



Description of a new species of *Phylloscopus* warbler from Vietnam and Laos

PER ALSTRÖM,^{1,2*} PETE DAVIDSON,^{3†} J. W. DUCKWORTH,³ JONATHAN C. EAMES,⁴ TRAI TRONG LE,⁴ CU NGUYEN,⁵ URBAN OLSSON,⁶ CRAIG ROBSON⁷ & ROB TIMMINS^{3‡}

¹Swedish Species Information Centre, Swedish University of Agricultural Sciences, PO Box 7007, SE-750 07 Uppsala, Sweden

²Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, PO Box 50007, SE-104 05 Stockholm, Sweden

³Wildlife Conservation Society Lao Program, PO Box 6712, Vientiane, Laos PDR

⁴BirdLife International in Indochina, N6/2 + 3, Lane 25, Lang Ha Street, Ba Dinh District, Hanoi, Vietnam

⁵Institute of Ecology and Biological Resources, Vietnam Academy of Science & Technology, 18 Hoang Quoc Viet Street, Cau Giay District, Hanoi, Vietnam

⁶Department of Zoology, University of Göteborg, PO Box 463, SE-405 30 Göteborg, Sweden

⁷63 Stafford Street, Norwich, Norfolk NR2 3BD, UK

A new species of *Phylloscopus* warbler, which we name *Phylloscopus calciatilis* Limestone Leaf Warbler, is described from central and northern Vietnam and central and northern Laos; it probably also breeds in southernmost China. In morphology, the new species is very similar to Sulphur-breasted Warbler *Phylloscopus ricketti*, but it is smaller with a proportionately larger bill and rounder wing. Its song and calls are diagnostic. Based on mitochondrial and nuclear DNA, the new species is most closely related to *P. ricketti* and Yellow-vented Warbler *Phylloscopus cantator*, and it is inferred to be sister to the latter. The mitochondrial divergences between these three species are at the low end of the variation found in other species of *Phylloscopus* and *Seicercus* warblers, but greater than in other taxa generally treated as subspecies. Possible introgressive hybridization between the new species and *P. ricketti* is discussed, but more data are needed to establish whether it does occur and, if it does, to what extent. The new species appears to have a restricted breeding range in limestone karst environments, where it is locally common and therefore not under any immediate threat. In view of the recognition of the new species, all previous records of *P. ricketti sensu lato* need to be re-evaluated.

Keywords: cryptic speciation, introgressive hybridization, limestone karst, phylogeny, Southeast Asia, taxonomy, vocalizations.

The genus *Phylloscopus* is distributed over much of Eurasia and more patchily in Africa, with one species reaching the northwestern corner of North America (Ticehurst 1938, Watson *et al.* 1986, Sibley & Monroe 1990, Dickinson 2003, Bairlein

et al. 2006). The number of recognized species has increased by more than 40% in the past two decades, from 45 (Watson *et al.* 1986) to 64 (Gill *et al.* 2009). This dramatic increase is mainly a result of increased knowledge of vocalizations and genetic relationships (Alström & Olsson 1990, 1992, 1995, Alström *et al.* 1992, 1997, Olsson *et al.* 1993, 2005, Helbig *et al.* 1995, 1996, Irwin *et al.* 2001, Martens *et al.* 2004, 2008, Saitoh *et al.* 2006, Päckert *et al.* 2009). Many species groups in the genus are cryptic, differing only in very subtle morphological features (Ticehurst 1938, Williamson 1967, Bairlein *et al.* 2006), while being diagnostic

*Corresponding author.
Email: per.alstrom@artdata.slu.se

Present addresses: [†]Bird Studies Canada, Pacific Wildlife Research Centre, 5421 Robertson Road, Delta, British Columbia, V4K 3N2, Canada; [‡]2313 Willard Avenue, Madison, WI 53704, USA.

vocally. Studies of DNA have also revealed that the genus *Phylloscopus* is not monophyletic, as a consequence of *Seicercus* warblers being nested within *Phylloscopus* (e.g. Olsson *et al.* 2004, 2005, Päckert *et al.* 2004).

The Sulphur-breasted Warbler *Phylloscopus ricketti* has been considered to breed in south and central China and winter in Thailand, Laos and Vietnam (Watson *et al.* 1986, Cheng 1987, Robson 2000, Bairlein *et al.* 2006, Fig. 1). In the mid-1990s, birds very similar in morphology to *P. ricketti* were observed, often commonly, in Vietnam and Laos under conditions suggesting local breeding. Localities included: (1) the Phong Nha sector of Phong Nha-Ke Bang National Park, Quang Binh Province, Central Annam, Vietnam, in July 1994 [N.C. (Cu Nguyen¹), J.C.E., L.T.T. (Trai Trong Le¹)] and again on 24 April 1995 (C.R., U.O.); and (2) the Nadi and Sayphou Loyang limestone areas, Bolikhamxai Province, on the border of north and central Laos during May 1995 (R.J.T.) (Fig. 1). A number of recordings of songs and calls were made, and later transcriptions of these were published under the name *P. ricketti* (Robson 2000). R.J.T. also recorded singing birds; the recordings were sent to P.A., who commented that, on the basis of these recordings, the birds appeared to represent a taxon distinct from *P. ricketti* (Duckworth *et al.* 1998).

Given the apparent novelty of the birds in central Indochina, further fieldwork was undertaken. On 29 June 1996, N.C., J.C.E. and L.T.T. collected two specimens, one adult male and one juvenile male, in Phong Nha-Ke Bang National Park. These were later donated to The Natural History Museum, Tring, UK. In mid- to late February 1998, within Hin Namno National Protected Area, Khammouan Province, central Laos (Fig. 1), P.D. found the putative new taxon to be locally common and territorial. He recorded some birds, and caught, measured and photographed one bird (Fig. 2d,g). Following this, P.A. visited the Phong Nha-Ke Bang National Park on 12–15 April 2004 to study the population discovered 10 years earlier. He observed approximately 15 birds: the songs of nine individuals were recorded and two individuals were caught, blood-sampled, measured and photographed (Fig. 2c).

¹In Vietnam, family names are placed before given names; however, to conform with the standards in the scientific literature, the order is reversed in the list of authors.

To evaluate whether the putative new taxon is consistently different from *P. ricketti* (which it resembles in plumage) and Yellow-vented Warbler *Phylloscopus cantator* (which is closely related to *P. ricketti*: Päckert *et al.* 2004, Olsson *et al.* 2005), as well as whether part of the Chinese breeding range of *P. ricketti* is in fact attributable to the unknown warbler, we have collected data from approximately 20 localities in southern Asia during the breeding season. These data show broadly congruent differences in morphometrics, vocalizations and mitochondrial DNA between the unknown Indochinese taxon, the Chinese *P. ricketti* and the more westerly distributed *P. cantator*. All three taxa are essentially allopatric/parapatric, although there are perhaps indications of range overlap and introgressive hybridization between the unknown warbler and *P. ricketti* in Guangxi Province in southern China (see below). Although there are still gaps in our knowledge regarding the distribution and level of interaction among these three taxa, we consider the Indochinese warbler to be sufficiently distinct to merit recognition as a new species, which we formally describe below. For reasons of clarity, in the intervening text we refer to this new species as the Limestone Leaf Warbler, the name alluding to the fact that this species is strongly associated with limestone karst.

