

## The radiation of the *Seicercus burkii* complex and its congeners (Aves: Sylviidae): molecular genetics and bioacoustics<sup>☆</sup>

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### Abstract

Cryptic species of passerine birds lack notable morphological differentiation and can best be identified by molecular and bioacoustic markers. Here we investigate seven cryptic species of the golden-spectacled warbler (*Seicercus burkii* complex) with respect to territorial song and cytochrome-*b* (*cyt-b*) sequences. Their phylogenetic relations to other *Seicercus* species and to members of the genus *Phylloscopus* are inferred by the same methods. Three separate lineages of *Seicercus* are nested within different branches of the molecular *Phylloscopus* tree. The *S. burkii* complex is a monophyletic unit comprising seven species (*S. burkii* s. str., *S. whistleri*, *S. valentini*, *S. soror*, *S. omeiensis*, *S. tephrocephalus* and *S. affinis*). *S. xanthoschistos* turned out to be a close relative of *Phylloscopus davisoni* within the *P. reguloides* group. Two isolated sister taxa, *S. grammiceps* and *S. castaniceps*, also branch together with the *P. reguloides* group. Within the *S. burkii* complex the overall haplotype and nucleotide diversity is highest in taxa from the Chinese middle and upper mountain belt (*S. valentini*, *S. omeiensis* and *S. soror*), indicating at least partially restricted gene flow in these species. This is explained by the fragmentation of high-altitude habitats in China while in the Himalayas the vicariant species *S. whistleri* inhabits a more continuous mountain belt at the same altitude. For the Chinese species from medium and high altitudes, past range expansion is indicated by significantly negative Tajima *D<sub>s</sub>*. According to pairwise genetic distances, most species of the *S. burkii* complex have diverged 5 myr ago, the most recent split between *S. burkii* and *S. tephrocephalus* is dated 2 myr ago. Coalescence times for haplotype lineages of the different species range from 9 up to 12 myr, and between 5 and 6 myr for *S. burkii* and *S. tephrocephalus*.

Within *Seicercus* divergence of song features such as frequency parameters and syntax structures correlate with genetic distances between taxa. The three *cyt-b* lineages of *Seicercus* correspond to different clusters in a discriminant analysis by acoustic parameters. Common syntax structures of territorial song in the *Phylloscopus/Seicercus* assemblage are: (1) an introductory element derived from specific calls and (2) a syntax of trills and repeated element groups or a combination of both. There are clear indications that these song structures have repeatedly emerged, were lost or were altered in different branches of the phylogenetic tree at different times. Absolute differences between taxa in frequency parameters or in an acoustic divergence index increase significantly with growing genetic distances.

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However, due to multiple parallel evolution phylogenetic information provided by single acoustic traits decreases with increasing numbers of taxa involved in the investigation.

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## Introduction

Recently, the use of modern techniques such as bioacoustics and molecular genetics in avian systematics has revealed speciation processes, radiation patterns and even unexpected species limits in cases where morphological variation at best had indicated subspecific differentiation. Vocalizations often show marked differences between closely related bird taxa or even between neighbouring populations and have thus been considered useful in avian systematics (review in Alström and Ranft 2003). Compared to molecular data, passerine vocalizations present marked intraspecific levels of differentiation and radiation, as was shown in crests and kinglets (Martens et al. 1998; Päckert et al. 2003), treecreepers (Baptista and Krebs 2000; Martens et al. 2002), and larks (Alström 1998). In Old World warblers (genus *Phylloscopus*), “cryptic species” were unmasked by means of molecular and bioacoustic analyses (Helbig et al. 1995, 1996; Irwin et al. 2001a). A case of “speciation in a ring” was documented in the greenish warbler, *Phylloscopus trochiloides* (Irwin 2000; Irwin et al. 2000, 2001b). Finally, new avian species were recently described or at least confirmed by bioacoustic data in the genera *Phylloscopus* (Alström et al. 1992; Alström and Olsson 1995; Olsson et al. 1993), *Certhia* (Martens et al. 2002), and *Pnoepyga* (Martens and Eck 1991), to mention only the Palearctic and adjacent areas.

Another challenging case of cryptic speciation was discovered in the Old World warbler genus *Seicercus* Swainson. In 1999, two treatises on the golden-spectacled warbler, *Seicercus burkii* (Burton) s. l., were published (Alström and Olsson 1999; Martens et al. 1999). On grounds of morphology and bioacoustics they independently insisted that the widespread *S. burkii* s. l. (Fig. 1) consists of at least five distinct biospecies. Two of them were described as new to science: *S. soror* Alström & Olsson and *S. omeiensis* Martens, Eck, Päckert & Sun. Most spectacularly, these two species co-occur on the same mountain, the Emei Shan in Sichuan, China. Mitochondrial DNA sequences (cytochrome-*b*, *cyt-b*) revealed a marked genetic divergence among all five taxa investigated (Martens et al. 1999), a result later confirmed by Alström and Olsson (2000). Despite the largely congruent morphological, bioacoustic, and molecular results of both papers, several minor differences in nomenclature emerged (see reviews by Alström

and Olsson 2000; Martens and Eck 2000; Martens et al. 2003).

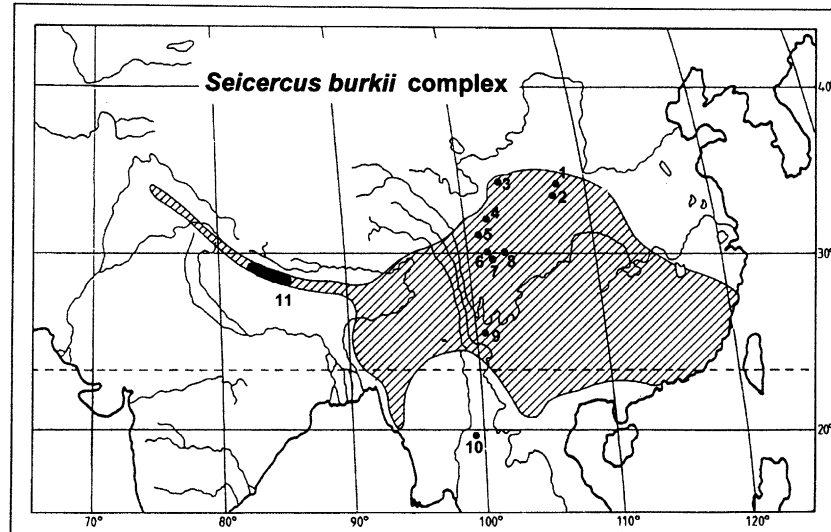
Intragenetic relations within *Seicercus* are complex, as based on bioacoustic and molecular data. Other members of the genus do not seem to be closely related to the *S. burkii* complex (Martens and Eck 1995; Martens et al. 1999). Further molecular analyses revealed that three *Seicercus* lineages represent different clades within the genus *Phylloscopus*, suggesting paraphyly of both genera (Alström 2002). Bioacoustic studies comparing the three lineages of *Seicercus* or representatives of *Seicercus* and *Phylloscopus* were lacking.

Our molecular study concentrates on the intergeneric comparison between *Seicercus* and *Phylloscopus* with emphasis on taxa of the *P. reguloides* group. We present a molecular phylogeny based on 658 bp of the *cyt-b* gene for 10 *Seicercus* and 13 *Phylloscopus* species. We have enlarged the molecular data set of our earlier study (Martens et al. 1999) and provide new data on intraspecific DNA diversity and population structure, based on most species of the *S. burkii* complex and the largest sample size available to date. Differentiation of frequency and time parameters of territorial song was investigated by discriminant analysis. Character tracing and homoplasy indices were used to infer phylogenetic information from song characters.

## Materials and methods

### Taxonomy

On the species level, nomenclature in the *S. burkii* complex follows Alström and Olsson (2000) and Martens et al. (2003). According to present knowledge six biospecies are distinguished (see also Dickinson 2003): *S. burkii* (Burton) s. str., *S. whistleri* Ticehurst, *S. valentini* (Hartert), *S. soror* Alström & Olsson, *S. omeiensis* Martens et al., and *S. tephrocephalus* (Anderson). The neotype designation for *S. tephrocephalus* by Alström and Olsson (1999) was seen as incorrect by Martens et al. (2003). For the sake of nomenclatural stability in this “difficult” group of warblers we do not consider the objections as expressed in Martens et al. (2003) here. Martens et al. (1999) described the song of an unidentified *Seicercus* species which was later



**Fig. 1.** Distribution area of the *S. burkii* complex, and sampling sites (voucher skins, tissue samples, song recordings). The latter represent local breeding populations, except for No. 10. Sites 1–9: China; 1=Taibai Shan, Shaanxi; 2=Foping, Shaanxi; 3=Lianhua Shan, Gansu; 4=Longxi-Honkou Reserve and Qingchen Shan near Dujiangyan, Sichuan; 5=Yaoji, Sichuan; 6=Labahe Reserve, Sichuan; 7=Wawu Shan, Sichuan; 8=Omei Shan, Sichuan; 9=Jizu Shan, Yunnan. Site 10=Thailand, Mae Hong Son Province, Tham Lot. Area 11=Nepal, various sites from extreme western to eastern border (map altered from Martens et al. 1999). Not figured: two localities in Cambodia (see Table 1).

recognized as *S. affinis* (Hodgson) by Alström and Olsson (2000) but not affiliated to the *S. burkii* complex. However, molecular analyses have shown that *S. affinis* and *S. poliogenys* (Blyth) belong to a monophyletic assemblage including all members of the traditional *S. burkii* complex (Alström 2002).

### Molecular genetics

We studied 81 blood or tissue samples from 10 *Seicercus* species mostly belonging to the *S. burkii* complex (Table 1). The study site locations are shown in Fig. 1. An according voucher specimen was available for 51 samples. In addition, we sequenced 21 samples from 13 species in the genus *Phylloscopus* (Table 1).

As for the close genetic relation between *S. xanthoschistos* (Gray & Gray) and *P. occipitalis* (Blyth) (see Alström 2002), we focussed the choice of *Phylloscopus* species for this study on the members of the *P. reguloides* group: we chose *P. reguloides* (Blyth), *P. occipitalis*, *P. davisoni* (Oates), *P. cantator* (Tickell), and *P. ricketti* (Slater). Additional *Phylloscopus* species were selected in order to investigate a broad variety of song structures in our comparative analyses (song structure highly similar/dissimilar to structures represented in the *S. burkii* complex). As outgroups we used the goldcrest, *Regulus regulus* (Linnaeus); the golden-crowned kinglet, *R. satrapa* Lichtenstein; and the green-backed tit, *Parus monticolus* Vigors.

Extraction of DNA, PCR conditions and sequencing followed Päckert et al. (2003). Sequences were analysed on an ABI<sup>TM</sup> 377 and automatically aligned with the Sequence Navigator software (Perkin-Elmer). Alignments were slightly adjusted by eye. Where possible our *Phylloscopus* sequences were compared to corresponding sequences in GenBank. For phylogenetic reconstructions we used only our own sequences.

An adequate substitution model was estimated via hierarchical Likelihood Ratio Tests (Modeltest 3.04, Posada and Crandall 1998). All *Seicercus* taxa plus one outgroup sequence (*P. collybita* (Vieillot)) were included in the input data set. The selected model was the Tamura–Nei model (TrN+I) for all *Seicercus* haplotypes. Likelihood settings were as follows: empirical base frequencies:  $\pi_A=0.275$ ,  $\pi_C=0.355$ ,  $\pi_G=0.145$ ,  $\pi_T=0.225$ ; proportion of invariable sites  $I=0.7188$ ; substitution rates:  $R=1$ , except  $R_{[A-G]}=14.4$ ,  $R_{[C-T]}=9$ . In a second run with Modeltest 3.04, we included the most common haplotypes of *Seicercus* species (one haplotype each) and 15 *Phylloscopus* haplotypes. The selected model was TVM+G (Posada and Crandall 1998) with:  $\pi_A=0.283$ ,  $\pi_C=0.384$ ,  $\pi_G=0.134$ ,  $\pi_T=0.1995$ ; proportion of invariable sites  $I=0.638$ ; gamma shape parameter  $\alpha=0.167$ ; substitution rates:  $R_{[A-C]}=1.01$ ,  $R_{[A-G]}=7.96$ ,  $R_{[A-T]}=0.75$ ,  $R_{[C-G]}=0.09$ ,  $R_{[C-T]}=8.4$ ,  $R_{[G-T]}=1$ .

