi-dschuang, Qilian Mts.		W. Beick, 15.5.1931	ZMB 35.513		FJ384551
06640	<i>Phylloscopus fuscatus</i>	<i>robustus</i>	China	Gansu	Lau-hu-kou, South Tetung Mts.		W. Beick, 16.5.1921	ZMB 29.545		FJ384553

ish-yellowish breast and belly, no marked contrast, no wing bars; freshly moulted birds slightly more vividly coloured. Taxon displays unique synapomorphies of base pairs of cytochrome-*b* and 16Sr RNA genes. Territorial song: an introductory note followed by a trill of identical notes. On the species level, *P. affinis* is split into two distinct populations, an eastern one (nominate *affinis*) and a western one representing an un-described subspecies (see below).

Description of neotype: Upperside from forehead to lower back blackish grey-brown (schwärzlichgrau-braun 11-2-8), a greenish tinge hardly recognizable; on underside throat and breast light brownish olive (hellbräunlicholiv 7-10-3), belly vivid olive-yellow (lebhaftolivgelb 5-7-3); general impression of underside is a dull yellowish green, bright tones are lacking. No distinct breast band but difference in colour between throat/breast and belly marked; remiges and rectrices blackish olive-brown (schwärzlicholiv-braun 11-7-8). Supercilium marked, does not reach base of bill, more vividly yellowish green in front of eye, less beyond. Eye stripe broad, marked, only slightly contrasting with supercilium, little contrast with cheeks; upper mandible dark brown horn, lower light horn, distal part slightly darker; legs medium brown.

Measurements (in mm): wing 58.5, tail 45.5, tarsus 18; mensural characterisation of the East Himalayan (Nepal) populations of *affinis* in DIESSELHORST (1962) and MARTENS & ECK (1995).

Variability: Newly moulted birds (from September) are only slightly brighter below. They have an extended greenish-beige breast band which runs down to the flanks leaving only reduced parts of lower breast and belly of nearly pure yellow-green; lightest parts close to dirty yellow (schwärzlichgelb 5-0-8), throat markedly lighter; upper parts with slight greenish tinge (needs comparison with June/July birds).

Distribution of taxon: *P. affinis* is restricted to the Himalayan chain (Fig. 2) with proven breeding records (at least birds from late May to end of August; see LU 2008) from Pakistan (ROBERTS, 1992), Nepal (DIESSELHORST, 1968; MARTENS & ECK, 1995) to Bhutan (LUDLOW & KINNEAR, 1944) and probably further east and from Southeast Tibet (Lhasa region: LU, 2008). The eastern border is unknown, but may be located somewhere in the eastern Himalayas or in southwestern China; in West China it is replaced by *Phylloscopus occisinensis* nov. sp. *P. affinis* sensu stricto is split into two subspecies-level taxa, nominate *affinis* and *P. a. perflavus* nov. ssp. (see below).

Phylloscopus occisinensis nov. sp.

Holotype: Male, China, Qinghai, western shore of lake Donggi Cona, 4200 m, in *Salix* bushes on rocky slope (35°21'N 98°19'E), J. Martens leg. 01. IX. 2002 (MTD C 62005; tissue sample MAR 3647, song recording of this specimen by A. Gebauer, vocal archive JM 33700, 33701 and 33702, Fig. 12b).

Paratypes: Male, China, Qinghai, Balong, 3650 m, open *Juniperus* forest with few bushes, (35°54'N 97°34'E), J. Martens leg. 27. VII. 2002 (MTD C 62004, tissue sample MAR 3627, Fig. 12b).

Male, China, Qinghai; south of Heimahe, Koko Nor (Qinghai Lake), appr. 3400 m (36°45'N 99°46'E), J. Fiebig & M. Kaiser leg. 26. VI. 2001 (ZMB 2001/3036; tissue sample MAR 1797).

Male, China, Gansu, Lianhua Shan Nature Reserve, above Shahetan, 2900m, in *Salix*, *Rhododendron* and *Berberis* bushes near coniferous forest edge (34°55'N 103°43'E), J. Martens leg. 30. VII. 2007 (IZAS; tissue sample MAR 6469).

Male, China, Sichuan, Pamuling Monastery W of Kangding, 4050 m, bushes and low pine (*Pinus*) trees near forest edge (30°06'N 101°10'E), J. Martens & Sun Yue-Hua leg. 24. V. 2008 (MTD C 63177; tissue sample MAR 6986).