MATERIAL AND METHODS

Morphology

The Limestone Leaf Warbler was studied in the field mainly in: (1) the Phong Nha sector of Phong Nha-Ke Bang National Park, Vietnam (c. 17°24'N, 106°23'E), (2) Nadi limestone (c. 18°20'N, 104°35'E) and Sayphou Loyang (c. 18°17'N, 104°40'E), Bolikhamxai Province, north/central Laos, and (3) Hin Namno National Protected Area, Khammouan Province, central Laos (17°15'–17°40'N, 105°43'–106°09'E) (Fig. 1, Supporting Information Table S1).

One adult and one juvenile male (aged by plumage, sexed internally) were collected in Phong Nha-Ke Bang on 29 June 1996. These specimens are deposited at The Natural History Museum, Tring, UK (BMNH), where we compared them with a series of *P. ricketti*. Further, two males (sexed by song and strong response to playback) were caught at the same locality on 14 April 2004, and one male (sexed by song and strong response

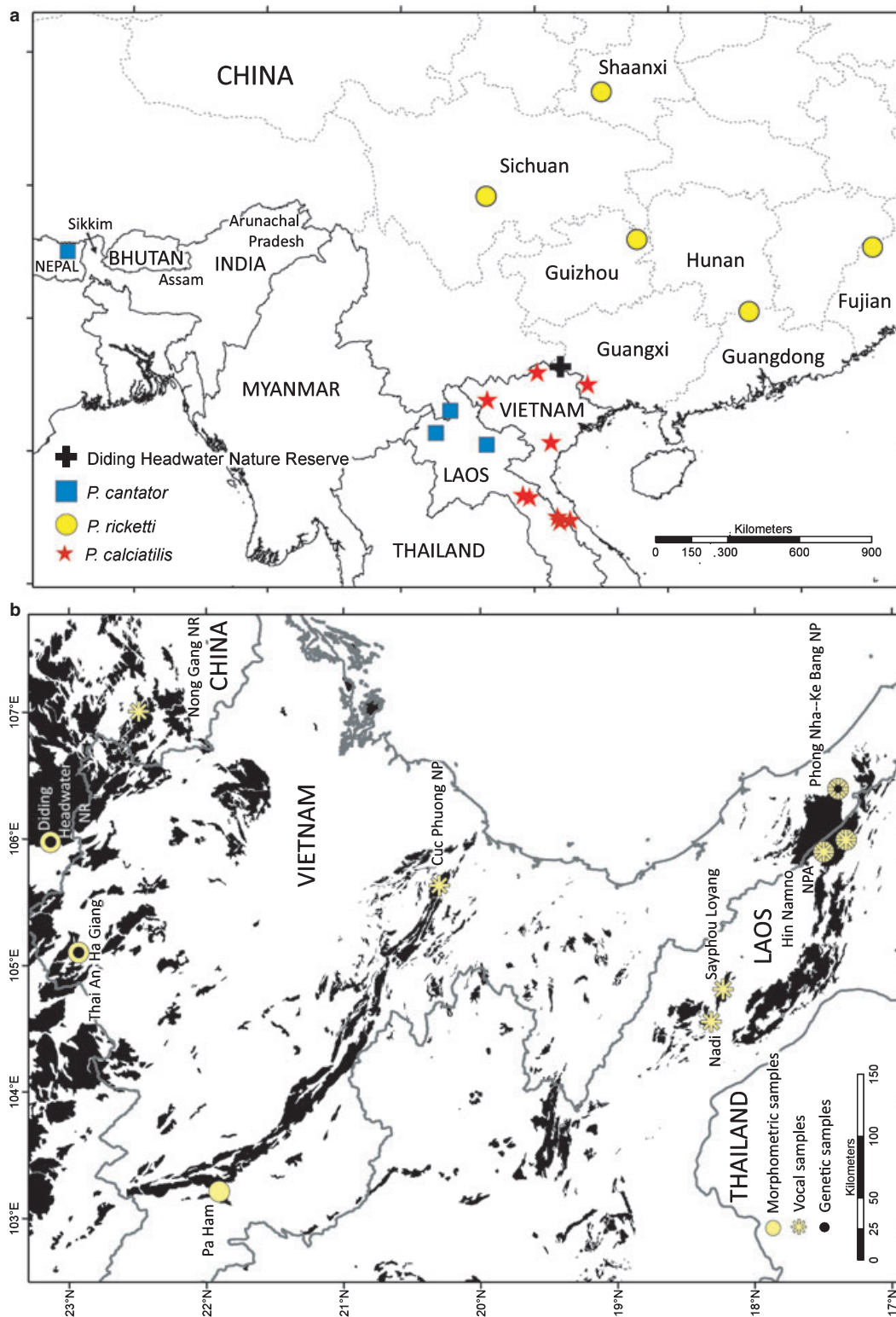


Figure 1. (a) Distribution of verified breeding season samples of *Phylloscopus calciatilis* and *Phylloscopus ricketti*, and breeding record samples of *Phylloscopus cantator pernotus* (Laos) and a DNA sample of *Phylloscopus cantator cantator* (Nepal). Records from Diding Headwater Nature Reserve are from the non-breeding season. (b) Distribution of records of *Phylloscopus calciatilis*. Black areas represent karst limestone.



Figure 2. *Phylloscopus calciatilis*: (a) Ha Giang Province, Vietnam, 1 June (NRM 20086529; U. Johansson/Swedish Museum of Natural History); (b) Ha Giang Province, Vietnam, 5 June (NRM 20086571; U. Johansson/Swedish Museum of Natural History); (c) Phong Nha-Ke Bang National Park, Quang Binh Province, Vietnam, mid-April (NRM 20047134; P. Alström, from video); (d) Hin Namno National Protected Area, mid/late February (P. Davidson); (e) *Phylloscopus ricketti*, Sichuan, China, early May (NRM 20037006; U. Olsson); (f) *Phylloscopus goodsoni goodsoni* Ba Bao Shan, 1 May (P. Leader); (g) same individual as in d; (h) same individual as in a; (i) same individual as in e; (j) same individual as in f.

to playback) was caught in the Hin Namno National Protected Area on 28 February 1998. One further specimen (male), collected at Thai An Commune, Quan Ba district, Ha Giang Province, East Tonkin, Vietnam (22°57'N, 105°06'E), on 31 May 2008, and photographs of three additional birds caught at the same locality between 1 and 5 June 2008 (Fig. 1, Supporting Information Table S1), are deposited in the Swedish Museum of Natural History, Stockholm, and were also examined. One female appearing to match the Limestone Leaf Warbler based on measurements and wing formula, collected at Pa Ham, Lai Chau Province, West Tonkin, Vietnam (c. 21°55'N, 103°14'E), on 7 April 1929, lodged in The Field Museum, Chicago (FMNH), was also examined (Fig. 1, Supporting Information Table S1).