Phylogenetic trees were constructed under different approaches: Neighbor Joining (NJ, Saitou and Nei 1987; PAUP 4.0.1, Swofford 2001), Maximum

**Table 1.** Reference data on blood and tissue samples for PCR and sequencing

Haplotype	<i>n</i>	Locality	Sample no.	GenBank accession no.
<i>Seicercus</i>				
<i>S. whistleri</i> 1	1	Nepal: Dolakha Distr.	NEP 31	AY 606136
<i>S. whistleri</i> 2	1	Nepal: Dolakha Distr.	NEP 36	AY 606159
<i>S. whistleri</i> 3	8	Nepal: Dolakha Distr., Mustang Distr., Bhojpur Distr., Maharigaon	NEP 58, 97, 103; MAR 2132, 2651, 2660, 2728, 2729	AY 606162
<i>S. whistleri</i> 4	1	Nepal: Maharigaon	MAR 2131	AY 606152
<i>S. whistleri</i> 5	1	Nepal: Bhojpur Distr.	MAR 2652	AY 606154
<i>S. whistleri</i> 6	1	Nepal: Bajura Distr.	MAR 2926	AY 606158
<i>S. burkii</i> 1	1	Nepal: Bhojpur Distr.	MAR 2730	AY 606155
<i>S. burkii</i> 2	1	Nepal: Bhojpur Distr.	MAR 2731	AY 606156
<i>S. valentini</i> 1	1	China: Taibai Shan	MAR 777	AY 606168
<i>S. valentini</i> 2	6	China: Taibai Shan, Lianhua Shan, Omei Shan	MAR 780, 790, 795, 806, 949, 2825	AY 616180
<i>S. valentini</i> 3	1	China: Taibai Shan	MAR 787	AY 606169
<i>S. valentini</i> 4	1	China: Taibai Shan, Omei Shan	MAR 801	AY 606137
<i>S. valentini</i> 5	1	China: Omei Shan	MAR 919	AY 606174
<i>S. valentini</i> 6	3	China: Wawu Shan, Omei Shan	MAR 943, 3268, 3283	AY 616179
<i>S. valentini</i> 7	1	China: Omei Shan	MAR 2051	AY 606150
<i>S. valentini</i> 8	1	China: Wawu Shan	MAR 2072	AY 606151
<i>S. omeiensis</i> 1	1	China: Taibai Shan	MAR 712	AY 606163
<i>S. omeiensis</i> 2	1	China: Taibai Shan	MAR 713	AY 606164
<i>S. omeiensis</i> 3	1	China: Taibai Shan	MAR 714	AY 606165
<i>S. omeiensis</i> 4	5	China: Taibai Shan, Qincheng Shan, Labahe Reserve, Omei Shan	MAR 715, 910, 925, 2021, 3004	AY 606176
<i>S. omeiensis</i> 5	1	China: Omei Shan	MAR 922*	AY 606138
<i>S. omeiensis</i> 6	2	China: Wawu Shan, Omei Shan	MAR 927, 3258	AY 606177
<i>S. omeiensis</i> 7	3	China: Qincheng Shan, Wawu Shan	MAR 2027, 2039, 2071	AY 606143
<i>S. omeiensis</i> 8	1	China: Omei Shan	MAR 2049	AY 606149
<i>S. omeiensis</i> 9	1	SW Cambodia: Mt. Kmaach	NHM 2000.5.42	AY 606161
<i>S. omeiensis</i> 10	1	China: Labahe Reserve	MAR 3302	AY 616190
<i>S. soror</i> 1	1	China: Taibai Shan	MAR 761	AY 606166
<i>S. soror</i> 2	1	China: Taibai Shan	MAR 765	AY 606167
<i>S. soror</i> 3	1	China: Omei Shan	MAR 904	AY 606173
<i>S. soror</i> 4	1	China: Omei Shan	MAR 921	AY 606175
<i>S. soror</i> 5	2	China: Omei Shan	MAR 929, 2040	AY 606178
<i>S. soror</i> 6	6	China: Qincheng Shan, Omei Shan	MAR 2010, 2013, 2020, 2031, 2043, 2045	AY 616141
<i>S. soror</i> 7	1	China: Omei Shan	MAR 2046	AY 606147
<i>S. soror</i> 8	1	SW Cambodia: Mt. Kmaach	NHM 2000.5.41	AY 606160
<i>S. soror</i> 9	1	China: Labahe Reserve	MAR 3288	AY 616189
<i>S. affinis</i> 1	1	China: Omei Shan	MAR 2042	AY 606146
<i>S. affinis</i> 2	1	China: Omei Shan	MAR 2047	AY 606148
<i>S. tephrocephalus</i> 1	7	China: Taibai Shan, Qincheng Shan; Thailand: Mae Hong Son Prov.	MAR 819, 830, 861, 2005, 2019, 2028, 2030	AY 606170
<i>S. tephrocephalus</i> 2	3	China: Taibai Shan	MAR 820, 823, 825	AY 606171
<i>S. tephrocephalus</i> 3	1	China: Taibai Shan	MAR 2004	AY 606140
<i>S. tephrocephalus</i> 4	1	China: Qincheng Shan	MAR 2029	AY 606144
<i>S. xanthoschistos</i> 1	1	Nepal: Kaski Distr.	MAR 76	AY 606135
<i>S. xanthoschistos</i> 2	1	Nepal: Myagdi Distr.	MAR 194	AY 606134
<i>S. grammiceps</i>	1	unknown, from aviary; fide R. Pfeifer	MAR 1944	AY 606139
<i>S. castaniceps</i>	1	Nepal: Bhojpur Distr.	MAR 2636	AY 606145
<i>Phylloscopus</i>				
<i>P. davisoni</i> 1	1	China: Taibai Shan	MAR 764	AY 616183
<i>P. davisoni</i> 2	1	China: Jizu Shan	MAR 3240	AY 616184
<i>P. ricketti</i>	1	China: Omei Shan	MAR 903	AY 606172

**Table 1.** (continued)

Haplotype	<i>n</i>	Locality	Sample no.	GenBank accession no.
<i>P. cantator</i>	1	Nepal: Bhojpur Distr.	MAR 2781	AY 606157
<i>P. reguloides</i> 1	2	China: Taibai Shan; Nepal: Bhojpur Distr.	MAR 701, 2635	AY 616181
<i>P. reguloides</i> 2	1	China: Taibai Shan	MAR 767	AY 616185
<i>P. occipitalis</i>	1	Nepal: Humla Distr.	MAR 3320	AY 616188
<i>P. armandii</i>	1	China: Taibai Shan	MAR 2003	AY 616182
<i>P. schwarzi</i>	4	Russia: middle Amur	MAR 1377–1380	AY 616187
<i>P. trochilus</i>	1	Germany: Lake Constance	MAR 3092	AY 616191
<i>P. collybita</i>	1	France: Pyrénées-Orientales	MAR 535	AF 551766
<i>P. proregulus</i>	1	Russia: sources of Ussuri	MAR 1399	AY 583243
<i>P. kansuensis</i> 1	1	China: Qinghai, Bei Shan	MAR 353	AY 583245
<i>P. kansuensis</i> 2	1	China: Qinghai, Bei Shan	MAR 357	AY 583246
<i>P. chloronotus</i> 1	1	Nepal: Bhojpur Distr.	MAR 2646	AY 583254
<i>P. chloronotus</i> 1	1	Nepal: Bhojpur Distr.	MAR 2648	AY 583255
<i>P. yunnanensis</i> 1	1	China: Taibai Shan	MAR 733	AY 583264
<i>Regulus regulus</i>				AF 551786
<i>R. satrapa</i>				AF 551790
<i>Parus monticolus</i>				AF 551785

Sample size *n* = number of individuals carrying a given haplotype; sample code NEP/MAR = from tissue collection of J. Martens; \* = type specimen. Most samples collected by J.M. and Y.-H.S., additional material provided by M. Fischer (MAR 2131, 2132, 2926, 3320), Y. Fang and Z.-L. Bi (MAR 2825), F. Steinheimer (NHM Tring 2000.5.41, 2000.5.42), A.A. Nazarenko (MAR 1399, 1377–1380), and D.T. Tietze (MAR 3092).

Likelihood (ML; Tree Puzzle, Schmidt et al. 2000), and Bayesian inference of phylogeny with MrBayes 2.01 (Huelsenbeck and Ronquist 2001). Robustness of clades was estimated by 1000 bootstrap replicates (Felsenstein 1985), via quartet puzzling in ML, 1000 puzzling steps (Strimmer and von Haeseler 1996) and via Bayesian posterior probabilities using the Markov Chain Monte-Carlo method (Huelsenbeck and Ronquist 2001; 500,000 generations, burnin=3000). To verify the molecular clock hypothesis for our data set, a constant rate test was carried out with Tree Puzzle (Schmidt et al. 2000). We calculated uncorrected p-distances (with PAUP 4.0.1) and corrected distance values according to the models chosen by Modeltest: Tamura–Nei distances (TrN-distances, Tamura and Nei 1993) and ML distances (with Tree Puzzle). Saturation effects of multiple hits were inferred by plotting uncorrected vs. corrected distance values for each codon position. Divergence times between *Seicercus* species were estimated according to a widely accepted substitution rate of 1.6%/myr in *cyt-b* of passerine birds (Fleischer et al. 1998).

We used DnaSP 4.0 (Rozas et al. 2003) for estimating nucleotide diversities ( $\pi$ , Nei 1987, Eq. 10.5), haplotype diversities (HDs, Nei 1987, Eqs. 8.4 and 8.12) and Tajima's *D*s (Tajima 1989, Eq. 38). Coalescence ratios (CRs) and coalescence times (*t*) were calculated according to Palumbi et al. (2001), with CR = branch length *L* divided by the intraspecific nucleotide diversity ( $\pi$ ), and with  $t = 2N_f$  (CR + 1), where  $N_f$  is the effective population size of females. The latter was estimated according

to Avise et al. (1988) with  $N_f = 0.5 \times 10^8 p/g$ , where *p* is the mean intraspecific genetic distance and *g* the generation time. For great and willow tits (*Parus major*, *P. ater*) generation times of 1.97 and 2.26 years were calculated from lifetime tables by Kvist et al. (1998, 1999). Referring to these values, we used an approximate value of 2 years for generation time (*t*) of small Old World warblers.

## Bioacoustics

### Definition of terms

Song (territorial s.): Continuous and complex vocalization of males given during the breeding season.

Element: Particular sound event, continuous line on a sonagram (Catchpole and Slater 1995, p. 10), also “note”; different element types can either be described by shape in the sonagram (e.g., hooks, downstrokes, unmodulated lines, modulated elements with broad frequency range and at least two frequency maxima) or by their tonal quality (clicks, whistles, etc.).

Introductory element: A single, mostly short element of a fixed type that precedes each verse of a species' territorial song. In contrast to verse elements, pauses between introductory elements may be of irregular length. In *S. valentini*, *S. whistleri*, and *S. soror*, verses are started with a specific hook-shaped element, whereas a similar verse introduction is missing in *S. omeiensis* and *S. burkii* s. str. (Alström and Olsson 1999; Martens et al. 1999).



**Element group:** Fixed group of two or more different element types, often repeated several times.

**Verse:** Complex vocalization of males consisting of various elements or subunits given during/within territorial song; verses are separated by pauses; different verse types can be distinguished by syntax and element composition.

**Syntax:** Denotes order of elements or element groups within a verse. In general, verses in the *S. burkii* complex can either consist of “trills” or repeated “element groups” (Alström and Olsson 1999; Martens et al. 1999). We will show that there are several substructures of these two general syntax patterns characterizing distinct syntax types present or lacking in species repertoires. Syntax patterns are schematized by combinations of different letters, each letter represents a distinct element type: AAAAA=element type A repeated; ABABAB=element types A and B alternated, element group AB repeated.

**Trill:** Multiple repetition of an element of a given type (AAAAA); a single repetition is called “double element” or “double note” (AA, BB, etc.).

**Repertoire:** Comprises the different verse types which are used by a given male or within a given population. To characterize repertoires of different verse types in the *S. burkii* complex we analysed every unique verse type of all recorded males. Each unique verse type was sonagraphed and characterized by its syntax structure. Numbers of unique verse types in one taxon range from 48 (10 males of *S. affinis*) to 272 (25 males of *S. omeiensis*; only recordings from the years 1998, 2000, and 2002 were used).

**Song specimen:** Voucher specimen (skin) of which a vocalization recording is documented; for most song specimens treated in this paper a tissue sample is preserved as well. Our sample size comprises 21 song specimens (see Appendix A, Table 7).

## Frequency and time parameters

We analysed sound recordings of 156 males from 10 *Seicercus* species and of 49 males from seven *Phylloscopus* species. Equipment used for field recordings until 1998 is listed in Martens et al. (1999). Recordings in China 1999, 2000, and 2002 and in Nepal 2001 were made with a portable Nagra SN and Telinga Pro III microphone with plastic reflector  $\varnothing = 60$  cm. Sonographic measurements were taken from the recordings of 140 males for the following verse parameters: maximum frequency ( $F_{\max}$ ), minimum frequency ( $F_{\min}$ ), frequency span (df), and duration ( $t$ ). Provenance of all recordings is listed in Appendix A (see Table 7). Each verse type of a single male was measured once and individual means were calculated. From the recordings of 1998, 2000, and 2001, the same measurements were

taken for trill parts of each verse. Speed of a trill ( $F_{\text{mod}}$ ) was defined by the number of elements ( $n$ ) per time unit (1 s). Acoustic differentiation between the investigated taxa was verified by discriminant analysis (SPSS 8.0). Absolute differences between taxa in song parameters (maximum frequency  $\Delta F_{\max}$ , frequency span  $\Delta f$ , and verse length  $\Delta t$ ) were taken as a measure for acoustic divergence.