Female, China, Yunnan, Jizu Shan, 2200 m, from a small flock of migrating birds, clearing in open forest (25°57'N 100°23'E), J. Martens leg. 26. IV. 2002 (MTD C 62006; tissue sample MAR 3204, Fig. 12b).

Additional material: The classical material of the Weigold and Beick collections is not incorporated into the type series. Genotypes of these birds are unknown; affiliation to *P. occisinensis* nov. sp. was achieved by skin comparison only. – Male, China, Sichuan, Kwan-hsien (presently: Dujiangyan), H. Weigold leg. 5. V. 1914 (MTD C 23262). – Male, China, Sichuan, Tatsienlu (presently: Kangding), H. Weigold leg. 12. VI. 1915 (MTD C 23261). – Male, China, Sichuan, Sunpan-ting, H. Weigold leg. 10. VI. 1914 (MTD C 23263). – Unsexed juvenile, China, Sichuan, Sungpan, H. Weigold leg. 9. VII. 1914 (MTD C 23264). – Female, China, Sichuan, Hoanglung, near Sungpan, H. Weigold leg. 16. VI. 1914 (MTD C 23265). – Male, China, Gansu, Sining-fu Gebiet (Xining area), Umgebung des Dorfes Lassa (close to village Lassa), W. Beick leg. 18. IX. 1928 (MTD C 33584).

Diagnosis of taxon: Medium-sized *Phylloscopus* leaf warbler of the *P. affinis* group with long bright-yellow supercilium, eye-stripe broad, distinct in front of eye, plumage dark brownish green above, bright yellow below, breast band brownish beige on lateral sides, tending to orange in centre of breast band in fresh plumage, sides of flanks darkened to greyish beige, no wing-bar, tips of rectrices with narrow light fringes. Taxon displays unique synapomorphies in the base pairs of cytochrome-*b* and 16S rRNA genes; based on the two mitochondrial markers forms a well supported monophyletic group with *P. griseolus* and *P. affinis*.

Description of holotype (Fig. 12b, left): Upper side from forehead to lower back blackish olive-brown

(schwärzlich-olivbraun 11-7-8), includes a slight greenish tinge; on underside throat light greenish yellow, breast on the sides brownish-olive tinged, tending to slightly orange in the centre of breast, dark yellow (dunkelgelb 5-0-7) on lower breast and belly, flanks marked olive brownish. Supercilium marked bright yellow in front, more greenish at rear, contrasting with dark head plate, eye stripe dark brownish to blackish, contrasting with supercilium, also with chin in front of eye, less with cheeks behind eye; rectrices and remiges dark grey blackish, outer webs with a greenish (basal half) or a narrow whitish part (distal half). Upper mandible of bill dark horn brown, lower light horn, legs dark brown. – Measurements (in mm): wing 62 mm, tail 50 mm, tarsus 21 mm.

Paratypes: Marked variation in coloration, depending on month; after autumn moult most vividly coloured, in breeding birds (April to July) brightness of yellow is reduced, but always remains more vivid than in nominate *affinis* but less so than in *P. affinis perflavus* nov. ssp. from western Himalayas (from same season). One apparently juvenile bird (Fig. 12b, C 62004) just starting moult of breast feathers is quite contrasting: whitish with yellow tinge on belly, breast band with grey tinge. Also in summer birds greenish margins in remiges and rectrices persist.

Measurements (in mm): wing, male: 53, 59, 60, 62, female: 60; -- tail: male: 41, 48, 48.5, 50; female: 50; -- tarsus, male: 18.5, 19.5, 20, 21, female: 20.

Derivatio nominis: The name is composed of (abbreviated) Latin “occidentalis”: western, belonging to the West, and “sinensis”: Chinese or related to China and refers to the distribution of this taxon in West China. As an English name we propose “West Chinese Leaf Warbler” which translates as “Hua Xi Liu Ying” in Chinese.

Habitat: *Caragana* and *Salix* bushes in open *Juniperus* forest, in alpine steppe facies (Qinghai), bushes close to *Abies/Picea* forest and open meadows (Gansu) or bushes near birch (*Betula*) and pine (*Pinus*) forest edge (Sichuan). Weigold (in STRESEMANN, 1923) indicates as habitat the high-alpine bush belt, where it is a common breeding bird between 3.000 and 4.500 m, song activity reduced on 6 July, feeding young on July 24.