Phylloscopus ricketti was studied on the breeding grounds in China on Emei Shan (mountain), Sichuan Province (c. 29°35'N, 103°11'E; May 1986, 1987, 1989, 1992, 1994), on Wuyi Shan, Fujian Province [at the species' type locality, Guadun (= Kuatun); c. 27°40'N, 117°40'E; May 1993], and on Qinling Shan, Shaanxi Province (c. 33°30'N, 107°30'E; June 1995) (Fig. 1, Supporting Information Table S1); one bird was caught on Emei Shan (Supporting Information Table S1). Eighteen males of *P. ricketti* collected during the breeding season in China (17 from the species' type locality in Fujian, one from Guizhou) were measured in the BMNH (Fig. 1, Supporting Information Table S1). In addition, the following material was studied: two males and one female matching *P. ricketti* in morphometrics and wing formula, two of these also matching *P. ricketti* in cytochrome-*b* haplotype (third not sequenced), from the Diding Headwater Nature Reserve, Guangxi Province, China (23°07'N, 105°58'E; Fig. 1, Supporting Information Table S1), lodged in the Kansas University Natural History Museum, Lawrence, Kansas (KUNHM); and four specimens matching Chinese *P. ricketti* in morphometrics and wing formula, collected in Southeast Asia between October and March (Supporting Information Table S1). We also obtained measurements of one male caught on Emei Shan, Sichuan (H. Jännes unpubl. data). Further, two females with uncertain identity (having a Limestone Leaf Warbler cytochrome-*b* haplotype, but ambiguous in morphology) were examined from the Diding Headwater Nature Reserve (KUNHM).

Several *P. cantator cantator* were observed in Assam and Arunachal Pradesh Provinces, India, in

February 1994, and *P. cantator pernotus* (identified to subspecies by geographical location) at several places in north and central Laos during 1994–98 and in 2004 (Davidson 1998, Thewlis *et al.* 1998, Fuchs *et al.* 2007; Fig. 1). Ten males of *P. c. cantator* from the University of Michigan Museum of Zoology, Ann Arbor (UMMZ) were measured (Supporting Information Table S1).

The following measurements were taken: wing-length (maximum length; flattened and stretched), bill-length (culmen length from bill-tip to skull), bill-width (at distal edge of nostril) and tail-length. Tail and wing measures were recorded to the nearest 0.5 mm; bill measures were estimated to the nearest 0.1 mm. Wing formula was also examined; the primaries were numbered in descending order. With a few exceptions, all birds were measured by the same person (P.A.). Standard statistical analyses were conducted in EXCEL (Microsoft, Redmond, WA, USA) and STATISTICA version 8 (StatSoft, Inc., Tulsa, OK, USA), and the principal component analysis was performed in MATLAB (MathWorks, Inc., Natick, MA, USA) using the 'princomp' function.

Vocalizations

Recordings were obtained of songs from 10 individuals and calls from five birds in Phong Nha-Ke Bang; songs of three birds and calls of one individual from Hin Namno National Protected Area; songs of three birds at Nadi; and the song of one bird at Cuc Phuong National Park, Ninh Binh Province, East Tonkin, Vietnam (c. 20°19'N, 105°36'E; Supporting Information Table S1). Most of the recordings from Phong Nha-Ke Bang are deposited with The British Library Sound Archive, London (BL; Nos. WA 05/14 DAT 13/13 refs 27, 35, 36, 40, 44, 50, 51, 60, 64, 65), and three songs and two calls are included as part of the Supporting Information accompanying this paper. In addition, songs of two birds (one in the background to another) matching Limestone Leaf Warbler song were obtained from Nong Gang Nature Reserve, Guangxi Province China (c. 22°30'N, 107°N; G.J. Carey unpubl. data; Supporting Information Table S1).

For *P. ricketti*, songs of seven males and calls of three birds were recorded on Emei Shan, Sichuan Province, China; all of these are deposited with the BL (WA 94/82 DAT 45/74 refs 623, 648, 658–656; WA 94/82 DAT 69/74 refs 03, 07, 11–13; WA 94/82 DAT 28/74 refs 407, 555). One singing male

was also recorded in Wuyi Shan, Fujian Province (species' type locality). Additional recordings of song were obtained from Emei Shan, Sichuan (three birds recorded by H. Jännes), Fanjing Shan, Guizhou (c. 27°57'N, 108°50'E; one bird by M. Crosby), Wuyi Shan, Fujian (three birds by G. J. Carey; one bird by T. Woodward), and Nanling/Babao Shan, Guangdong Province, China (24°42'N, 113°03'E; one bird by Van Lu, five birds by G. J. Carey; these arrived too late to be included in detailed analyses in Supporting Information Tables S2 and S3), and of calls from Wuyi Shan (one bird G. J. Carey). See Supporting Information Table S1.

For *P. cantator cantator*, song and call were recorded from one male in Arunachal Pradesh, India (WA 94/82 DAT 67/74 cuts 06–09), and additional recordings of song were obtained from Arunachal Pradesh (two birds recorded by P. Singh), Assam, India (BL No. 100475), Bhutan (three birds by P. Holt, BL Nos. 65110, 65112, 65113; one by D. Fisher, BL No. 100916; one by H. Jännes; one from Connop 1995). For *P. cantator pernotus* (identified to subspecies by geographical location) one song was recorded in Phou Louey National Protected Area (Nam Et-Phou Loey National Protected Area), northern Laos (c. 20°15'N, 103°1'E). See Supporting Information Table S1.

The sonograms were made using RAVEN 1.1 (Charif *et al.* 2003). The following measurements were taken on the sonograms: (1) total duration of strophe(s), (2) frequency span (delta frequency) of strophe (kHz), (3) top frequency of strophe (kHz), (4) bottom frequency of strophe (kHz), (5) mid-frequency of strophe (kHz), (6) number of elements and syllables, and (7) the number of 'introductory elements'. The proportion of unique strophes per recorded song was calculated by dividing the number of unique strophes per recording by the natural logarithm of the total number of strophes in the recording (to correct for different length of recordings).

The following voice terminology has been used: song strophe – a continuous flow of notes, separated from other strophes by pauses (silent or filled with calls); element – a discrete, unbroken unit in a sonogram; note – a sound that may or may not consist of more than one element; syntax – the arrangement of elements in a strophe; syllable – a unit consisting of two (or more elements) that are separated by very short pauses, and that always occur together in the same sequence.