## Syntax structures

While measurements of frequency and time parameters provide objective values, syntax characters are often treated in a purely descriptive analysis. We carried out a quantitative analysis by tracing a character matrix (Appendix A, Table 6) on the molecular phylogenetic tree for the *Seicercus/Phylloscopus* assemblage, using MacClade 3.0 (“character tracing”, Maddison and Maddison 1992). The same program was used to calculate the homoplasy indices consistency index (CI, Kluge and Farris 1969), retention index (RI, Farris 1989), and rescaled consistency index ( $RC = CI \times RI$ , Farris 1989). High values of these indices indicate a low level of homoplasy, i.e. reliable phylogenetic information of a distinct character. We reconstructed various phylogenies differing in the number of taxa by removing various *Phylloscopus* species from the acoustic data set to verify a possible correlation between the CI and the number of taxa ( $n$ ) as had been found in molecular data sets (Klassen et al. 1991). For character tracing we used the Bayes tree. All poorly supported nodes were collapsed and treated as polytomies, only clades with support higher than 0.90 in Bayes reconstruction and with more than 70% ML bootstrap support were retained. As a measure of acoustic divergence between taxa we calculated a divergence index ( $\Delta_{\text{ac}}$ , mean character difference with PAUP 4.01) using the same character matrix (Appendix A, Table 6). We examined seven discrete acoustic characters for all 23 taxa of the molecular *Seicercus/Phylloscopus* tree, e.g. syntax types (verses consist of “element groups”, “trills” or a combination of both; syntax: AAAABCCCC; see the “Definition” section) or element types (presence and shape of the introductory note). Among taxa of the *P. proregulus* group, songs can also be distinguished by the length of verses. In several species, males display long continuous songs (“endless song”) instead of series of short verses separated by pauses (Martens et al. 2004). We also included this character into our matrix (Appendix A, Table 6). Information on syntax characters within this group and the well-studied European *P. collybita* (Vieillot) and *P. trochilus* (Linnaeus) was inferred from the literature (Martens 1980; Helbig et al. 1996; Martens et al. 2004).

Similar analyses were carried out to infer phylogenetic information in vocalizations of crests and kinglets (Päckert et al. 2003) and of heron species (McCracken and Sheldon 1997).

## Results

### Molecular genetics

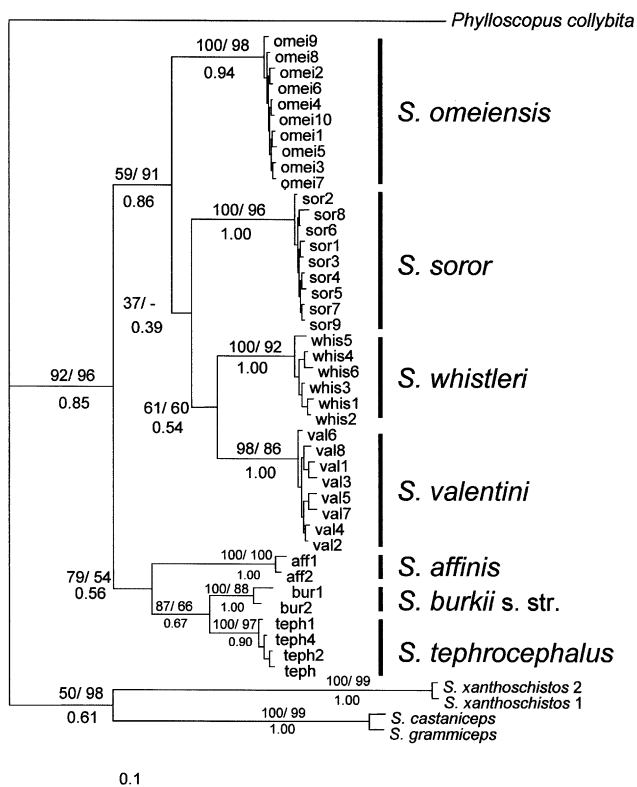
Consistently, 658 bp of the *cyt-b* fragment could be analysed for all haplotypes. Within each species of the *S. burkii* complex we found up to 10 out of a total of 41 different haplotypes. Most of these were rare and have been found only in one individual; only a single haplotype each occurred at a higher frequency in most species (Table 1).

In the Bayes tree (Fig. 2), seven monophyletic haplotype clusters represent seven clearly separated entities: *S. omeiensis*, *S. soror*, *S. whistleri*, *S. valentini*,

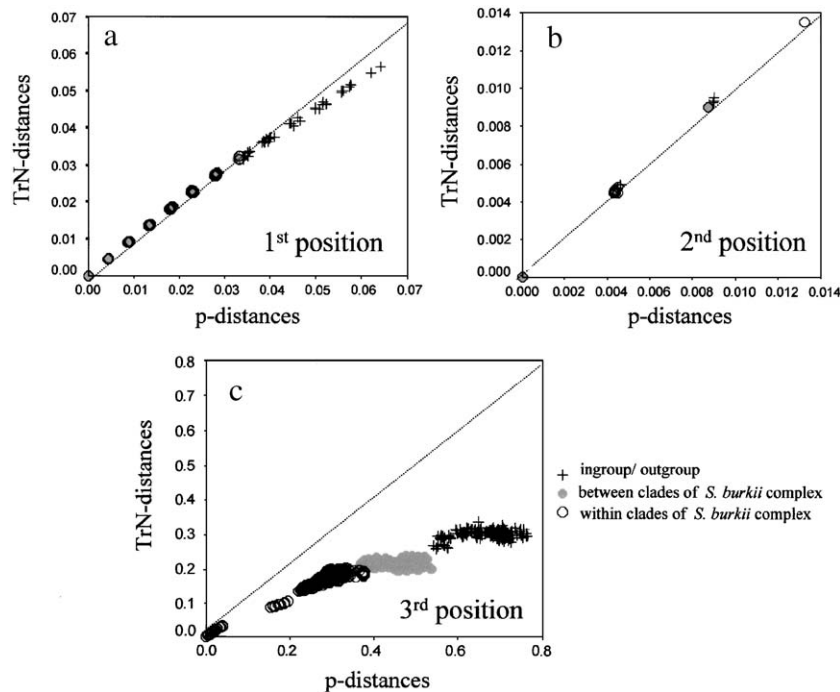
*S. affinis*, *S. burkii* s. str., and *S. tephrocephalus*. The complex is divided at its base into two separate monophyletic branches. One of them includes *S. omeiensis* and *S. soror*, which inhabit medium altitudes, and the two taxa from the upper mountain belt, *S. whistleri* and *S. valentini* (Fig. 2). Phylogenetic relations between these four species are not well resolved. *S. whistleri* and *S. valentini* appear as sister taxa in all reconstructions with weak branch support. Sister-group relation between these two and *S. soror* is poorly supported only in NJ and Bayesian reconstruction; in ML analysis these taxa form a polytomy with *S. omeiensis*. The second branch in Fig. 2 comprises three species from low-mountain altitudes and from a tropical/subtropical distribution range: *S. affinis*, *S. burkii* s. str., and *S. tephrocephalus*. Monophyly of the entire *S. burkii* complex including *S. affinis* is well supported in all phylogenetic reconstructions. Comparison of uncorrected *p*- and TrN-distances in Fig. 3 indicates that multiple hits can be excluded for the first two codon positions, while at the third position there is a strong saturation effect within and between the two major clades of the *S. burkii* complex as well as between the ingroup (*S. burkii* complex) and the outgroup taxa (other *Seicercus* and *P. collybita*). We therefore weighted codon positions in the NJ analysis as: position 1:2:3 = 3:5:1. The Bayes tree for 10 *Seicercus*, 13 *Phylloscopus* species, and *R. regulus*, *R. satrapa* and *P. monticolus* as outgroups is shown in Fig. 4. All taxa of the *S. burkii* complex appear as a well-supported monophyletic lineage within the genus *Phylloscopus* (Fig. 4, clade II). Robustness of clade 1 is higher than in the haplotype tree, clade 2 is poorly supported by Bayesian posterior probabilities.

Three other members of *Seicercus* branch together with various taxa of the *P. reguloides* group (Fig. 4, clade I). *S. grammiceps* (Strickland) and *S. castaniceps* (Hodgson) form the sister group to a moderately supported clade which includes all taxa of the traditional *P. reguloides* group and *S. xanthoschistos* as sister taxon to *P. davisoni*. Phylogenetic relations between clades of the *P. reguloides* group are poorly supported.

Uncorrected *p*-distances within the *S. burkii* complex range between 3.5% (*S. burkii* s. str. vs. *S. tephrocephalus*) and 7.9% (*S. affinis* vs. *S. whistleri* and *S. valentini*). TrN-distances under the selected model (shown in Table 2) are slightly higher. Sequences differ by 6.2–7.7% for most pairwise comparisons within the *S. burkii* complex. All members of this group are highly divergent from other species of the genus. Genetic distances between the three *Seicercus* lineages range between 10.0% and 13.0%. Lowest distance values were found between allopatric sister species, e.g. *S. grammiceps*/*S. castaniceps* (<1%), between *S. xanthoschistos*/*P. davisoni* (about 3%) and between *P. cantator*/*P. ricketti* (4%). On the intraspecific level, distance values between



**Fig. 2.** Haplotype tree for seven taxa of the *S. burkii* complex, three additional *Seicercus* species, and one outgroup taxon (*P. collybita*); 658 bp, Bayesian inference of phylogeny, all haplotypes included, see Table 1; robustness of clades is indicated by NJ and ML bootstrap (upper values, 1000 replicates/puzzle steps) and Bayesian posterior probabilities (lower values, Markov Chain over 500,000 generations); “-” = ML bootstrap support < 50%; codon positions weighted in NJ analysis: 1:2:3 = 3:5:1.



**Fig. 3.** Genetic divergence in the *S. burkii* complex: (a–c) scatter plots for the three codon positions, p-distances (*X*-axis) vs. TrN-distances (*Y*-axis).

haplotypes of *Seicercus* species range from 0.2% to 0.8%.

For all haplotypes of the genus *Seicercus* the clocklike tree cannot be rejected on the  $p < 0.05$  level in the constant rate test ( $\log L$  without clock =  $-2646.83$ ;  $\log L$  with clock =  $-2667.83$ ).

If we consider a widely accepted substitution rate for *cyt-b* of 1.6%/myr (Fleischer et al. 1998), the two major branches of the *S. burkii* complex separated about 5 myr ago. Most recent events—both about 2 myr ago—are the splits between the allopatric sister taxa *S. burkii* s. str. (Himalayas) and *S. tephrocephalus* (W/S China, Vietnam), and between *S. xanthoschistos* (Himalayas) and *P. davisoni* (W China, SE Asia).

DNA polymorphism data are shown in Table 3. HDs range between 0.64 and 0.90, except for *S. affinis* and *S. burkii* (HD = 1.00 because of the low sample size of two individuals in each species). Values for  $\pi$ , HD, and  $\theta$  are lowest in *S. tephrocephalus* and *S. whistleri*. In both these species and in *S. valentini* the observed distribution of pairwise distances does not match what is expected for a constant population (mismatch distributions, Fig. 5) but shows two peaks indicating geographical substructuring of species and at least two phases of differentiation among populations.

Unimodal left-skewed mismatch distributions in *S. soror* and *S. omeiensis* indicate recent population expansions (Harpending and Rogers 2000), which is additionally supported by significantly negative Tajima's *D*-values. A significantly negative Tajima's *D* is

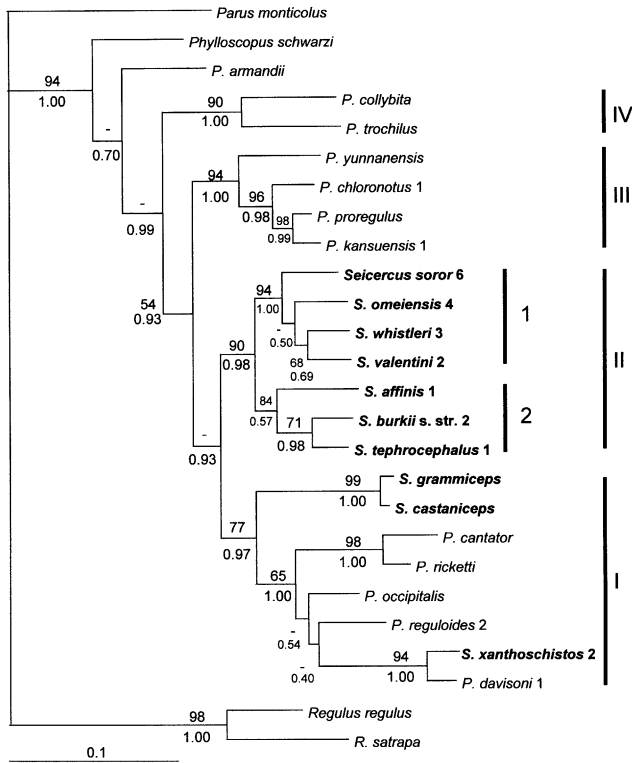
also found in *S. valentini* (Table 3). CRs reach relatively high values between 10.9 and 13.7, except for lowest values in *S. valentini* (7.3) and *S. burkii* (2.0, Table 3). Coalescence times range between 8.8 and 12.2 myr for haplotypes of most species of the *S. burkii* complex, the most recent divergence of haplotype lineages occurred between the sister species pair *S. tephrocephalus* and *S. burkii* s. str., between 4.7 and 6.2 myr ago.