Distribution of taxon: Records during the breeding season are from Sichuan (Weigold collection; STRESEMANN, 1923; Martens collection), Qinghai and Gansu (Martens collection); a record from Yunnan (April) refers to a migrating bird accompanied by three additional birds. Cytb and 16S genes were proven for all type specimens. Habitat and breeding are described by

STRESEMANN *et al.* (1923, including Weigold's observations) and by STRESEMANN (1937; using W. Beick's collection and diaries).

P. affinis perflavus nov. ssp.

Holotype: Male, India, Kashmir, Lopara, Kiar-Maran Janet, Maran river, 6,000 ft., H. Whistler leg. 29. V. 1931 (BMNH 1949.Whi.1.12276, tissue sample MAR 3940).

Paratypes: Female, India, Kashmir, Warwan valley, 8,300 ft., H. Whistler leg. 07. VI. 1931 (BMNH 1949.Whi.1.12278, tissue sample MAR 3941).

Female, India, North Kashmir, Suru, Rungdom Plain, below Zuildo, 13,000 ft., H. Whistler leg. 01. VII. 1931 (BMNH 1949.Whi.1.12282, tissue sample MAR 3942).

Additional material: Male, India, Kashmir, N base, Diobani Mt., 12,000 ft., V.S. La Personne lg. 04. VII. 1928 (BMNH 1949.Whi.1.12279).

Male, Pakistan, Kashmir, Turmik Nallah, Baltistan, 10,000 ft., F. Ludlow leg. 21. V. 1928 (BMNH 1928.11.20.64).

Male, India, Kashmir, Aliabad terai, W.E. Brooks leg. 18. VI. 1871 (BMNH 1886.7.8.753).

Male, India, Kashmir, 10,000 ft., collector unknown, 02. VII. 1908 (BMNH 1941.5.30.4889).

Diagnosis of taxon: Medium-sized *Phylloscopus* leaf warbler of the *P. affinis* group with long supercilium bright yellow all over its length, eye-stripe broad, distinct but short in front of eye, not reaching base of bill, broad behind eye, more or less concolorously fusing with dark neck and slightly lighter cheeks, plumage dark brownish green above, bright yellow below, without breast band and without differently coloured flanks (a difference to nominate *affinis* and *P. occisinensis* nov. sp.), no wing-bar. Upper mandible light horn brown, slightly darker than lower mandible, legs light horn brown. Taxon displays unique synapomorphies in the base pairs of cytochrome-*b*, sister taxon to nominate *affinis* from the eastern Himalayas.

Description of holotype: Upper side from forehead to lower back dull brownish olive, greenish tinge distinct (schwärzlich-braunoliv 7-11-8). Lower side (Fig. 12, BMNH 1949.Whi.1.12276) from chin to throat, breast and belly bright uniform yellow with greenish touch (schwärzlich-gelb 5-0-8), no breast band and flanks not darkened. Supercilium marked bright yellow, more vividly coloured than underside, slightly lighter behind eye, contrasting with dark head plate, eye stripe short and rather indistinct, does not reach base of bill, blackish, contrasting with supercilium, broader behind eye, fusing with neck and cheeks; rectrices and remiges dark brown blackish, outer webs (after some abrasion) with a greenish to whitish tinge; upper mandible of bill dark horn brown, lower light horn brown,

legs light horn. – Measurements (in mm): wing: 61 mm, tail: 59 mm, tarsus: 20 mm.

Variability: The bright yellow underside makes it a very characteristic form within the *affinis* complex. Of the seven specimens available (all from the breeding season), five are very similar to each other; one specimen is slightly more greenish (BMNH 1949.Whi.1.12279) and one is much lighter yellow-greenish below (BMNH 1941:5:30-4889, collected July 2, 1908).

Measurements: wing, male: 55, 56, 59.5, 60, 61, female: 54, 55.5; -- tail, male: 43, 46, 47, 48, 59, female: 42, 43; -- tarsus, male: 18.5, 19, 19, 19.5, 20; female: 19, 19.

Derivatio nominis: The name is derived from Latin *flavus* 'yellow' and *per* 'very', 'throughout' referring to the all bright yellow underside of this taxon, which distinctly differs from the other representatives of the *affinis* complex.

Distribution of taxon: Restricted to the western Himalayas, but area not known in detail. The material available here originates from the Vale of Kashmir and surroundings in the Northwest. The eastern limit presumably is far west of the West Nepal border. Habitat and breeding are described by BATES & LOWTHER (1952) and ROBERTS (1992).