Molecular sequence data

Blood or muscle from fresh samples or toepads from museum specimens were obtained for eight Limestone Leaf Warblers, one *P. cantator cantator*, six *P. ricketti* and two Limestone Leaf Warbler/*P. ricketti* individuals (originally identified as *P. ricketti*, but having a Limestone Leaf Warbler cytochrome-*b* haplotype); one further *P. ricketti* cytochrome-*b* sequence was downloaded from GenBank (AY606172). Eastern Bonelli's Warbler *Phylloscopus orientalis* and Yellow-browed Warbler *Phylloscopus inornatus* were chosen as outgroups, as they have been shown to be closely related to, but not part of, the clade that *P. ricketti* and *P. cantator* belong to (Olsson *et al.* 2005, Johansson *et al.* 2007).

DNA was extracted using the QIA Quick DNEasy Kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. We sequenced the mitochondrial cytochrome-*b* gene and introns 6–7 of the nuclear ornithine decarboxylase (ODC) gene. Cytochrome-*b* was sequenced for all individuals; ODC was sequenced for two individuals of the Limestone Leaf Warbler and one each of the other species (Supporting Information Table S1). Amplification and sequencing of cytochrome-*b* followed the protocols described in Olsson *et al.* (2005), and that of ODC as described in Allen and Omland (2003). Cytochrome-*b* was amplified as a single fragment to decrease the risk of amplifying nuclear pseudogenes (cf. Sorensen & Quinn 1998). No stop codons or distinct double peaks in the chromatograms that would indicate the presence of nuclear pseudogenes were found in the coding cytochrome-*b* sequences. The sequences have been deposited in GenBank (cytochrome-*b* GU045613–25, ODC GU045626–31, Supporting Information Table S1).

Sequences were easily aligned using MEGALIGN 4.03 (DNASTAR Inc., Madison, WI, USA) with small changes being made by eye. Molecular phylogenies were estimated by Bayesian inference (BI) using MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). Posterior probabilities were calculated for cytochrome-*b* using all unique haplotypes under a general time-reversible (GTR) model (Lanave *et al.* 1984, Tavaré 1986, Rodríguez *et al.* 1990), assuming rate variation across sites according to a discrete gamma distribution with four rate categories (Γ ; Yang 1994). Posterior probabilities for the ODC data were estimated under the Hasegawa *et al.* (1985) (HKY) model and an estimated proportion of invariant sites (I; Gu *et al.* 1995). The choice of

model was determined based on the Akaike Information Criterion (Akaike 1973) calculated by MRMODELTEST2 (Nylander 2004) in conjunction with PAUP*4.0b10 (Swofford 2002). Moreover, for one individual of each species all data were combined so that the concatenated cytochrome-*b* and ODC sequences were partitioned and each locus was allowed to have partition-specific model parameters (Ronquist & Huelsenbeck 2003, Nylander *et al.* 2004). Default priors in MRBAYES were used. Four Metropolis-coupled MCMC chains with incremental heating temperature of 0.2 were run for 50 million generations (cytochrome-*b*) and 10 million generations (other analyses), respectively, and sampled every 1000 generations. Two runs were run simultaneously, starting from random trees, and the results compared to ascertain that the chains had reached the same target distributions. For the cytochrome-*b* data, stationarity was probably never reached, despite the many replicates, as there were several plateaus in the likelihood plot. However, the tree topology and posterior probabilities were identical irrespective of whether 10% or 90% of the samples were discarded as burn-in. For the ODC and combined ODC and cytochrome-*b* analyses the first 50% of the generations, long after the chain reached apparent stationarity, were discarded and the posterior probability estimated for the remaining topologies. The samples from the stationary phases of the independent runs were pooled to obtain the final results. Maximum likelihood (ML) bootstrapping (1000 replicates) was performed in TREEFINDER (Jobb *et al.* 2004, Jobb 2008) using default settings with the same models used in the Bayesian inference analyses with the exception of the combined ODC and cytochrome-*b* analysis, which was performed under the GTR + Γ model, as proposed by TREEFINDER. Clade support for the unweighted data set was also assessed by maximum parsimony (MP) bootstrapping in PAUP*: branch and bound search strategy, starting trees obtained via random stepwise addition, 10 replicates, followed by TBR branch swapping, 1000 replicates. Uncorrected-p pairwise sequence divergences were calculated in PAUP*.

RESULTS

Morphology

In plumage, the Limestone Leaf Warbler (Fig. 2a–d, g,h) appears to be indistinguishable from *P. ricketti*

(Fig. 2e,i), although as a result of the small number of specimens available for the Limestone Leaf Warbler and their rather poor quality, detailed comparisons are difficult to make. The only two specimens of the Limestone Leaf Warbler (the types, see below), which have been directly compared with a series of *P. ricketti* are marginally colder yellow below and more greyish-tinged above, and show marginally greyer lateral crown-stripes than *P. ricketti*. The Limestone Leaf Warbler is easily separable from *P. cantator* by its yellow belly (white in *P. cantator*, sometimes with a thin yellow stripe centrally; similar in both the nominate and *pernotus* subspecies), and from the only other strongly yellowish-bellied ‘crowned’ leaf warbler, *P. goodsoni goodsoni* (*sensu* Olsson *et al.* 2005) by having a more contrasting and better defined crown pattern, especially anteriorly, showing blacker lateral crown-stripes and a paler median crown-stripe (Fig. 2f,j).

The Limestone Leaf Warbler is smaller and proportionately larger-billed than *P. ricketti* (Figs 2 and 3, Table 1). In our small sample of males there is no overlap in the bill-length/wing-length ratio (Fig. 3a), and little overlap in wing-length (Fig. 3b) or bill-width (Table 1). The above variables, as well as tail-length and bill-length, are statistically significantly different (Table 1). However, a larger sample, especially of the Limestone Leaf Warbler, might reveal more overlap. The only female examined that is believed to represent the Limestone Leaf Warbler (FMNH 79799; from Pa Ham, West Tonkin, Vietnam) matches this taxon in all measurements (taking into account that females are likely to be smaller than males, as in other *Phylloscopus* species; Ticehurst 1938, Williamson 1967, Cramp 1992) and wing formula, and also has a bill-length/wing-length ratio that is non-overlapping with that of *P. ricketti* (including our only female *P. ricketti* that fits this species in both morphology and DNA; KUNHM 93365). Compared with *P. cantator*, the Limestone Leaf Warbler has a proportionately longer bill and shorter tail (Fig. 3, Table 1).

A principal component analysis (PCA) including all males of the Limestone Leaf Warbler, *P. ricketti* and *P. cantator* for which we have measurements of wing-, tail- and bill-length and bill-width shows clear separation between the Limestone Leaf Warbler and *P. ricketti*, whereas *P. cantator* occupies a somewhat intermediate position in multidimensional space (Supporting Information Fig. S1).

Table 1. Measurements and wing formula of *Phylloscopus calciatilis*, *Phylloscopus ricketti* and *Phylloscopus cantator* (in mm).