### Bioacoustics

Structural variation of territorial song is high in all analysed species. Repertoires in the *S. burkii* complex consist of a large amount of verse types differing in syntax and element composition. Maximum repertoire sizes range from 44 verse types (of 51 verses given in a series) in *S. omeiensis*, through 35 verse types (of 68 in a series) in *S. soror*, to 25 verse types (of 63 in a series) in *S. affinis*. Also notably small repertoires have been found, for instance in one male of *S. soror* (eight within a total of 41 verses).

We found eleven different syntax patterns of verses in the *S. burkii* complex, which are shown in Table 4. Verses of patterns 1–4 include only repeated element groups, whereas patterns 5–10 also include trills. Syntax patterns 7–9 do not include repeated element groups at all. In *S. whistleri*, *S. valentini*, and *S. soror* individual repertoires usually do not contain trill structures. In this group, almost 100% of all verse types found in a single





**Fig. 4.** Molecular phylogeny for 10 *Seicercus* species (bold-face) and 13 mainly Asian *Phylloscopus* species; 658 bp, Bayesian inference of phylogeny; robustness of clades is indicated by ML bootstrap (upper values, 1000 puzzle steps) and Bayesian posterior probabilities (lower values, Markov Chain over 500,000 generations); “-” = ML bootstrap support < 50%; major clades: I = *P. reguloides* complex, II = *S. burkii* complex, III = *P. proregulus* complex; IV = chiffchaff and willow warbler; clades of the *S. burkii* complex indicated by Arabic numerals 1 and 2; number of haplotypes indicated behind species names.

population contain repeated element groups only (Table 4, syntax patterns 1–4). In *S. valentini* only one male sang a single trill verse (Table 4, syntax 7) including the typical introductory element (Fig. 6b<sub>4</sub>). Trill structures have also been found in *S. whistleri* (two verses of a single male, syntax pattern 10), but never in *S. soror*. Double notes in the two rare verse types in *S. whistleri* are due to the repetition of element groups which start and end with the same element type (element group ABA repeated: verse syntax = ABAABA).

Repertoires of *S. burkii* s. str., *S. omeiensis*, *S. tephrocephalus*, and *S. affinis* are strongly dominated by trills (Fig. 6, Table 4). Only in *S. burkii* s. str. more than 50% of all analysed verses consist of groups of two alternating elements (Table 4, syntax 1). *S. omeiensis* is the only species in which all eleven syntax types have been found; a single male displayed one triple trill verse (syntax: AAABBBCCC). In *S. burkii* s. str., almost all syntax types are present (Table 4).

Verse and element structure of *S. xanthoschistos* (Fig. 6h) is similar to that of its close relative, *P. davisoni* (Fig. 7a). In both species, songs include verses of irregular and complex structure including highly modulated elements. Repeated element groups (Table 4, patterns 1–4) are less abundant; most of them include three or more elements. Trills are uncommon in the *P. reguloides* group and have not been found in any individual repertoire of *S. xanthoschistos*, *P. occipitalis* and *P. reguloides* (rarely in *P. davisoni*). In this group, only the highly similar songs of *P. ricketti* and *P. cantator* include trills (Fig. 7d and e). Territorial song of *S. castaniceps* and *S. grammiceps* consists of series of high-pitched, mostly unmodulated whistles (Fig. 6i–k) otherwise unknown in *Seicercus*.

Repertoires of *P. schwarzi* (Radde) and *P. armandii* (Milne–Edwards) contain several syntax patterns typical of the *S. burkii* complex (Fig. 7; Table 4, patterns 5 and

**Table 2.** Genetic differentiation between *Seicercus* species

Species	<i>whis</i>	<i>val</i>	<i>sor</i>	<i>omei</i>	<i>teph</i>	<i>burk</i>	<i>aff</i>	<i>gram</i>	<i>cast</i>	<i>xant</i>
<i>S. whistleri</i>	<b>0.005</b>	47	52	54	58	59	57	73	72	79
<i>S. valentini</i>	0.088	<b>0.0056</b>	62	56	59	61	62	78	78	86
<i>S. soror</i>	0.103	0.118	<b>0.0041</b>	61	59	62	61	80	79	84
<i>S. omeiensis</i>	0.104	0.105	0.120	<b>0.0037</b>	57	59	61	81	80	85
<i>S. tephrocephalus</i>	0.146	0.134	0.143	0.132	<b>0.0026</b>	28	46	67	68	69
<i>S. burkii</i>	0.138	0.135	0.141	0.129	0.064	<b>0.0078</b>	46	70	71	77
<i>S. affinis</i>	0.141	0.144	0.143	0.143	0.123	0.115	<b>0.003</b>	75	73	81
<i>S. grammiceps</i>	0.204	0.206	0.211	0.219	0.194	0.202	0.221	—	6	74
<i>S. castaniceps</i>	0.201	0.207	0.208	0.217	0.198	0.206	0.227	0.015	—	75
<i>S. xanthoschistos</i>	0.224	0.231	0.226	0.228	0.200	0.225	0.247	0.223	0.228	<b>0.0015</b>

*whis* = *S. whistleri*, *val* = *S. valentini*, *sor* = *S. soror*, *omei* = *S. omeiensis*, *teph* = *S. tephrocephalus*, *burk* = *S. burkii*, *aff* = *S. affinis*, *gram* = *S. grammiceps*, *cast* = *S. castaniceps*, *xant* = *S. xanthoschistos*.

Lower left section: Mean pairwise interspecific genetic distances (among all respective haplotype pairs) according to the selected substitution model (Tamura–Nei distances, TrN); table diagonal (boldface): mean pairwise intraspecific genetic distances among haplotypes; upper right section: pairwise numbers of mutations.

**Table 3.** DNA polymorphism in five species of the *S. burkii* complex

Species	<i>n</i>	<i>n<sub>h</sub></i>	HD	$\pi$	$\theta$	<i>L</i>	CR	<i>t</i>	<i>D</i>	<i>N<sub>f</sub></i>
<i>S. whistleri</i>	13	6	0.641	0.00226	0.00329	0.0253	11.2	12.2	-1.639	500,000
<i>S. valentini</i>	15	8	0.829	0.00330	0.00608	0.0240	7.3	9.3	-1.797*	560,000
<i>S. soror</i>	15	9	0.848	0.00298	0.00608	0.0343	11.5	10.3	-2.003*	410,000
<i>S. omeiensis</i>	17	10	0.897	0.00260	0.00540	0.0345	13.3	10.6	-1.949*	370,000
<i>S. tephrocephalus</i>	12	4	0.636	0.00180	0.00151	0.0197	10.9	6.2	+0.622	260,000
<i>S. affinis</i>	2	2	1.000	0.00304	0.00304	0.0416	13.7	8.8	—	300,000
<i>S. burkii</i>	2	2	1.000	0.00760	0.00760	0.0155	2.0	4.7	—	780,000

*n*=sample size, *n<sub>h</sub>*=number of haplotypes, HD=haplotype diversity,  $\pi$ =nucleotide diversity,  $\theta=2N\mu$ , *L*=branch length (NJ tree), CR=coalescence ratio, *t*=coalescence time (in myr), *D*=Tajima's *D*-value (\*=*p*<0.05), *N<sub>f</sub>*=effective population size of females.

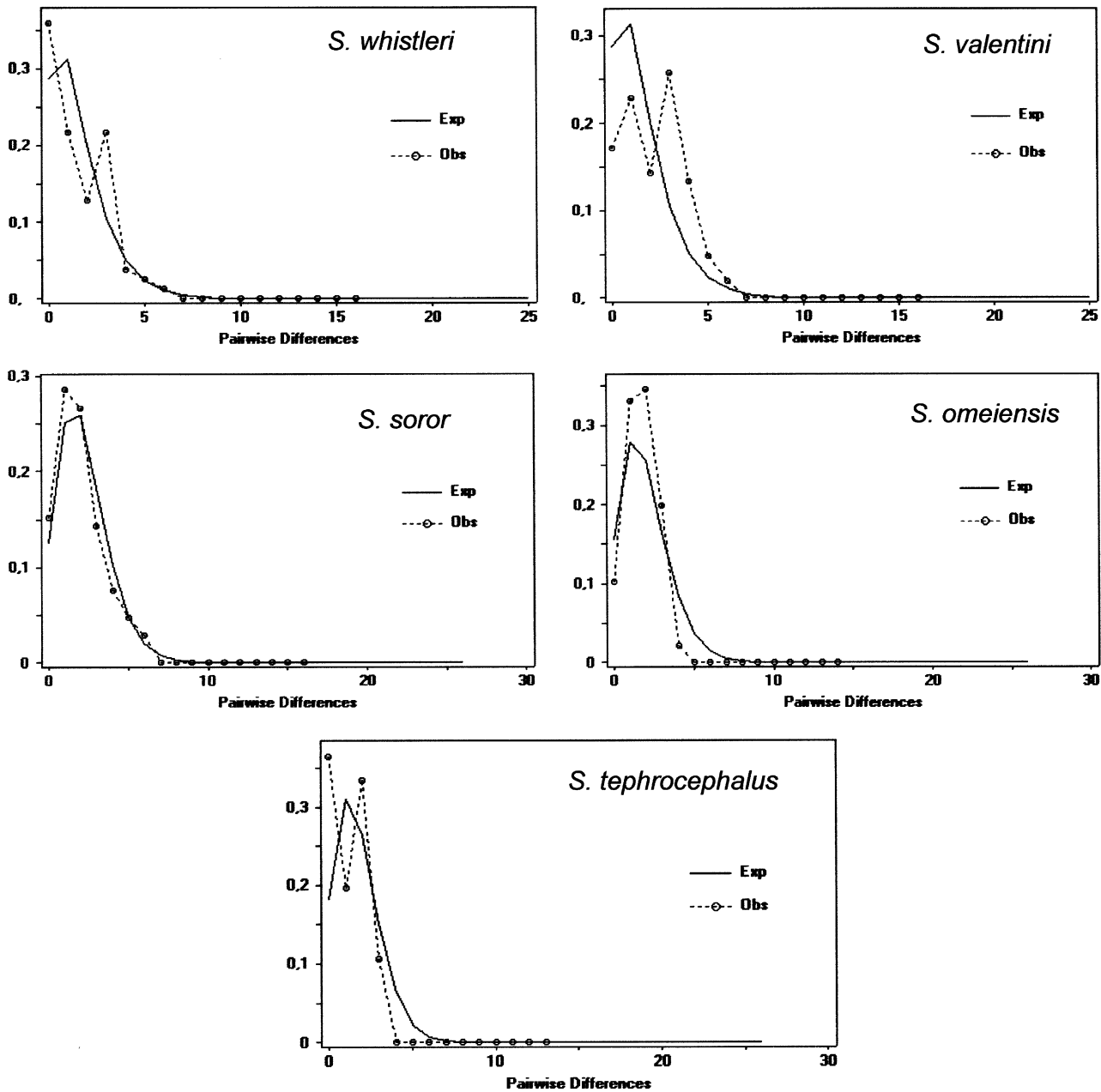
6). Unlike all other *Phylloscopus* taxa in this study, *P. armandii* also displays combined verses with element groups and trills (Fig. 7f); in *P. schwarzi* this syntax is rare (Table 4).

The specific introductory note in songs of *S. whistleri*, *S. valentini*, and *S. soror* may be replaced in single verses by an excitement call in the latter species (Fig. 6c<sub>3</sub>, white arrows). Similarly, in *S. tephrocephalus* verses often start with the specific double click call (Fig. 6f<sub>3</sub>). Within a variety of introductory elements of *S. tephrocephalus*, one element type strongly resembles these calls. Song of the remaining species (*S. burkii* s. str., *S. omeiensis*, *S. affinis*) lacks the introductory element. A few males of *S. omeiensis* rarely started verses with a call-like element. In songs of most investigated *Phylloscopus* species an introductory note is present as well. Verses can be started with modified calls (*P. schwarzi*, *P. armandii*), short whistles (*P. cantator*, *P. ricketti*), or short hooks (*P. reguloides*). In *P. occipitalis*, the shorter element of the repeated group precedes the verse in a short trill (syntax: iiii AiAiAiAi, Fig. 7b). Verses in *P. collybita*, *P. trochilus*, *P. davisoni*, and all members of the *P. proregulus* group lack an introductory note.