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References

- ALSTRÖM, P. (2006): Species concepts and their application: insights from the genera *Phylloscopus* and *Seicercus*. – *Acta Zoologica Sinica*, **52**, Suppl.: 429–434.
- ALSTRÖM, P. & OLSSON, U. (1990): Taxonomy of the *Phylloscopus proregulus* complex. – *Bull. Brit. Orn. Club*, **110**: 38–43.
- ALSTRÖM, P. & OLSSON, U. (1999): The Golden-spectacled Warbler: a complex of sibling species, including a previously undescribed species. – *Ibis*, **141**: 545–568.
- ALSTRÖM, P., ERICSON, P.G.P., OLSSON, U. & SUNDBERG, P. (2006): Phylogeny and classification of the avian superfamily Sylvioidae. *Mol. Phyl. Evol.*, **38**: 381–397.
- AMADON, D. (1966): The superspecies concept. – *Systematic Zoology*, **15**: 245–249.
- BATES, R.S.P. & LOWTHER, E.H.N. (1952): Breeding birds of Kashmir. – Oxford. Univ. Press, Delhi.
- BENSCH, S., IRWIN, D.E., IRWIN, J.H., KVIST, L. & ÅKESSON, S. (2006): Conflicting patterns of mitochondrial and nuclear DNA diversity in *Phylloscopus* warblers. *Mol. Ecol.*, **15**: 161–171.
- CHENG, T.-H. (1987): A synopsis of the Avifauna of China. – Parey, Hamburg and Berlin: i-xvi, 1223 pp.
- CHIRABRYJ, W.M., W.M. LOSKOT & VIETINGHOFF-SCHEEL, E. (1989): *Phylloscopus fuscatus* (Blyth). Dunkellaubsänger. – Atlas der Verbreitung paläarktischer Vögel, Lieferung **16**. Akademie-Verlag Berlin. 6 pp.
- CLEMENT, P. (2006): *Phylloscopus affinis*. *Phylloscopus fuscatus*. *Phylloscopus fulgiventis*. *Phylloscopus armandi*. *Phylloscopus subaffinis*. In: DEL HOYO J., A. ELLIOT & CHRISTIE, D.A. [eds]. Handbook of the birds of the World, **Vol. 11**. Old World Flycatchers to Old World Warblers. – Lynx Edicions, Barcelona: 655–657.
- DEL HOYO, J., A. ELLIOT & CHRISTIE, D.A. [eds] (2006): Handbook of the Birds of the World, **Vol. 11**, Old World Flycatchers to old World Warblers. Species Accounts. – Lynx Edicions, Barcelona: 647–672.
- DICKINSON, E.C. [ed.] (2003): The Howard and Moore complete checklist of the birds of the world. 3rd ed. – Christopher Helm, London, 1039 pp.
- DIESSELHORST, G. (1968): Beiträge zur Ökologie der Vögel Zentral- und Ost-Nepals. – Khumbu Himal. Ergebnisse des Forschungsunternehmens Nepal-Himalaya **2**: 1–417.
- ECK, S. & QUAISER, C. (2004): Verzeichnis der Typen der Vogelsammlung des Museums für Tierkunde in den Staatlichen Naturhistorischen Sammlungen Dresden. – *Zool. Abh. Dresden*, **54**: 233–316.
- EDWARDS, S.V., P. ARCTANDER & WILSON, A.C. (1991): Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. – *Proc. R. Soc. London B*, **243**: 99–107.
- ERICSON, P.G.P., A.-L. JANSÉN, U.S. JOHANSSON & EKMÄN, J. (2005): Inter-generic relationships of the crows, jays, magpies and allied groups (Aves: Corvidae) based on nucleotide sequence data. – *J. Avian Biol.*, **36**: 222–234.
- FELSENSTEIN, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution*, **39**: 783–791.
- GLUTZ VON BLITZHEIM, U.N. & BAUER, K.M. (1991): Handbuch der Vögel Mitteleuropas, **12/II**, Passeriformes (Part 3). – Aula Verlag, Wiesbaden: 1147–1161.
- HARPENDING, R.C. (1994): Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. – *Hum. Biol.*, **66**: 591–600.
- HARRISON (1982): An atlas of the birds of the western Palearctic. Collins, London.
- HELB, H.-W., H.-H. BERGMANN & MARTENS, J. (1982): Acoustic differences between populations of western and eastern Bonelli's Warblers (*Phylloscopus bonelli*, Sylviidae). – *Experientia*, **38**: 356–357.
- HUELSENBECK, J. P. & RONQUIST, F. (2001): MRBAYES: Bayesian inference of phylogenetic trees. – *Bioinformatics*, **17**: 754–755.
- ICZN, INTERNATIONAL COMMISSION OF ZOOLOGICAL NOMENCLATURE (1999): International Rules for Zoological Nomenclature. 4th ed. – London. XXIX + 306 pp.
- IRWIN, D.E. (2000): Song variation in an avian ring species. – *Evolution*, **54**: 998–1010.
- IRWIN, D.E., S. BENSCH & PRICE, T.D. (2001): Speciation in a ring. *Nature*, **409**: 333–337.
- IRWIN, D.E., S. BENSCH, J.H. IRWIN & PRICE, T.D. (2005): Speciation by distance in a ring species. – *Science*, **307**: 414–416.
- JOHANSSON, U.S., P. ALSTRÖM, U. OLSSON, P.G.P. ERICSON, P. SUNDBERG & PRICE, T.D. (2007): Build-up of the Himalayan avifauna through immigration: A biogeographical analysis of the *Phylloscopus* and *Seicercus* Warblers. – *Evolution*, **61**: 324–333.
- KIMURA, M. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. – *J. Mol. Evol.*, **16**: 111–120.
- KIMURA, M. (1981): Estimation of evolutionary distances between homologous nucleotide sequences. – *Proceedings of the National Academy of Sciences USA*, **78**: 454–458.
- KOCHER, T.D., K.W. THOMAS, A. MEYER, S.V. EDWARDS, S. PÄÄBÖ, F.X. VILLABLANCA & WILSON, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. – *Proc. Nat. Acad. Sci. USA*, **86**: 6196–6200.