	<i>Phylloscopus calciatilis</i> M	<i>Phylloscopus ricketti</i> M	<i>Phylloscopus cantator</i> M	<i>Phylloscopus calciatilis</i> F FMNH 79799	<i>Phylloscopus ricketti</i> F KUNHM 93365	<i>Phylloscopus calciatilis/ricketti</i> F KUNHM 93362	<i>Phylloscopus calciatilis/ricketti</i> F KUNHM 96954
Wing	52.0–55.0 (53.6; 1.05; 9)	54.5–60.5 (57.3; 1.52; 22)***	52.0–56.0 (54.6; 1.22; 10) ns	51.0	53.5	53.5	52.5
Tail	37.0–40.0 (38.1; 1.17; 7)	37.5–43.0 (40.1; 1.50; 22)**	38.0–42.0 (40.4; 1.39; 10)**	c. 37.5	36.5	38.5	37.5
Bill-length	13.3–13.9 (13.6; 0.26; 6)	12.2–14.0 (13.0; 0.51; 22)*	12.1–13.7 (12.8; 0.55; 9)**	13.0	11.7	12.7	11.8
Bill-width	2.7–3.2 (3.0; 0.24; 4)	2.6–3.1 (2.8; 0.18; 19)*	2.6–3.1 (2.8; 0.16; 9)*	–	2.7	2.9	2.7
Bill-length/wing	0.25–0.27 (0.25; 0.007; 6)	0.22–0.24 (0.23; 0.005; 22)***	0.23–0.26 (0.24; 0.01; 9)***	0.25	0.22	0.24	0.23
P10 > p.c.	7.5–9.0 (8.5; 0.87; 3)	5.0–9.0 (7.5; 1.15; 16) ns	(not checked)	8.0	8.0	8.0	(not checked)
P9	8.5–9.0 (8.8; 0.29; 3)	5.5–9.5 (7.4; 1.12; 18) ns	6.5–9.0 (7.1; 1.14; 5) *	9.0	5.0	8.0	7.0
P8	1.0–2.0 (1.5; 0.5; 3)	0–2.0 (0.8; 0.57; 18) ns	0.5–1.5 (1.1; 0.42; 5) ns	1.5	0	1.5	0.5
P7	0 (3)	0–0.5 (0.03; 0.12; 18) ns	0 (5) ns	0.4	0.5	0	0
P6	0 (3)	0–1.0 (0.4; 0.34; 17) ns	0–0.5 (0.1; 0.22; 5) ns	0	0.5	0	0
P5	0.5–1.0 (0.7; 0.29; 3)	1.0–2.5 (1.7; 0.46; 18)**	0.5–2.5 (1.4; 0.89; 5) ns	0.5	2.0	0.5	1.5
P4	3.0–3.5 (3.2; 0.29; 3)	4.5–7.0 (5.5; 0.93; 17)***	3.0–6.0 (4.5; 1.06; 5) ns	2.5	5.0	3.0	4.5
Relative length P9	≤ss (100%)	P2/P3 (n = 3); = P3 (n = 4); P3/P4 (n = 12); = P4 (n = 1)	(not checked)	<ss	P5/4	≈ss	(not checked)

Values given are: minimum–maximum (mean; sd; sample size). Samples of *Phylloscopus ricketti* males are from Fujian (17), Guizhou (1), Guangxi (2) and Sichuan (2) Provinces, China (all March–May, except Guangxi, from September), and of *Phylloscopus cantator* from Assam and Manipur, India. Females tentatively identified to species based on measurements and wing formula (KUNHM 93365 also by cytochrome-*b* haplotype), and two unidentified *Phylloscopus calciatilis/ricketti* specimens (possible hybrids) having *Phylloscopus calciatilis* cytochrome-*b* haplotypes, but measurements and wing formula either matching *Phylloscopus ricketti* (KUNHM 96954) or measurements seeming to indicate *Phylloscopus ricketti* but with wing formula matching *Phylloscopus calciatilis* (KUNHM 93362).

M, male; F, female. Primaries (P) numbered in descending order; p.c., primary coverts; ss, secondaries. In all three species, primaries numbers 5–8 are emarginated. FMNH – The Field Museum, Chicago; KUNHM – Kansas University Natural History Museum, Lawrence.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant (*t*-test); comparisons with *Phylloscopus calciatilis*.

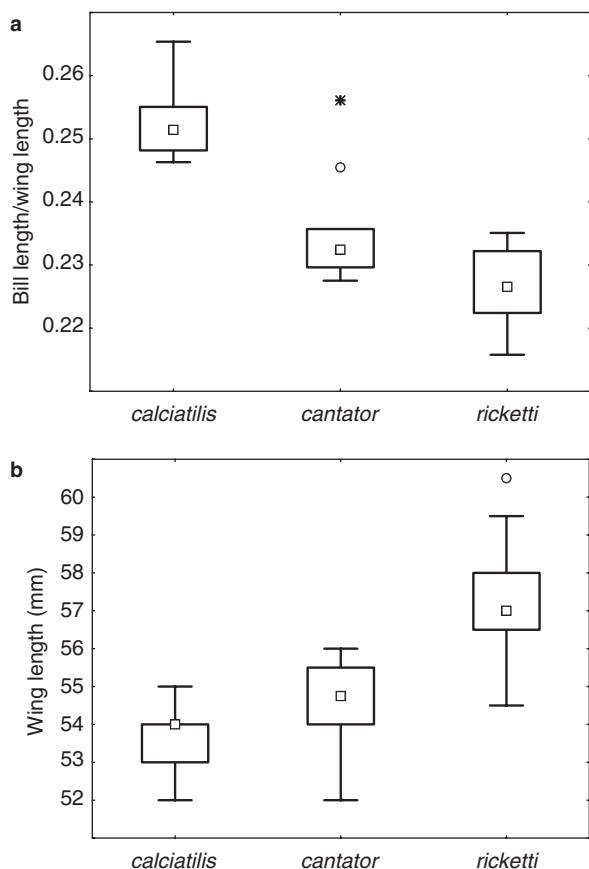


Figure 3. Box plots of (a) bill-length (to skull) divided by wing-length of males of *Phylloscopus calciatilis* ($n = 6$), *Phylloscopus cantator* ($n = 9$) and *Phylloscopus ricketti* ($n = 23$); (b) wing-length of males of *Phylloscopus calciatilis* ($n = 9$), *Phylloscopus cantator* ($n = 10$) and *Phylloscopus ricketti* ($n = 23$). Small squares represent the median; box, 50% of samples; bars, maximum and minimum, respectively, of non-outliers; circle, outlier; star, extreme outlier.