### Frequency and time parameters

Sonographic analysis produces three main clusters of song types within the *S. burkii* complex (Fig. 8). Four taxa from medium (*S. burkii* s. str., *S. omeiensis*, and *S. soror*) to low altitudes (*S. tephrocephalus*) share songs with a broad frequency range of 4.0–4.6 kHz and high frequency maxima up to 7.0 kHz (Table 5; Fig. 8, cluster 1). Songs in the two species from the upper mountain belt, *S. valentini* and *S. whistleri*, occupy lower frequency levels (maximum frequency at 4.6–5.0 kHz, Table 5) and have narrow frequency ranges of about 2.5 kHz (Fig. 8, cluster 2). However, males of *S. whistleri* sing significantly higher than those of *S. valentini* (about 0.5 kHz difference in maximum and minimum frequency, Mann–Whitney *U*-test, *p*<0.05). In *S. affinis* (Fig. 8, cluster 3), the song has similar frequency

parameters but differs significantly from the previous two taxa in its long verses and an extremely narrow frequency range of 2.3 kHz (Mann–Whitney *U*-test, *p*<0.05). In discriminant analysis, highest correlations occurred between function 1 and *F<sub>min</sub>*, between function 2 and *F<sub>max</sub>*, *df*, and between function 3 and *t*. All three functions were used for analysis. Function 1 explains 67.0% of the overall variance (eigenvalue=34.8), and 93.9% are explained by the first two discriminant functions (function 2: eigenvalue=21.1). Without exception, all measured verses of *S. whistleri*, *S. valentini*, *S. xanthoschistos*, *S. affinis*, and *S. castaniceps* have been correctly assigned to the appropriate group. Data for the sole individual of *S. grammiceps* have been pooled with those of *S. castaniceps* (Fig. 8, cluster 5). Among species in cluster 1, discrimination between songs is less accurate. Only 78.0–94.0% of all verses from *S. burkii* s. str., *S. omeiensis*, *S. tephrocephalus*, and *S. soror* have been correctly assigned to the appropriate group. About 15.0% of all songs in *S. burkii* s. str. and *S. tephrocephalus* have been misassigned to the respective other species. A second analysis was run to discriminate between the lowland species in cluster 1, using only measures of trill parts of verses (function 1: eigenvalue=1.08, 77.0% of the overall variance explained, highest correlations with *F<sub>max</sub>* and *df*; function 2: eigenvalue=0.29, 98.0% of the variance explained by functions 1 and 2, highest correlations with number of elements [*n*] and *F<sub>min</sub>*). Among all trill singers, only trills of *S. affinis* (Fig. 8, cluster 3) can be discriminated from all species in cluster 1 (*S. omeiensis*, *S. tephrocephalus*, *S. burkii* s. str.), due to their low frequency parameters (analogous to entire verses, see Table 5 and Fig. 8). In *S. affinis*, 95.0% of all trills were correctly assigned to the group, while assignment within species in cluster 1 was much less accurate (only 49.0–65.0% of all measured trills correctly assigned). Moreover, there seems to be no notable difference between trill elements sung by the four trill singer species, as all of them use the same element types in trill parts of the song. Nevertheless, subtle frequency differences between species in cluster 1 can be detected by non-parametric tests, for instance



**Fig. 5.** Observed and expected distribution of pairwise differences (mismatch distributions) under the model of stable populations for five species of the *S. burkii* complex.

between songs of the locally syntopic species *S. omeiensis* and *S. tephrocephalus* ( $\Delta F_{\max}$ ,  $\Delta df$  and  $\Delta t$ ; Mann–Whitney *U*-test,  $p < 0.01$ ), or between extremely similar songs in the geographic vicariants *S. omeiensis* and *S. burkii* ( $\Delta F_{\max}$ ,  $\Delta df$  and  $\Delta t$ ; Mann–Whitney *U*-test,  $p < 0.01$ ; see also Table 5).

Songs in three other *Seicercus* species differ considerably from those of their congeners. The song of *S. xanthoschistos* males covers a frequency range similar to that in *P. davisoni* and other members of the *P. reguloides* group (between 3.4 and 8.2 kHz; Table 5; Fig. 8, cluster 4). The high-pitched songs in *S. castaniceps* and *S. grammiceps* are rather similar in time

and frequency parameters (Table 5, only *S. castaniceps*; Fig. 8, cluster 5).

### Acoustic divergence

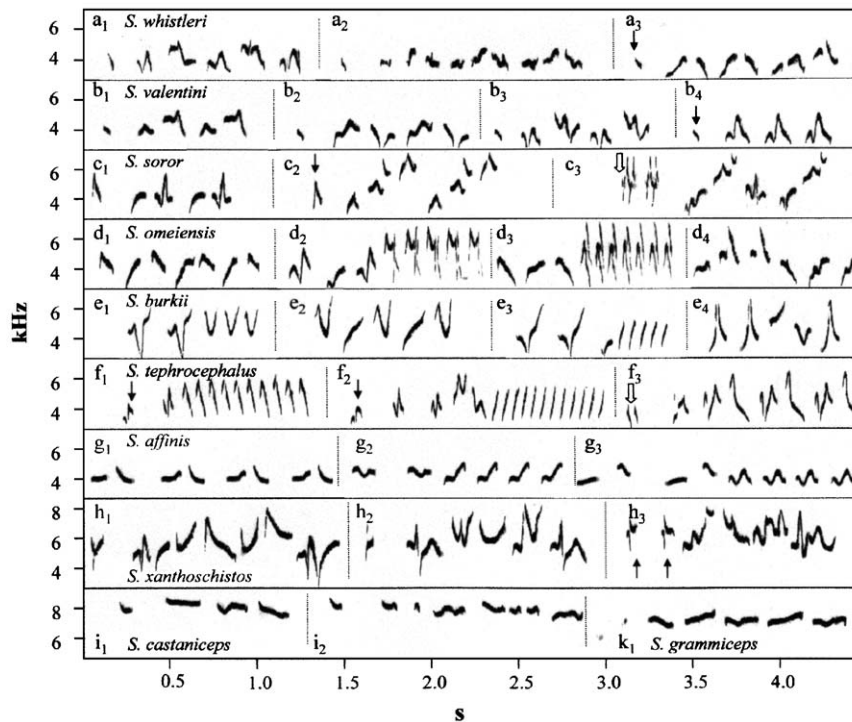
We found a strong correlation between genetic and acoustic divergence for some song parameters in the examined *Seicercus* species. Differences in maximum frequency ( $\Delta F_{\max}$ ), frequency span ( $\Delta df$ ), and verse length (*t*) increase with genetic distance (Fig. 9a). Correlations are significant for the frequency parameters ( $\Delta F_{\max}$ :  $r^2 = 0.159$ ,  $p < 0.01$ ;  $\Delta df$ :  $r^2 = 0.104$ ,  $p < 0.05$ ), but

**Table 4.** Percentage representation of eleven syntax types in populational repertoires of eight *Seicercus* and two *Phylloscopus* species

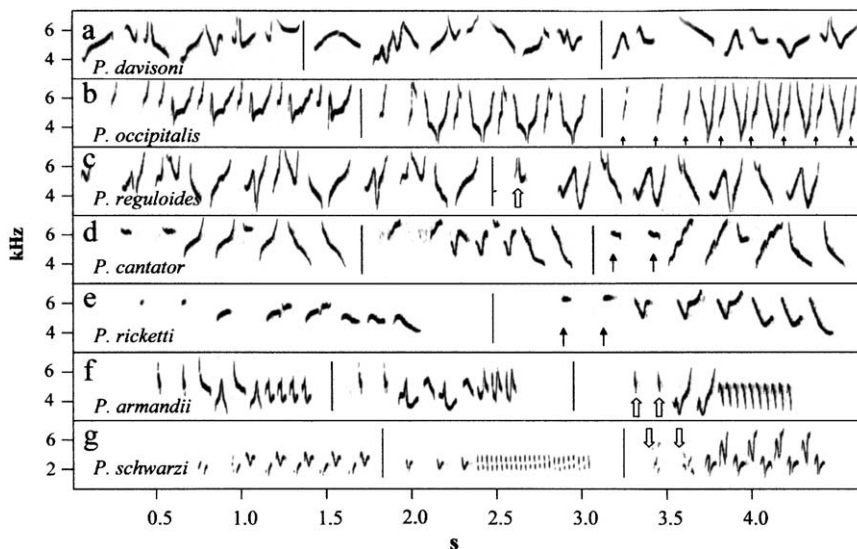
Syntax		Sonagram	<i>whis</i> <i>n</i> = 106	<i>val</i> <i>n</i> = 112	<i>sor</i> <i>n</i> = 86	<i>burk</i> <i>n</i> = 179	<i>omei</i> <i>n</i> = 272	<i>teph</i> <i>n</i> = 55	<i>aff</i> <i>n</i> = 48	<i>xant</i> <i>n</i> = 87	<i>schwa</i> <i>n</i> = 118	<i>arma</i> <i>n</i> = 86	
1	Group 2 elements	ABAB	<b>a</b>	31	53	14	55	15	47	11	2	36	6
2	Group 3 elements	ABCABC	<b>b</b>	44	36	37	—	5	—	—	18	1	—
3	Group 2 ascending	A <sup>B</sup> C <sub>A</sub> B <sup>C</sup>	<b>c</b>	10	8	47	—	4	—	—	1	—	—
4	Double group	ABABCD <sub>CD</sub>	<b>d</b>	9	—	9	2	1	2	6	—	—	5
5	Group+double note	ABABCC	<b>e</b>	—	—	—	7	25	18	34	2	—	5
6	Group+trill	ABABCCCC	<b>f</b>	—	—	—	17	6	18	4	—	1	41
7	Trill	AAAAAA	<b>g</b>	—	1	—	9	6	29	15	—	61	12
8	Double trill	AAA BBBB	<b>h</b>	1	—	—	3	4	4	15	—	2	31
9	Complex double trill	AAABCCCC	<b>i</b>	—	—	—	3	11	—	13	—	—	—
10	Group+trill+group		<b>k</b>	2	—	—	—	7	—	—	—	—	—
11	Complex		<b>l</b>	3	1	1	2	9	—	—	76	—	1

*whis* = *S. whistleri*, *val* = *S. valentini* (sonagram a), *sor* = *S. soror* (sonagram c), *burk* = *S. burkii* (sonagrams b, e–i), *omei* = *S. omeiensis* (sonagram k), *teph* = *S. tephrocephalus*, *aff* = *S. affinis* (sonagram d), *xant* = *S. xanthoschistos* (sonagram l), *schwa* = *P. schwarzi*, *arma* = *P. armandii*. Syntax types: repetitions of element groups only (types 1–4), verses include trill structures (types 5–10), verses of irregular and complex syntax (type 11); sonagrams unscaled.

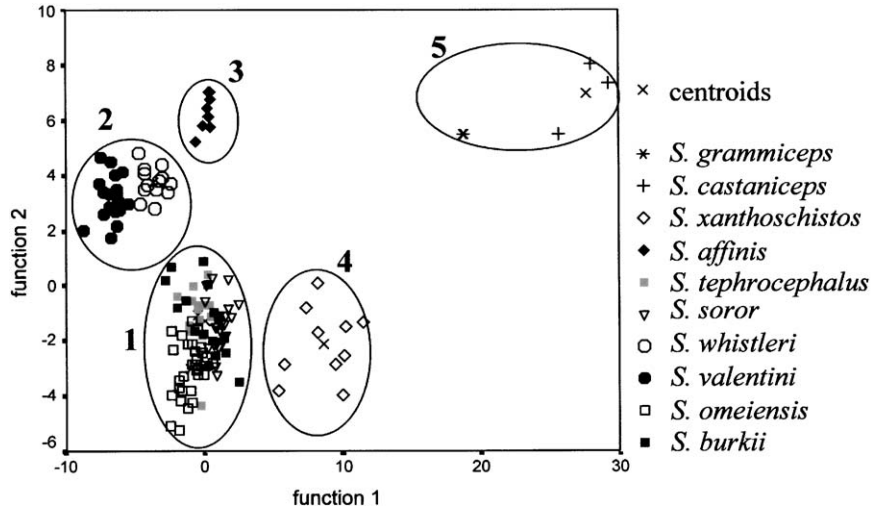




**Fig. 6.** Territorial song in the genus *Seicercus*. Introductory notes indicated by arrows: black = song element, white = specific call. *S. whistleri*: (a<sub>1</sub>–a<sub>3</sub>) three verses of one male, Nepal, Bhojpur Distr., 2001. *S. valentini*: (b<sub>1</sub>–b<sub>3</sub>) three verses of one male, (b<sub>4</sub>) rare verse with trill of another male, both China, Sichuan, 2002. *S. soror*: (c<sub>1</sub>–c<sub>3</sub>) three verses of one male, China, Sichuan, 2002. *S. omeiensis*: (d<sub>1</sub>–d<sub>4</sub>) four verses of one male, China, Sichuan, 2002. *S. burkii* s. str.: (e<sub>1</sub>–e<sub>4</sub>) four verses of two different males, Nepal, Bhojpur Distr., 2001. *S. tephrocephalus*: (f<sub>1</sub>–f<sub>3</sub>) three verses of one male, China, Sichuan, 2000. *S. affinis*: (g<sub>1</sub>–g<sub>3</sub>) three verses of one male, China, Sichuan, 2000. *S. xanthoschistos*: (h<sub>1</sub>–h<sub>3</sub>) three verses of one male, Nepal, Sankhua Sabha Distr., 1988. *S. castaniceps*: (i<sub>1</sub>–i<sub>2</sub>) two verses of one male, China, Sichuan, 1998. *S. grammiceps*: (k<sub>1</sub>) Indonesia, Java, 1997. *S. grammiceps* from Scharringa (1999), all other recordings by J. Martens.