- LOSKOT, W.M. & VIETINGHOFF-SCHEEL, E.V. (1991): *Phylloscopus griseolus* (Blyth) Felsenlaubsänger. – Atlas der Verbreitung palaearktischer Vögel, Lieferung 17. 7 pp. – Akademie-Verlag Berlin.
- LU, X. (2008): Breeding ecology of an Old World high-altitude warbler, *Phylloscopus affinis*. – J. Ornithol., **149**: 41–47.
- LUDLOW, F. & KINNEAR, N.B. (1944): The birds of south-eastern Tibet. – Ibis, **86**: 43–86, 176–208, 348–389.
- MARTENS, J. (1980): Lautäußerungen, verwandtschaftliche Beziehungen und Verbreitungsgeschichte asiatischer Laubsänger (*Phylloscopus*). – Advances in Ethology, **22**: 1–71.
- MARTENS, J. & ECK, S. (1995): Towards an Ornithology of the Himalayas. Systematics, ecology and vocalizations of Nepal birds. – Bonner Zool. Monogr., **38**: 1–445.
- MARTENS, J. & PÄCKERT, M. (2003): Disclosure of songbird diversity in the Palearctic/Oriental transition zone. In: Legakis, A., S. Sfentourakis, R. Polymeni & M. Thessalou-Lagaki (eds). The New Panorama of Animal Evolution. – Proc. 18th Int. Congr. Zoology, Athens 2000; Pensoft, Sofia: 551–558.
- MARTENS, J. & TIETZE, D.T. (2006): Sino-himalayanisches Verbreitungsmuster. Abstracts. 138. Jahresversammlung DO-G Stuttgart 2005. – Vogelwarte, 44: 56–57.
- MARTENS, J., D.-T. TIETZE, S. ECK, & VEITH, M. (2004): Radiation and species limits of the Asian Pallas's warbler complex (*Phylloscopus proregulus* s.l.). – J. Ornithol., **145**: 206–222.
- MARTENS, J., D.-T. TIETZE & SUN, Y.H. (2006): Molecular phylogeny of *Parus* (*Periparus*), a Eurasian radiation of tits (Aves: Passeriformes: Paridae). – Zool. Abh. Mus. Tierk. Dresden, **55**: 103–120.
- MICHEL Farbenführer (2000): 37th ed. – Schwaneberger Verlag, München. 47 pp.
- OLSSON, U., P. ALSTRÖM, & SUNDBERG, P. (2004): Non-monophyly of the avian genus *Seicercus* (Aves: Sylviidae) revealed by mitochondrial DNA. – Zoologica Scripta, **33**: 501–510.
- OLSSON, U., P. ALSTRÖM, P.G.P. ERCISON, & SUNDBERG, P. (2005): Non-monophyletic taxa and cryptic species – Evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*: Aves). – Mol. Phyl. Evol., **36**: 261–275.
- PÄCKERT, M., J. MARTENS, J. KOSUCH, A.A. NAZARENKO & VEITH, M. (2003): Phylogenetic signal in the songs of Crests and Kinglets (Aves: *Regulus*). – Evolution, **57**: 616–629.
- PALUMBI, S., A. MARTIN, S. ROMANO, W.O. MCILLAN, L. STICE & GRABOWSKI, G. (1991): The simple fools guide to PCR. – Kewalko Marine Laboratory, University of Hawaii, Honolulu, HI.
- POSADA, D. & CRANDALL, K.A. (1998): MODELTEST: testing the model of DNA substitution. – Bioinformatics, **14**: 817–818.
- PRICE, T., HELBIG, A. & RICHMAN, A. (1997): Evolution of breeding distributions in the Old World leaf warblers (genus *Phylloscopus*). Evolution, **51**: 552–561.
- RASMUSSEN, P.C. & ANDERTON, J.C. (2005): Birds of South Asia. The Ripley guide. 2. Attributes and Status. – Lynx Edicions, Barcelona.