The Limestone Leaf Warbler has a rounder wing-tip than *P. ricketti* (Table 1). This is in agreement with its presumed more sedentary habits (Ticehurst 1938, Marchetti *et al.* 1995). In our small sample of males there is no overlap between the Limestone Leaf Warbler and *P. ricketti* in the length of primary number 4 in relation to the wing-tip and the length of primary number 9 relative to the other remiges, and only marginal overlap between the two species in the length of primary number 5. All these variables, as well as the length of primary number 8, are statistically significantly different between the Limestone Leaf Warbler and *P. ricketti*. The only unquestionable female of Limestone Leaf Warbler (FMNH 79799)

agrees with males of this taxon in wing formula (whereas the only undoubted female *P. ricketti* (KUNHM 93365) agrees with male *P. ricketti*). The wing formulae of the Limestone Leaf Warbler and *P. cantator* are very similar (Table 1).

One female from Diding Headwater Nature Reserve, Guangxi Province, China (KUNHM 93362), appears to be morphologically somewhat intermediate between the Limestone Leaf Warbler and *P. ricketti*, although it has a cytochrome-*b* haplotype matching the former. Another female from the same locality (KUNHM 96954) appears to match *P. ricketti* in morphology, but has a cytochrome-*b* haplotype matching the Limestone Leaf Warbler.

Song

The song of the Limestone Leaf Warbler (Fig. 4a, Supporting Information Tables S2 and S3) consists of short (1.2–1.8 s) strophes of seven to nine soft whistled notes/elements of varying pitch, duration and structure that are generally given on a slightly falling scale. At Phong Nha (Fig. 4a, ii–iv) the song generally has a fairly regular syntax: (1) an ‘introductory element’, which is usually shorter and thinner than the others (marked by A* in Fig. 4a and Supporting Information Table S3); (2) one to three elements (either all different from each other or two successive elements similar); and (3) a series of elements that are frequently given in the order X, Y, X, Z, Y, Z [e.g. D, E, D, F, E, F and B, D, B, E, D, E in two of the strophes in Fig. 4a(ii); the last element sometimes different from others or missing; sequence underlined in Supporting Information Table S3]. Our only recording from Cuc Phuong has the same syntax, except that there is no introductory element. Birds from Laos (Fig. 4a, i) have on average less structured songs, although most individuals have the sequence Z, Y, Z at the end (Supporting Information Table S3). At Phong Nha, from where most of our recordings come, each male in our sample had a repertoire of one to seven unique strophes (mean 2.6; proportion of unique strophes per recorded song = 1.07); the same strophe was sometimes repeated many (up to 34) times. Certain strophes are shared by up to four males, often with slight variations.

Recordings made by G. J. Carey from the Nong Gang Nature Reserve, Guangxi Province,

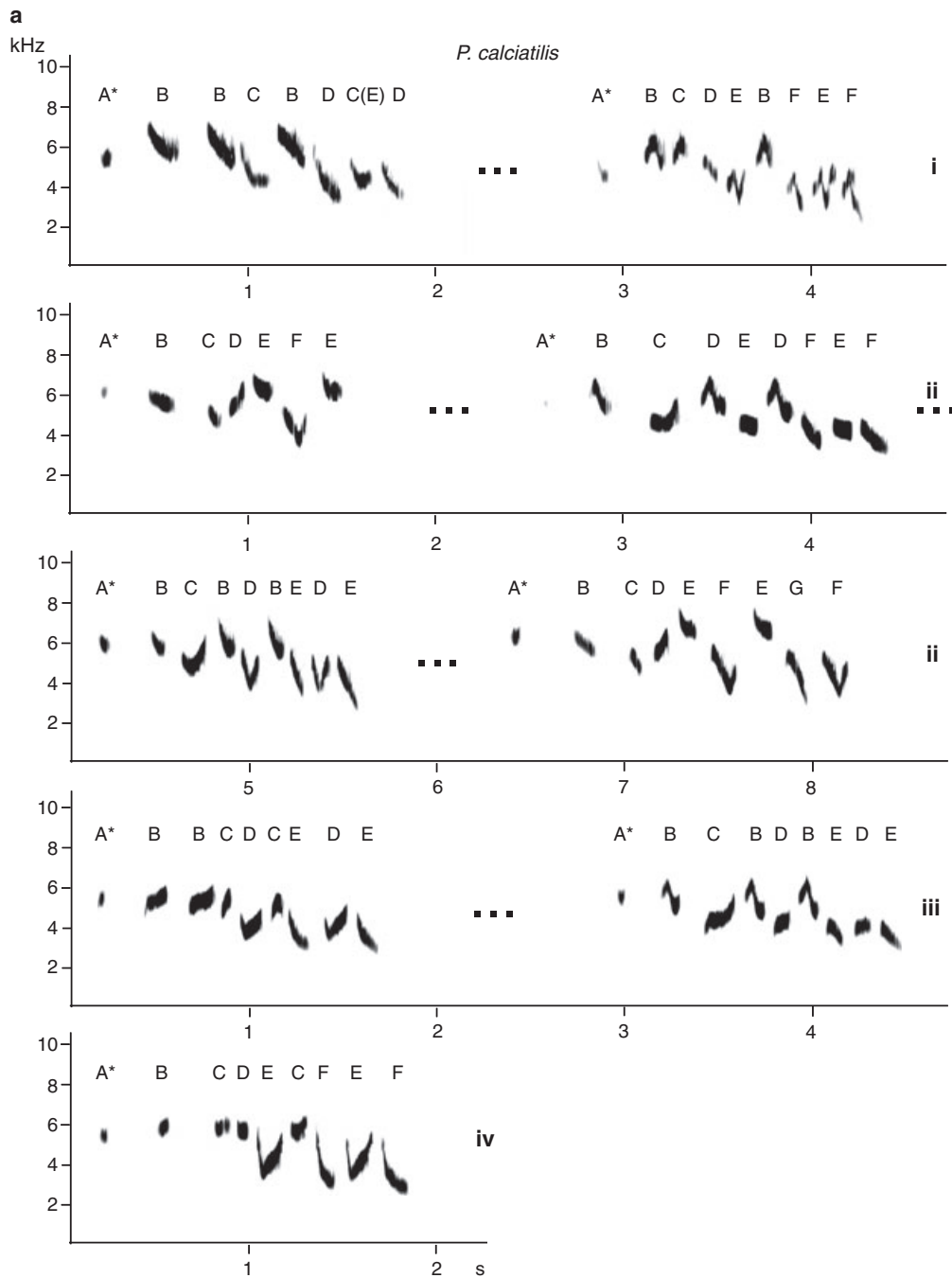


Figure 4. Songs of: (a) *Phylloscopus calciatilis* from (i) Hin Namno National Protected Area, mid/late February (P. Davidson) and (ii–iv) Phong Nha-Ke Bang National Park, Quang Binh Province, Vietnam, mid-April (P. Alström); (b) *Phylloscopus ricketti* from (i) Fujian Province, China, mid-May (G. J. Carey); (ii) Sichuan Province, China, mid-May (P. Alström); and (iii) Sichuan, China, late April (H. Jännes); (c) *Phylloscopus cantator cantator* from (i) Bhutan, mid-April (P. Holt/The British Library Sound Archive); (ii,iii) Arunachal Pradesh, India, late May and early June, respectively (P. Singh); (iv) Bhutan, mid-April (H. Jännes); and (v) *Phylloscopus cantator pernotus* from Phou Louey (N. Loeuy), Laos, early May (P. Davidson). The letters A, B, C, etc., indicate the syntax as in Supporting Information Table S3; A* refers to ‘introductory element’; the dots between strophes indicate that the pauses have been artificially shortened; the bold letters above the elements in (b) and (c) indicate where an element is repeated, either singly or in ‘syllables’ (cf. Supporting Information Table S3).