**Fig. 7.** Territorial song in the genus *Phylloscopus*. Introductory notes indicated by arrows: black = song element, white = specific call. *P. davisoni*: (a) China, Yunnan, 2002. *P. occipitalis*: (b) India, Kashmir, 1976. *P. reguloides*: (c) China, Sichuan, 2000. *P. cantator*: (d) Nepal, Bhojpur Distr., 2001. *P. ricketti*: (e) China, Sichuan, 1998 (song specimen: MAR903), and China, Shaanxi, 2000 (another male). *P. armandii*: (f) China, Shaanxi, 2000. *P. schwarzi*: (g) lower Ussuri valley, 1996. Recordings (a–f) by J.M. and (g) by M.P.



**Fig. 8.** Acoustic divergence within the genus *Seicercus*; scatterplot, discriminant analysis based on five song parameters. Acoustic clusters: 1 = medium- and low-altitude species: *S. burkii* s. str., *S. omeiensis*, *S. soror*, *S. tephrocephalus*; 2 = high-altitude species: *S. valentini*, *S. whistleri*; 3 = *S. affinis* (W China); 4 = *S. xanthoschistos* (Himalayas); 5 = *S. grammiceps* (SE Asia), *S. castaniceps* (W China). Data on the latter two species were pooled for discriminant analysis.

**Table 5.** Frequency and time parameters of territorial songs in the *S. burkii* complex, the *P. reguloides* group, and of *S. castaniceps*, *P. armandii*, and *P. schwarzi*

Species	$F_{max}$ (kHz)	$F_{min}$ (kHz)	df (kHz)	$t$ verse (s)	$t_1$ trill (s)	$F_{mod}$ (el./s)
<i>S. burkii</i> complex						
<i>S. whistleri</i>	5.05 ( $\pm 0.46$ )	2.59 ( $\pm 0.28$ )	2.46 ( $\pm 0.55$ )	0.89 ( $\pm 0.24$ )	—	—
<i>S. valentini</i>	4.63 ( $\pm 0.40$ )	2.14 ( $\pm 0.29$ )	2.44 ( $\pm 0.68$ )	0.86 ( $\pm 0.14$ )	—	—
<i>S. soror</i>	6.97 ( $\pm 0.54$ )	2.58 ( $\pm 0.30$ )	4.38 ( $\pm 0.81$ )	1.12 ( $\pm 0.18$ )	—	—
<i>S. omeiensis</i>	6.97 ( $\pm 0.58$ )	2.33 ( $\pm 0.29$ )	4.65 ( $\pm 0.78$ )	0.91 ( $\pm 0.16$ )	0.55 ( $\pm 0.17$ )	9.2 ( $\pm 3.98$ )
<i>S. burkii</i>	6.75 ( $\pm 0.65$ )	2.73 ( $\pm 0.30$ )	4.01 ( $\pm 0.86$ )	0.79 ( $\pm 0.13$ )	0.73 ( $\pm 0.24$ )	6.5 ( $\pm 3.62$ )
<i>S. tephrocephalus</i>	6.57 ( $\pm 0.59$ )	2.40 ( $\pm 0.29$ )	4.17 ( $\pm 0.69$ )	1.15 ( $\pm 0.18$ )	0.71 ( $\pm 0.19$ )	9.9 ( $\pm 4.19$ )
<i>S. affinis</i>	5.16 ( $\pm 0.19$ )	2.94 ( $\pm 0.20$ )	2.25 ( $\pm 0.27$ )	1.42 ( $\pm 0.19$ )	0.63 ( $\pm 0.15$ )	8.7 ( $\pm 3.83$ )
<i>S. castaniceps</i>	9.4 ( $\pm 0.17$ )	7.1 ( $\pm 0.39$ )	2.3 ( $\pm 0.41$ )	1.20 ( $\pm 0.03$ )	—	—
<i>S. reguloides</i> group						
<i>S. xanthoschistos</i>	8.2 ( $\pm 0.68$ )	3.4 ( $\pm 0.30$ )	4.8 ( $\pm 0.71$ )	1.26 ( $\pm 0.21$ )	—	—
<i>P. davisoni</i>	7.9 ( $\pm 0.44$ )	3.0 ( $\pm 0.47$ )	4.6 ( $\pm 0.41$ )	1.42 ( $\pm 0.18$ )	—	—
<i>P. ricketti</i>	7.5 ( $\pm 0.37$ )	3.3 ( $\pm 0.39$ )	4.2 ( $\pm 0.53$ )	1.59 ( $\pm 0.03$ )	—	—
<i>P. cantator</i>	8.3 ( $\pm 0.25$ )	3.4 ( $\pm 0.34$ )	4.9 ( $\pm 0.49$ )	1.27 ( $\pm 0.17$ )	—	—
<i>P. reguloides</i>	8.0 ( $\pm 0.41$ )	3.2 ( $\pm 0.54$ )	4.9 ( $\pm 0.59$ )	1.90 ( $\pm 0.37$ )	—	—
<i>P. occipitalis</i>	8.6 ( $\pm 0.59$ )	3.2 ( $\pm 0.43$ )	5.2 ( $\pm 0.68$ )	1.80 ( $\pm 0.29$ )	—	—
<i>P. armandii</i>	7.5 ( $\pm 0.55$ )	3.3 ( $\pm 0.39$ )	4.3 ( $\pm 0.59$ )	1.59 ( $\pm 0.13$ )	n.m.	n.m
<i>P. schwarzi</i>	5.4 ( $\pm 0.82$ )	1.6 ( $\pm 0.29$ )	3.9 ( $\pm 0.80$ )	1.10 ( $\pm 0.15$ )	n.m.	n.m

$F_{max}$  = maximum frequency,  $F_{min}$  = minimum frequency, df = frequency span,  $t$  = verse length,  $t_1$  = length of trill,  $F_{mod}$  = speed of trill (number of elements per second), — = trills are not displayed by the species concerned, n.m. = not measured.

not for the time parameter  $t$ . The mean acoustic character difference ( $\Delta_{ac}$ ) based on six syntax features also is correlated with genetic distance (Fig. 9b,  $r^2 = 0.86$ ,  $p < 0.05$ ). Acoustic divergence ( $\Delta_{ac}$ ) ranges from 0.00 (identical syntax in *S. valentini*, *S. whistleri*, and *S. soror*; also between *S. castaniceps* and *S. grammiceps*) to 0.83 (between members of the *S. burkii*

complex and various *Phylloscopus* species). Homoplasy indices (CI, RI, and RC) of the investigated syntax characters produce highest values in the phylogeny for all 10 *Seicercus* species plus *P. davisoni* as sister to *S. xanthoschistos*. However, homoplasy of syntax characters increases with the number of taxa enclosed in the underlying phylogeny, as indicated by decreasing CI.

This is shown for two characters in Fig. 9c: the CI for “element groups” and the “introductory element” decreases with an increasing number of taxa ( $X$ -axis). Most consistent with the molecular data is the presence and shape of the introductory note, even in the full phylogeny including 23 taxa (CI ranges from 1.00 to 0.78, Fig. 9c).

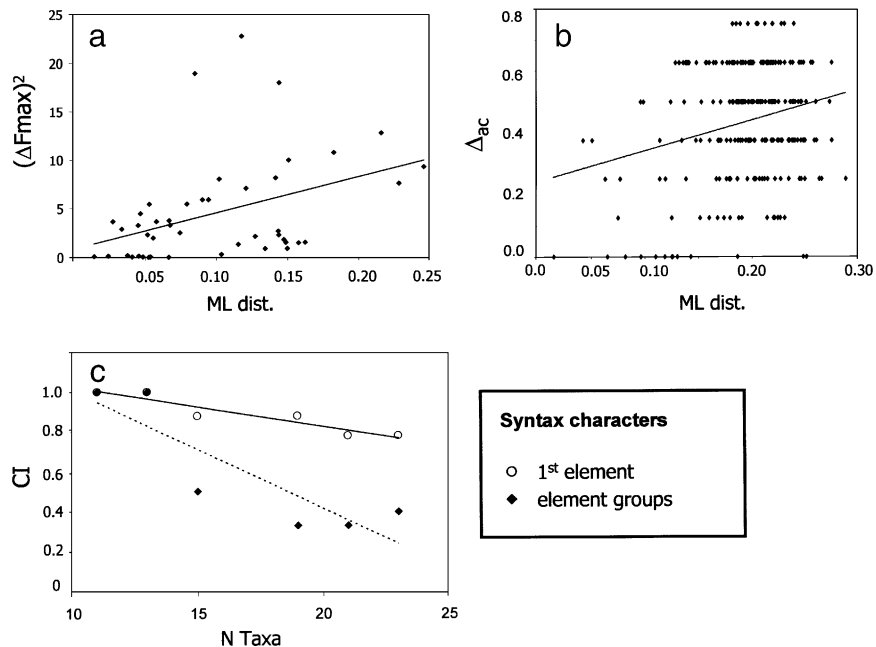
## Discussion

### Phylogeography

Molecular and bioacoustic analyses confirm that, despite the high level of interspecific differentiation, the species of the *S. burkii* complex are a monophyletic assemblage and share a common evolutionary history (see also Alström 2002). However, DNA polymorphism reveals marked differences in population structure of species among and within the two major clades of the *S. burkii* complex. Mismatch distributions indicate population substructuring and at least two phases of differentiation within three species only: *S. whistleri*, *S. valentini* and *S. tephrocephalus*. In these species, subspecific differentiation is reflected by morphological characters (Alström and Olsson 1999; Martens et al. 1999) and by slight but marked genetic divergence between the subspecies pairs *S. whistleri whistleri*/*S. w. nemoralis* and *S. valentini valentini*/*S. v. latouchei*,

respectively (Alström 2002). For *S. tephrocephalus* intraspecific differentiation was suggested as well, based on morphological and acoustic data (Alström and Olsson 1999), but one of three subgroups later turned out to be a separate species, *S. omeiensis* (Martens et al. 1999, “group 6, *tephrocephalus*” sensu Alström and Olsson 1999). Although in *S. tephrocephalus* multiple differentiation phases are indicated, intraspecific DNA diversity is low and the estimate of the effective population size is small. In contrast, the high level of intraspecific nucleotide and HD in *S. valentini*, *S. omeiensis*, and *S. soror* points to at least partially restricted gene flow between neighbouring populations of the more fragmented upper and middle mountain belt habitats. Throughout a large part of the investigated area in China, the breeding range of these species is fragmented into several small areas of isolated mountain tops like at Taibai Shan, Omei Shan, Jizu Shan, and Wawu Shan. Diversity values for *S. whistleri* from the higher elevations of the Himalayas are considerably lower, suggesting a higher degree of intraspecific gene flow in *S. whistleri* than in the vicariant Chinese species *S. valentini* from the same altitudes. Unlike in Chinese mountains, the area belt of *S. whistleri* between 2300 and 3800 m is continuous in the Himalayas, and large valleys seem to have no notable effect on population structure and DNA diversity of *S. whistleri* in the studied area.

Estimates of divergence time between members of the *S. burkii* complex range between 5 myr among taxa from



**Fig. 9.** Acoustic differentiation in *Seicercus/Phylloscopus*. Linear correlations: (a) ML distances ( $X$ -axis) vs. difference of mean maximum frequency ( $\Delta F_{\max}$ ),  $r^2=0.159$ ,  $p<0.01$ ; (b) ML distances ( $X$ -axis) vs. acoustic divergence index ( $\Delta_{ac}$ , seven syntax characters),  $r^2=0.053$ ,  $p<0.05$ ; (c) number of taxa ( $X$ -axis) vs. CI for two song characters, “1st element” (presence and shape of introductory note,  $r^2=0.79$ ,  $p<0.05$ ) and “element groups” ( $r^2=0.85$ ,  $p<0.05$ ). For character matrix see Appendix A (Table 6).

the two major clades and 2 myr between *S. burkii* and *S. tephrocephalus*. These datings perfectly match the time estimates by Alström (2002) and challenge the concept of late Pleistocene origin of avian species (LPO model; Hubbard 1973; Avise and Walker 1998; but see Johnson and Cicero 2004 for new evidence for the validity of the LPO model). Evidence for Pliocene radiation of passerine bird genera has also been documented for cardueline finches (Arnaiz-Villena et al. 1998), Nearctic warblers (Lovette and Bermingham 1999; Lovette et al. 1999), and several Holarctic avian species pairs (Zink et al. 1997; Zink and Klicka 2000). Coalescence times for single haplotype lineages (species) of the *S. burkii* complex are even considerably higher and range from 4.7–6.2 myr up to 8.8–12.2 myr ago. For species with high CR (> 10), approximately 95% of nuclear loci are predicted to be monophyletic (Palumbi et al. 2001). The fact that CRs for most species of the *S. burkii* complex (except *S. valentini* and *S. burkii*) are that high underlines the long time span of divergent evolution and corroborates their taxonomic status as separate species. However, the predictions of the three-times rule by Palumbi et al. (2001) have been criticized recently by Hudson and Turelli (2003) because of wide confidence intervals of the estimated probabilities.