- RHEINDT, F.E. (2006): Splits galore: the revolution in Asian leaf warbler systematics. – BirdingASIA, **5**: 25–39.
- RICHMAN, A. (1996): Ecological Diversification and Community Structure in the Old World Leaf Warblers (Genus *Phylloscopus*): A Phylogenetic Perspective. Evolution, **50**: 2461–2470.
- ROBERTS, T.J. (1992): The birds of Pakistan, **2**. Passeriformes. – Oxford University Press, Karachi. XXXV + 617 pp.
- ROZAS, J. & ROZAS, R. (1999): DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. – Bioinformatics, **15**: 174–175.
- SAITOU, N. & NEI, M. (1987): The neighbor-joining method: A new method for reconstructing phylogenetic trees. – Mol. Biol. Evol. **4**: 406–425.
- SCHMIDT, H.A., K. STRIMMER, M. VINGRON & VON HAESELER, A. (2000): TREE-PUZZLE, version 5.0. München.
- SPICER, G.S. & DUNIPACE, L. (2002): Molecular phylogeny of songbirds (Passeriformes) inferred from mitochondrial 16S ribosomal RNA sequences. – Mol. Phyl. Evol. **30**: 325–335.
- STRESEMANN, E. (1923): Zoologische Ergebnisse der Walter Stötznerschen Expeditionen nach Szetschwan, Osttibet und Tschili. 3 Teil. Aves. Passeres und Picariae I. – Abh. Ber. Mus. Tierk. Völkerk. Dresden, **16** (2): 11–21.
- STRESEMANN, E., W. MEISE & SCHÖNWETTER, M. (1937): Aves Beickianae. Beiträge zur Ornithologie von Nordwest-Kansu nach den Forschungen von Walter Beick (†) in den Jahren 1926–1933. Pt. 1. – J. Ornithol., **85** (3): 375–576.
- STRIMMER, K. & VON HAESELER, A. (1996): Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. – Mol. Biol. Evol., **13**: 964–969.
- TAJIMA, F. (1996): The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. – Genetics, **143**: 1457–1465.
- TICEHURST, C.B. (1938): A systematic review of the genus *Phylloscopus* (willow-warblers or leaf-warblers). British Museum, London. 195 pp.
- TICEHURST, C.B. (1934): Description of a new form of *Phylloscopus*: *Phylloscopus armandii perplexus*, subsp. nov. – Bull. Brit. Orn. Club **54**, 96–97.
- TIETZE, D.-T., J. MARTENS & SUN, Y.H. (2006): Molecular phylogeny of treecreepers (*Certhia*) detects hidden diversity. – Ibis, **148** (3): 477–488.
- VAURIE, C. (1954): Systematic notes on palearctic birds. No. 9. Sylviinae: the genus *Phylloscopus*. – Amer. Mus. Novit., **1685**. 23 pp.
- VAURIE, C. (1959): The birds of the palearctic fauna. A systematic reference. Order Passeriformes. – Witherby, London. 762 pp.
- VIETINGHOFF-SCHEEL, E. v. (1984): *Phylloscopus armandi* (Milne-Edwards). David-Laubsänger. – Atlas der Verbreitung palaearktischer Vögel, Lieferung 12. 4 pp. Akademie-Verlag Berlin.
- VIETINGHOFF-SCHEEL, E. v. (1986): *Phylloscopus affinis* (Tieckell). Himalaya-Laubsänger. – Atlas der Verbreitung palaearktischer Vögel, Lieferung 13. 5 pp. Akademie-Verlag Berlin.

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