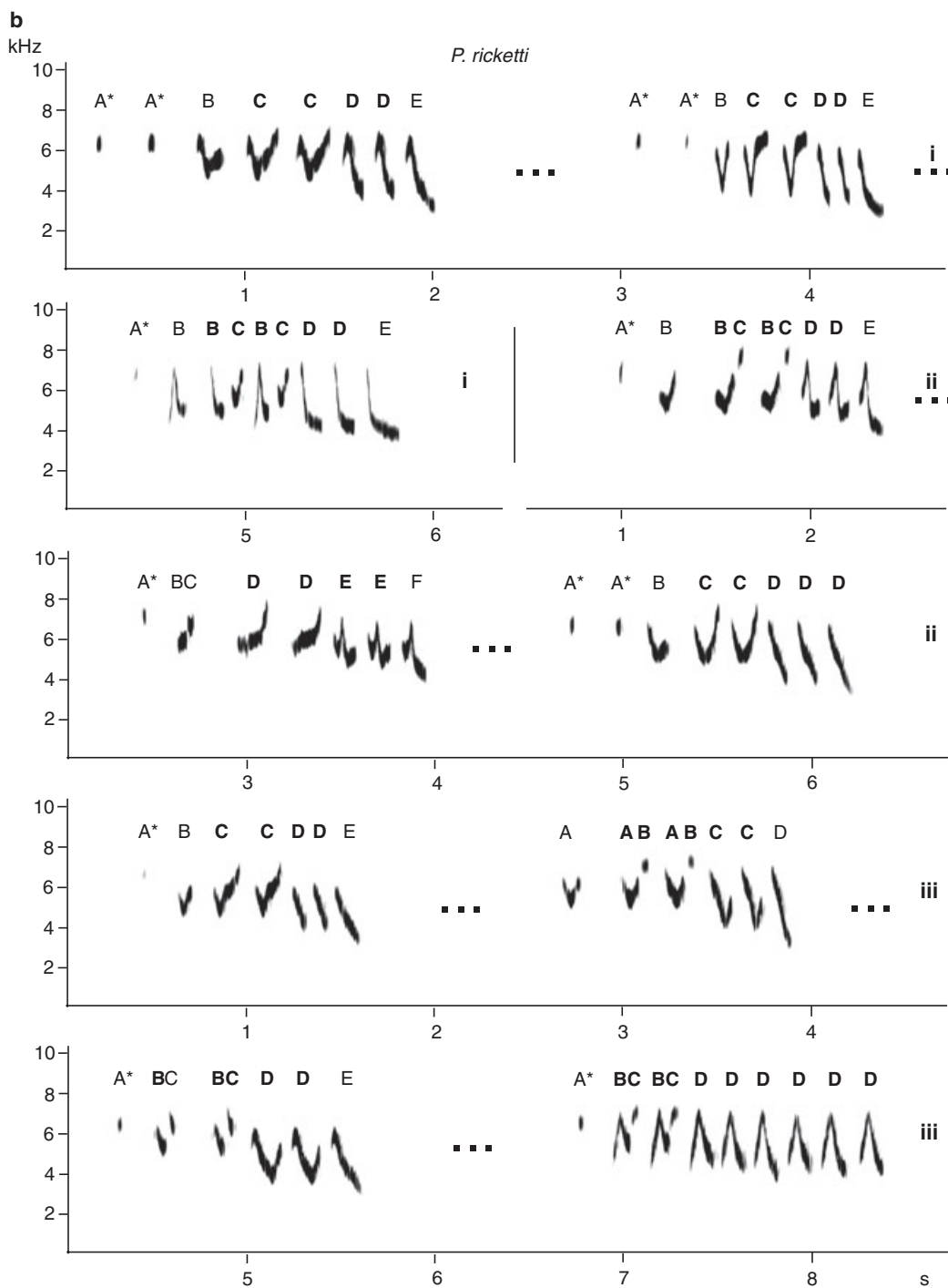


Figure 4. Continued

southern China, in late May 1998, of two singing males resemble the Limestone Leaf Warbler, but are more varied and less structured than birds from Vietnam and Laos, with a higher proportion of doubled elements. Moreover, the strophes have

a broader frequency span, due to a generally higher top frequency and on average a higher number of elements per strophe than our recordings from Vietnam and Laos (Supporting Information Tables S2 and S3; no sonograms shown,