Nevertheless, the start of intense radiation and species divergence is to be dated to the Pliocene period, while Pleistocene events certainly had an impact, too, on recent population structuring and DNA diversity. Presumably, adaptations to cold climates of the upper and central mountain region (in *S. valentini*, *S. whistleri*, and partly in *S. omeiensis*) are likely to have evolved in northern refuge areas. In these small, isolated areas reduction of population size by Pleistocene events might have been more severe than in the presumably less fragmented southern areas of *S. tephrocephalus*, and led to a higher diversity within species from the middle and upper mountain belt. Furthermore, past range expansion is indicated by significantly negative Tajima's *D*-values for only three species from that group (*S. valentini*, *S. omeiensis*, and *S. soror*), but not for *S. tephrocephalus*. We exclude *S. burkii* and *S. affinis* from these interpretations because of the small sample size (two samples of each species).

On the genus level, the results of our molecular and bioacoustic analyses confirm the parphyly of both genera, *Seicercus* and *Phylloscopus*, as suggested by Alström (2002). The seven biospecies of the traditional *S. burkii* complex (including *S. affinis*) are not closely related to other species so far combined with the same genus name, but form one of three *Seicercus* lineages nested in different branches of the *Phylloscopus* tree. *S. xanthoschistos* turns out to be a true member of the *P. reguloides* clade, with *P. davisoni* as its closest relative (see Alström 2002 for the close phylogenetic relation between *S. xanthoschistos* and *P. occipitalis* from this

clade). The third *Seicercus* lineage, including the sister species pair *S. grammiceps* and *S. castaniceps*, also clusters with the *P. reguloides* group but on a separate branch. The Indonesian species *S. montis* (Sharpe) has been shown to be a member of that third clade, too (Alström 2002). Accordingly, song in *S. montis* is very similar to that in *S. grammiceps* and *S. castaniceps* (sonagram made from Scharringa 1999). The species of the *burkii* complex are quite different in morphology and colouration from the brown-crested species *S. castaniceps* and *S. grammiceps*, on the one hand, and from *S. xanthoschistos*, on the other hand, suggesting that the three lineages are not closely related. Our acoustic data support this view. Therefore, nomenclatural consequences are inevitable in the *Phylloscopus/Seicercus* complex. The genus *Seicercus* Swainson, 1837 (type species *Cryptolopha auricapilla* Swainson, 1837 = *Sylvia burkii* Burton, 1836) would have to be synonymized with *Phylloscopus* Boie, 1826, unless the latter is split into several genera. However, definite decisions can be made only when a complete molecular phylogeny is established for *Phylloscopus* and closely allied genera such as *Abroscopus* Baker.

### Song evolution

Divergence of song features between *Seicercus* species increases significantly with genetic distance. Genetically highly divergent species differ in frequency, time, and syntax parameters, while songs of more closely related species cover a similar frequency range and share common syntax structures. Such co-occurrence of molecular and bioacoustic differentiation also has been shown for various subgroups of the genus *Phylloscopus*: *P. bonelli* group (Helbig et al. 1995), *P. collybita* group (Helbig et al. 1996), and *P. trochiloides* group (Irwin 2000; Irwin et al. 2001b). A linear correlation between molecular and acoustic divergence has been demonstrated for the genus *Regulus* Cuvier (Päckert et al. 2003).

Though acoustic clusters based on frequency parameters are well consistent with the molecular phylogeny, we have to consider effects of habitat structure on song parameters in co-existing taxa. In dense broad-leaved forests, bird songs cover a narrower and lower frequency band compared to songs of species from open-land habitats (Morton 1975; Hunter and Krebs 1979; Ryan and Brenowitz 1985). In neotropical antbirds, there is also an influence of perching height on song parameters (Nemeth et al. 2001). However, in *Seicercus/Phylloscopus* ecological influences on territorial song are not obvious. We find species with different song characteristics in the same habitat and altitude, as well as species with similar frequency bands from different mountain regions (*S. valentini* and *S. whistleri*



up to 3300 m vs. *S. affinis* up to 1200 m). But see Dubois and Martens (1984) and Martens and Geduldig (1988) for a well-supported example of environmental influence on the voice of a *Phylloscopus* species. All the species of the *S. burkii* complex are very similar in size and body mass (Martens et al. 1999), so that the differences in frequency cannot be explained by different size (see Roberts et al. 1983). On the contrary, in the *S. burkii* complex we find strong evidence that similarities in acoustic features are primarily caused by common ancestry rather than by ecology.

Among discrete syntax characters two superior song structures are present in various taxa of the *S. burkii* complex: (1) an introductory note, which can be either a specific call or a derived song element; (2) a general regular syntax structure of either trills or phrases or a combination of both. On a higher taxonomic level, e.g. within the whole *Phylloscopus/Seicercus* assemblage, homoplasy indices CI, RI, and RC decrease with the number of taxa investigated, and indicate that several song structures may have evolved homoplastically or were independently eliminated from an ancestral repertoire. CI values exceed the range for random data sets (Klassen et al. 1991) only for the character “first element”, indicating that presence and shape of the introductory note has a high phylogenetic information value. In *Phylloscopus/Seicercus*, this first distinct element of a verse obviously derived from specific calls in closely related taxa, e.g. *S. soror*, *S. valentini*, and *S. whistleri* (see Martens et al. 1999). Insertion of call-like elements into song is also known from treecreepers (*Certhia*, Thielcke 1964, 1965) and firecrests (*Regulus*, Päckert et al. 2001).

In summary, territorial song characters may carry phylogenetic information only within an assemblage of closely related taxa, whereas due to homoplasy the same characters are less informative in a wider phylogenetic context. Nevertheless, we point out that no more than five main frequency and time parameters of song were necessary for an acoustic delimitation of the three *Seicercus* clades, or even of single *Seicercus* species. And, in addition, the correlation of genetic and acoustic divergence in these taxa resulted from a small set of seven syntax characters only. For a more accurate distinction of specific song features in *Seicercus/Phylloscopus*, detailed bioacoustic studies on substructures of song, e.g. element types, are required.

### Species recognition

Although there are no difficulties with species-level delimitations within the *S. burkii* complex in the sense of diagnosability and monophyly (phylopecies, Cracraft 1983; Davis and Nixon 1992; Mayden 1997), the crucial verification of these taxa as biospecies (Mayr 1942,

1967) has been achieved only tentatively (Martens et al. 1999; Alström and Olsson 1999). The main difficulty in corroborating biospecies status, i.e. proof of reproductive isolation between each of the seven species-level taxa, is that most of them occur in allopatry or at least in vertical parapatry, e.g. up to four taxa on a single mountain slope in China. A convincing argument for all seven taxa (haplotype clusters) as biospecies is that, so far, we have not found any hint of introgression between them, i.e. incongruence of phenotype, song-type repertoire and haplotype, in our individual samples. Acoustic discontinuities remain stable wherever two or even three populations meet: no mixed singers occur, and this also holds true for the minor but well-developed morphological traits (for details see Alström and Olsson 1999; Martens et al. 1999). This concerns areas of horizontal and vertical allopatry as well as local contact zones, and also populations in two newly discovered areas of local sympatry and even syntopy in Sichuan (*S. tephrocephalus*, *S. soror*, and *S. omeiensis*, Martens et al. 2003). Repertoires of locally syntopic species which share a common frequency range of song can be distinguished by differences in syntax structures (trills missing in song of *S. soror*, different or missing introductory notes).

Differentiation of territorial song is supposed to reinforce reproductive isolation of closely related songbird taxa (Thielcke 1972; Martens 1996). In playback experiments, Alström and Olsson (1999) found that several species of the *S. burkii* complex do not react to songs of their closely related congeners. Interestingly, most species pairs with almost identical territorial songs are not only closest relatives (sister taxa) but also geographical vicariants. These pairs are: *S. whistleri/S. valentini*, *S. xanthoschistos/P. davisoni*, *S. grammiceps/S. castaniceps*, and *P. cantator/P. ricketti*. To date, potential horizontal contact zones between these vicariants are unknown. In contrast, songs of locally parapatric *Seicercus* species always show at least subtle divergence in syntax or frequency parameters. The extremely similar songs of *S. omeiensis* and *S. tephrocephalus* cover almost the same frequency range, include the same syntax structures and, in areas of local sympatry (Qinchen Shan and Longxi-Hongkou Reserve, Sichuan), distinction of their songs by auditory impression is difficult. However, songs of the two species differ slightly but significantly in some frequency and time parameters and also in the presence or absence of an introductory note. In contrast, element types in trills of all trill singers within *Seicercus* are very similar in shape, but element differences may be subtle and still undetected. In the *P. collybita* complex several species recognize conspecific song by small differences in downstrokes and upstrokes of elements (Becker et al. 1980; Martens and Hänel 1981; Martens and Meincke 1989). Finally, acoustic divergence of excitement calls within the *S. burkii* complex is marked (Alström and

Olsson 1999; Martens et al. 1999) and may play a key role in species recognition as well.

Moreover, we emphasize that the yellow eye ring—characteristic for members of the *S. burkii* complex—is not uniform in the seven species. It is broadest in *S. affinis* (Alström and Olsson 2000, photograph), narrower in *S. tephrocephalus*, very narrow in *S. omeiensis*, and of irregular breadth in *S. whistleri* and *S. burkii* s. str. (photographs in Martens and Eck 2000; Martens et al. 2003). Such slight differences in head pattern are important in species recognition of the European goldcrest, *R. regulus*, and its sibling, the firecrest, *R. ignicapillus* (Thaler 1981), and may play an important role in the *S. burkii* complex as well.

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**Appendix A**

See Tables 6 and 7.

**Table 6.** Matrix for seven acoustic characters of territorial song in the genera *Seicercus* and *Phylloscopus*

Species	Trills	Groups	Group + trill	AABCCCC	Intro	Intro <i>n</i>	Frequency	Verse length
<i>S. xanthoschistos</i>	0	1	0	0	4	1	0	0
<i>P. davisoni</i>	0	1	0	0	0	0	0	0
<i>P. occipitalis</i>	0	1	0	0	6	3	0	0
<i>P. reguloides</i>	0	1	0	0	1	1	0	0
<i>P. ricketti</i>	1	0	0	0	5	2	1	0
<i>P. cantator</i>	1	0	0	0	5	2	1	0
<i>S. whistleri</i>	0	1	0	0	2	1	0	0
<i>S. valentini</i>	0	1	0	0	2	1	0	0
<i>S. soror</i>	0	1	0	0	1 and 2	1	0	0
<i>S. omeiensis</i>	1	1	1	1	0 and 1	0 and 1	0	0
<i>S. burkii</i>	1	1	1	1	0	0	0	0
<i>S. tephrocephalus</i>	1	1	1	1	1 and 3	1 and 2	0	0
<i>S. affinis</i>	1	1	1	1	0	0	0	0
<i>S. grammiceps</i>	1	0	0	0	0	0	1	0
<i>S. castaniceps</i>	1	0	0	0	0	0	1	0
<i>P. proregulus</i>	1	1	1	0	0	0	0	1
<i>P. kansuensis</i>	1	0	0	0	0	0	0	0
<i>P. chloronotus</i>	1	1	0	0	0	0	0	1
<i>P. yunnanensis</i>	1	1	0	0	0	0	0	1
<i>P. armandii</i>	1	1	1	0	1	2	0	0
<i>P. schwarzi</i>	1	1	0	0	1	2	0	0
<i>P. collybita</i>	0	2	0	0	0	0	0	0
<i>P. trochilus</i>	1	0	0	0	0	0	1	0

Trills: 0 = absent, 1 = part of repertoire. Groups: 0 = absent, 1 = regularly repeated groups of 1–4 elements, 2 = 2–3 elements irregularly alternated (only in *P. collybita*). Group + trill: 0 = absent, 1 = combined verses of a group followed by a trill. Syntax scheme AABCCCC: 0 = absent, 1 = part of repertoire. Intro (= introductory element): 0 = absent, 1 = specific call, 2 = short hook, opened downwards, 3 = various: modulated, z-shaped; 4 = hook, opened upwards, v-shaped; 5 = short, unmodulated whistle; 6 = short element of group preceding the verse (syntax: iii AiAiAiAi). Intro *n* (= number of introductory elements): 0 = none; 1 = one; 2 = two; 3 = three and more. Frequency: 0 = unmodulated, 1 = descending to the end of the verse. Verse length: 0 = regular verses of about 1–3 s separated by pauses, 1 = “endless song”, passages longer than 20 s.

**Table 7.** Sound recordings of territorial song in the *S. burkii* complex

	Year	Tape MAR	Locality	Altitude (m)
<i>S. whistleri</i> (12)				
	1970	Green 6: 003-	Nepal, Mustang Distr.	3150
	1973	Green2: 422-	Nepal, Dolakha Distr.	2900
	1973	Green3: 479-	Nepal, Thodung	3200
	1988	Tape 6: 13,5-	Nepal, Dhopar Kharka	2700
	1988	Tape 9: 4,5-	Nepal, Paniporua	2800
	2001	Tape 6: 12-	Nepal, Dingla	2750
	2001	Tape 7: 7,5-	Nepal, Dingla	2850
	2001	Tape 7: 15-	Nepal, Dingla	2750
	2001	Tape 13: 6,5-	Nepal, Phedi	2750
	2001	Tape 13: 11-	Nepal, Phedi	2750
	2001	Tape 14: 23-	Nepal, Phedi	2280
	1985	Scharringa (1999)	India, Darjeeling	2350–2580
<i>S. burkii</i> (16)				
	1970	Red1: 002-	Nepal, Bobang/ S Dhorpatan	2500
	1970	Green16: 140-	Nepal, Bobang/ S Dhorpatan	2480
	1970	Green5: 103-	Nepal, Chadziou Chola	2650
	1988	Tape 4: 3-	Nepal, Mai Pokhari	2100
	1988	Tape 9: 3-	Nepal, Paniporua	2300
	1988	Tape 9: 5,5-	Nepal, Paniporua	2300
	1988	Tape 9: 8-	Nepal, Paniporua	2300
	1988	Tape 14: 6,5-	Nepal, Yamputhin	2000
	1988	Tape 14: 23-	Nepal, Yamputhin	1900
	1988	Tape 16: 14-	Nepal, Yamputhin	1900
	1988	Tape 23: 12-	Nepal, Omje Khola	2200
	1988	Tape 36: 5,5-	Nepal, betw. Mure and Hurure	2100
	1988	Tape 37: 8-	Nepal, betw. Mure and Hurure	2100
MAR 2730	2001	Tape 15: 3-	Nepal, Phedi	2220
MAR 2731	2001	Tape 15: 6-	Nepal, Phedi	2160
	2001	Tape 15: 11,5-	Nepal, Phedi	2140
<i>S. valentini</i> (30)				
	1997	Tape 21: 0-	China, Shaanxi, Taibai Shan	2500
	1997	Tape 21: 2-	China, Shaanxi, Taibai Shan	2500
	1997	Tape 17: 15-	China, Shaanxi, Taibai Shan	2500
	1997	Tape 17: 5-	China, Shaanxi, Taibai Shan	2500
	1998	Tape 9: 13-	China, Sichuan, Omei Shan	2880
	1998	Tape 9: 18-	China, Sichuan, Omei Shan	3020
MAR 919	1998	Tape 11: 4-	China, Sichuan, Omei Shan	3050
	1998	Tape 11: 25-	China, Sichuan, Omei Shan	2950
	1998	Tape 12: 4-	China, Sichuan, Omei Shan	2500
	1998	Tape 12: 22,5-	China, Sichuan, Omei Shan	2430
	1998	Tape 13: 7-	China, Sichuan, Omei Shan	2480
	1998	Tape 13: 16,5	China, Sichuan, Omei Shan	2320
	1998	Tape 19: 21,5-	China, Sichuan, Omei Shan	3000
*	1998	Tape 19: 24,5	China, Sichuan, Omei Shan	3000
	1998	Tape 21: 15-	China, Sichuan, Omei Shan	3000
	1998	Tape 22: 14-	China, Sichuan, Omei Shan	3000
*	1998	Tape 23: 15-	China, Sichuan, Omei Shan	2800
	1998	Tape 23: 21-	China, Sichuan, Omei Shan	2700
	2000	Tape 18: 5-	China, Sichuan, Omei Shan	2200
(bg)	2000	Tape 18: 10-	China, Sichuan, Omei Shan	2240
	2000	Tape 18: 12-	China, Sichuan, Omei Shan	2400
MAR 2051	2000	Tape 18: 13-	China, Sichuan, Omei Shan	2380
	2000	Tape 18: 23,5-	China, Sichuan, Omei Shan	3000
	2000	Tape 19: 5,5-	China, Sichuan, Omei Shan	3100
(bg)	2000	Tape 20: 14-	China, Sichuan, Omei Shan	3000

**Table 7.** (continued)

	Year	Tape MAR	Locality	Altitude (m)
MAR 2072	2000	Tape 23: 16,5-	China, Sichuan, Wawu Shan	2700
	2000	Tape 24: 20-	China, Sichuan, Wawu Shan	2700
	2002	Tape 2: 16-	China, Yunnan, Jizu Shan	2200
	2002	Tape 13: 24-	China, Sichuan, Wawu Shan	2700
	2002	Tape 18: 9,5-	China, Sichuan, Longxi-Hongkou	2700
	2002	Tape 24: 0-	China, Sihuan, Yaoji	2900
<i>S. soror</i> (25)				
	1997	Tape 2: 18-	China, Shaanxi, Taibai Shan	1450
	1997	Tape 3: 14-	China, Shaanxi, Taibai Shan	1500
MAR 904	1998	Tape 5: 14,5-	China, Sichuan, Omei Shan	1050
	1998	Tape 8: 23,5-	China, Sichuan, Omei Shan	1180
MAR 921	1998	Tape 12: 18-	China, Sichuan, Omei Shan	1020
MAR 929	1998	Tape 17: 14-	China, Sichuan, Omei Shan	1050
	1998	Tape 17: 22-	China, Sichuan, Omei Shan	850
	1998	Tape 17: 25-E	China, Sichuan, Omei Shan	850
	1998	Tape 18: 3-	China, Sichuan, Omei Shan	1120
	1998	Tape 18: 8,5-	China, Sichuan, Omei Shan	1170
	2000	Tape 2: 22-E	China, Shaanxi, Taibai Shan	1560
MAR 2010	2000	Tape 3: 0-	China, Shaanxi, Taibai Shan	2000
	2000	Tape 4: 15-	China, Shaanxi, Foping	900
MAR 2013	2000	Tape 5: 7-	China, Sichuan, Qincheng Shan	1200
	2000	Tape 6: 7,5-	China, Sichuan, Qincheng Shan	1300
	2000	Tape 6: 9,5-	China, Sichuan, Qincheng Shan	1300
	2000	Tape 7: 19-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 8: 7,5-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 9: 0-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 12: 8-	China, Sichuan, Omei Shan	700
MAR 2043	2000	Tape 12: 18,5- tape 13: 0-	China, Sichuan, Omei Shan	1140
call	2000	Tape 13: 16-	China, Sichuan, Omei Shan	1150
MAR 2045	2000	Tape 14: 12,5-	China, Sichuan, Omei Shan	1090
	2000	Tape 14: 14-	China, Sichuan, Omei Shan	1090
	2002	Tape 15: 3,5-	China, Sichuan, Longxi-Hongkou	970
	2002	Tape 15: 14,5-	China, Sichuan, Longxi-Hongkou	1300
<i>S. omeiensis</i> (37)				
	1997	Tape 5: 23-	China, Shaanxi, Taibai Shan	1950
	1997	Tape 2: 12,5-	China, Shaanxi, Taibai Shan	1450
MAR 901	1998	Tape 3: 14-	China, Sichuan, Omei Shan	1850
	1998	Tape 3: 17,5-	China, Sichuan, Omei Shan	1850
	1998	Tape 3: 23-E	China, Sichuan, Omei Shan	1950
	1998	Tape 4: 9-10	China, Sichuan, Omei Shan	2000
	1998	Tape 4: 13-	China, Sichuan, Omei Shan	2000
	1998	Tape 6: 7-	China, Sichuan, Omei Shan	1550
	1998	Tape 6: 14 -	China, Sichuan, Omei Shan	1700
	1998	Tape 6: 23-	China, Sichuan, Omei Shan	1750
MAR 922 (type specimen)	1998	Tape 13: 13,5-	China, Sichuan, Omei Shan	2330
	1998	Tape 14: 9-	China, Sichuan, Omei Shan	2100
MAR 925	1998	Tape 14: 22-	China, Sichuan, Omei Shan	2100
	1998	Tape 15: 17,5-	China, Sichuan, Omei Shan	2100
	2000	Tape 3: 4-	China, Shaanxi, Taibai Shan	2000
	2000	Tape 3: 7-	China, Shaanxi, Taibai Shan	2000
MAR 2027	2000	Tape 6: 17,5	China, Sichuan, Qincheng Shan	1250
	2000	Tape 7: 7-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 8: 19-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 8: 21-	China, Sichuan, Qincheng Shan	1250
	2000	Tape 9: 25-	China, Sichuan, Qincheng Shan	1350
	2000	Tape 10: 4-	China, Sichuan, Qincheng Shan	1450



Table 7. (continued)

	Year	Tape MAR	Locality	Altitude (m)
	2000	Tape 15: 18-	China, Sichuan, Omei Shan	1240
	2000	Tape 15: 21-	China, Sichuan, Omei Shan	1450
	2000	Tape 16: 9-	China, Sichuan, Omei Shan	1700
	2000	Tape 16: 24-	China, Sichuan, Omei Shan	
	2000	Tape 17: 6-	China, Sichuan, Omei Shan	1750
MAR 2049	2000	Tape 17: 10-	China, Sichuan, Omei Shan	1780
	2000	Tape 18: 10-	China, Sichuan, Omei Shan	2240
MAR 2071	2000	Tape 22: 5,5-	China, Sichuan, Wawu Shan	1480
	2000	Tape 22: 8-	China, Sichuan, Omei Shan	1980
	2000	Tape 22: 20-	China, Sichuan, Omei Shan	1980
	2000	Tape 25: 4-	China, Sichuan, Omei Shan	1310
	2000	Tape 25: 9-	China, Sichuan, Omei Shan	1310
	2002	Tape 10: 11-	China, Sichuan, Omei Shan	1950
	2002	Tape 16: 9,5-	China, Sichuan, Longxi-Hongkou	1850
	2002	Tape 19: 14-	China, Sichuan, Labahe Reserve	2000
	2002	Tape 20: 8-	China, Sichuan, Labahe Reserve	2200
	2002	Tape 21: 5-	China, Sichuan, Yaoji	2620
<i>S. affinis</i> (10)				
	1998	Tape 18: 9,5-	China, Sichuan, Omei Shan	1270
	1998	Tape 18: 20-	China, Sichuan, Omei Shan	1270
	1998	Tape 18: 23,5-E	China, Sichuan, Omei Shan	1280
MAR 2042	2000	Tape 12: 21-/13: 0-	China, Sichuan, Omei Shan	1140
	2000	Tape 13: 9-	China, Sichuan, Omei Shan	1230
	2000	Tape 13: 20-	China, Sichuan, Omei Shan	1250
	2000	Tape 14: 5-	China, Sichuan, Omei Shan	1200
(bg)	2000	Tape 14: 17,5-	China, Sichuan, Omei Shan	1250
(bg) MAR 2047	2000	Tape 15: 3-	China, Sichuan, Omei Shan	1250
	2000	Tape 15: 8-	China, Sichuan, Omei Shan	1260
<i>S. tephrocephalus</i> (11)				
	2000	Tape 1: 13-	China, Shaanxi, Taibai Shan	1300
	2000	Tape 1: 18,5-	China, Shaanxi, Taibai Shan	1300
	2000	Tape 1: 20,5-	China, Shaanxi, Taibai Shan	1300
MAR 2004	2000	Tape 1: 22-	China, Shaanxi, Taibai Shan	1300
	2000	Tape 3: 20-/4: 8,5-	China, Shaanxi, Taibai Shan	1300
	2000	Tape 4: 0-	China, Shaanxi, Taibai Shan	1300
	2000	Tape 7: 9,5-	China, Sichuan, Qincheng Shan	1250
	2002	Tape 1: 11-	China, Yunnan, Jizu Shan	2200
	2002	Tape 1: 20,5-	China, Yunnan, Jizu Shan	2200
	2002	Tape 15: 23,5-	China, Sichuan, Longxi-Hongkou	1300
	2002	Tape 16: 4-	China, Sichuan, Longxi-Hongkou	1850
<i>S. xanthoschistos</i> (11)				
	1988	Tape 3: 14,5-	Nepal, Mai Pokhari	1660
	1988	Tape 9: 20,5-	Nepal, Paniporua	2200
	1988	Tape 10: 16,5-	Nepal, Yektin	1500
	1988	Tape 17: 19-	Nepal, Omje Kharka	2400
	1988	Tape 23: 23-	Nepal, Yamputhin	2350
	1988	Tape 38: 20,5-	Nepal, betw. Mure and Hurure	2100
	1988	Tape 42: 15-	Nepal, Chichila	2000
	2001	Tape 3: 19,5-	Nepal, Bhojpur Distr.	1250
	2001	Tape 4: 0-	Nepal, Bhojpur Distr.	1350
	2001	Tape 4: 8,5-	Nepal, Bhojpur Distr.	1620
	2001	Tape 4: 15,5-	Nepal, Bhojpur Distr.	2100
<i>S. grammiceps</i> (1)				
	1986	Scharringa (1999)	Thailand, Chiang Mai Prov.	1700

**Table 7.** (continued)

	Year	Tape MAR	Locality	Altitude (m)
<i>S. castaniceps</i> (4)	1997	Tape 5: 14,5-	China, Shaanxi, Taibai Shan	1650
	1998	Tape 7: 0–2	China, Sichuan, Omei Shan	1680
(bg)	1998	Tape 7: 0–2	China, Sichuan, Omei Shan	1880
	1997	Scharringa (1999)	Indonesia, Java	1300–1500

Note: Song specimens are indicated in the far left column by either an asterisk or the corresponding sample number (MAR). (bg) = *Seicercus* song in the background of another recorded species.

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