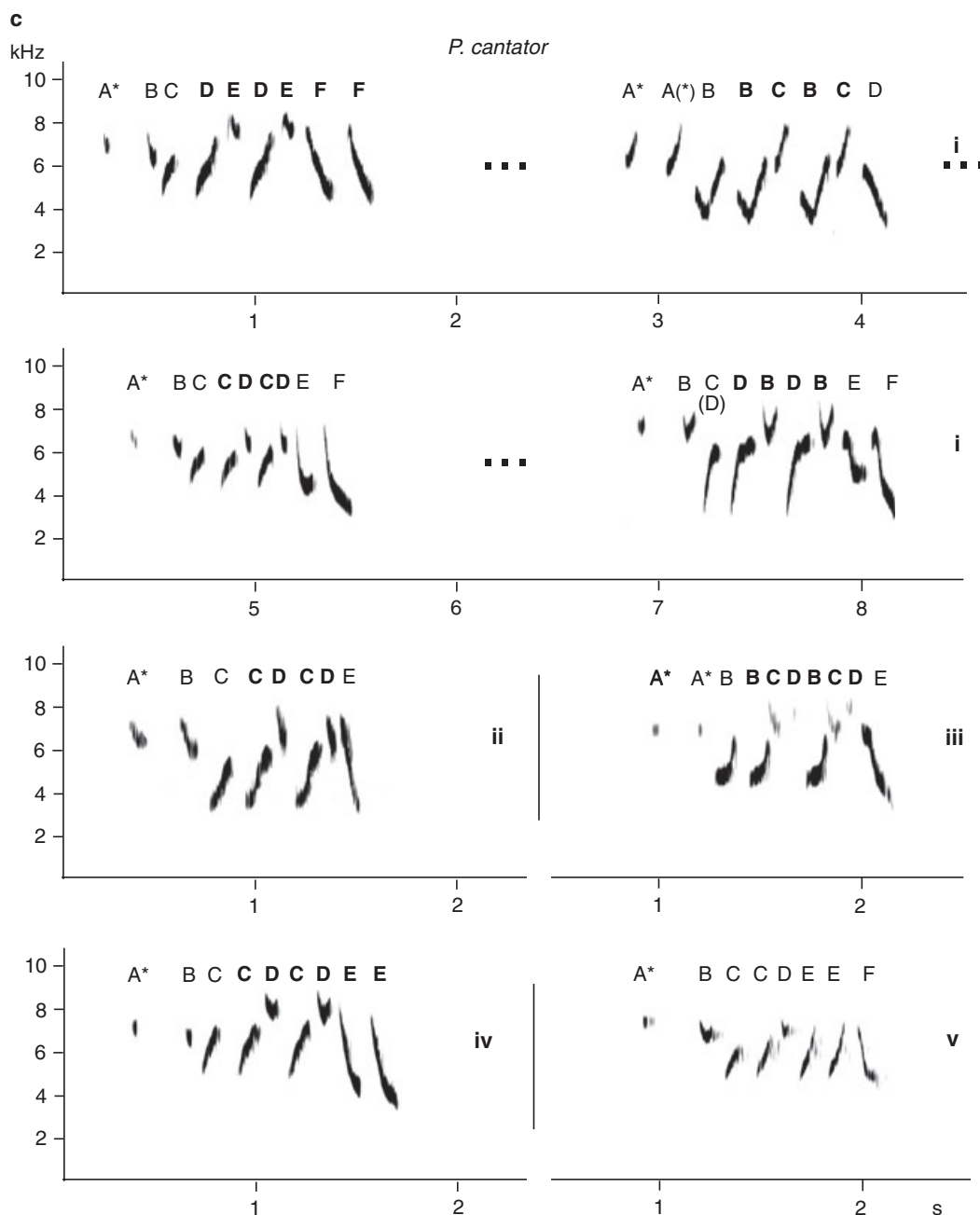


Figure 4. Continued

as quality unsuitable for reproduction). Unfortunately, there are no recordings of birds from the Ha Giang locality where the Limestone Leaf Warbler (based on morphology and DNA) has been found, which is only 200 km from Nong Gang, at a similar latitude, in limestone that on a landscape level appears to be contiguous between these two areas.

The song of *P. ricketti* (Fig. 4b, Supporting Information Tables S2 and S3) is higher-pitched and, as some of the elements are arranged in ‘blocks’, more regular and stereotyped than that of the Limestone Leaf Warbler. It usually has the following syntax: (1) one to two ‘introductory elements’ (marked by A* in Fig. 4b and Supporting Information Table S3); (2) zero to two elements (when

present, often reminiscent of, but not identical to, the main element in (3) (e.g. element B in all strophes of Fig. 4b(i)); (3) a single element given twice (e.g. element C in first two strophes of Fig. 4b(i)) or a two-element syllable (second element higher-pitched than first; e.g. elements BC in third strophe of Fig. 4b(i) or BC in first strophe of Fig. 4b(ii)) given twice (marked in bold in Fig. 4b and Supporting Information Table S3); (4) a different element given twice, lower in pitch than the previous ones (e.g. D in all strophes of Fig. 4b(i); marked in bold in Fig. 4b and Supporting Information Table S3); and (5) one element that usually resembles the pair succeeding it, but falling deeper in pitch and noticeably emphasized (e.g. element E in all strophes of Fig. 4b(i)). Occasionally, a 'block' of notes is repeated.

The song of *P. cantator cantator* (Fig. 4c(i–iv), Supporting Information Tables S2 and S3) is more reminiscent of that of *P. ricketti* than of the Limestone Leaf Warbler, though it is less stereotyped and even higher-pitched than in *P. ricketti*. It is usually built up of: (1) one to two 'introductory elements' (marked by A* in Fig. 4c and Supporting Information Table S3); (2) a single element that is similar to or identical to the main element in (3) (e.g. the first B in the second strophe in Fig. 4c(i)) or, more commonly, two elements of which the second is similar to, but not identical to, the main element in (3) (e.g. BC in all except the second strophes in Fig. 4c(i)); (3) a two-element syllable (second higher-pitched than first) given twice (e.g. DE in first strophe, BC in second strophe, CD in third strophe and DB in fourth strophe in Fig. 4c(i); exceptionally, a three-element syllable, BCD in Fig. 4c(iii); marked in bold in Fig. 4c and Supporting Information Table S3); and (4) one or more commonly two elements, at least the final element falling in pitch and stressed; when two, the elements usually differ from each other (marked in bold in Fig. 4c and Supporting Information Table S3 when identical, e.g. FF in Fig. 4c(i)). The song of *P. cantator pernotus*, for which we have only one song type from one individual, resembles the song of *P. c. cantator*, but with a slightly different syntax (Fig. 4c(v), Supporting Information Table S3).

Although no proper playback experiments have been carried out, songs of both *P. ricketti* and *P. cantator* were played to several males of the Limestone Leaf Warbler at Phong Nha in 2004. These elicited mutually similar curious responses,

but not as aggressive as the response elicited when a song from the local population was played.

Calls

The call of the Limestone Leaf Warbler (Fig. 5a–d) is a short, soft *pi-tsiu*; *pi-tsiu*; *pi-tsu* or similar; the first element can be almost inaudible. When the bird is alarmed, the call is repeated at short intervals. It differs clearly from the call of *P. ricketti* (Fig. 5f–j), which is higher-pitched and comprises a broader frequency range, and frequently consists of multiple elements. We have limited experience with the call of *P. cantator* (only nominate subspecies), but our only recording (Fig. 5e) suggests a close similarity with *P. ricketti* (see Supporting Information Table S4).

Intra-population genetic variation and relationships

Among the eight individuals sequenced of the Limestone Leaf Warbler, we recovered six different mitochondrial cytochrome-*b* haplotypes, which differ from each other by $\leq 0.37\%$ (Table 2). One of these haplotypes (No. 4, Supporting Information Table S1) is shared between individuals at Ha Giang and Phong Nha. A seventh haplotype matching the Limestone Leaf Warbler is represented by two individuals from the Diding Headwater Nature Reserve, Guangxi Province, China (KUNHM 93362 and KUNHM 96954). As mentioned above, one of these individuals appears to be morphologically intermediate between the Limestone Leaf Warbler and *P. ricketti*, whereas the other individual seems to match *P. ricketti* in morphology (see Taxonomy, below). Among the six unquestionable samples of *P. ricketti*, we identified five cytochrome-*b* haplotypes, which are $\leq 0.75\%$ divergent from each other (Table 2). Pairwise cytochrome-*b* distances between the Limestone Leaf Warbler–*P. ricketti*–*P. cantator* haplotypes are considerably higher, 3.35–4.09% (Table 2). As expected, the divergence among these three species in the ODC intron is much lower, 0.89–2.05% (Table 2), reflecting the slower evolution of nuclear DNA relative to mitochondrial DNA.

The cytochrome-*b* haplotypes fall into three clades (Fig. 6a) corresponding to the Limestone Leaf Warbler (including the haplotype from Diding Headwater, Guangxi Province, which is

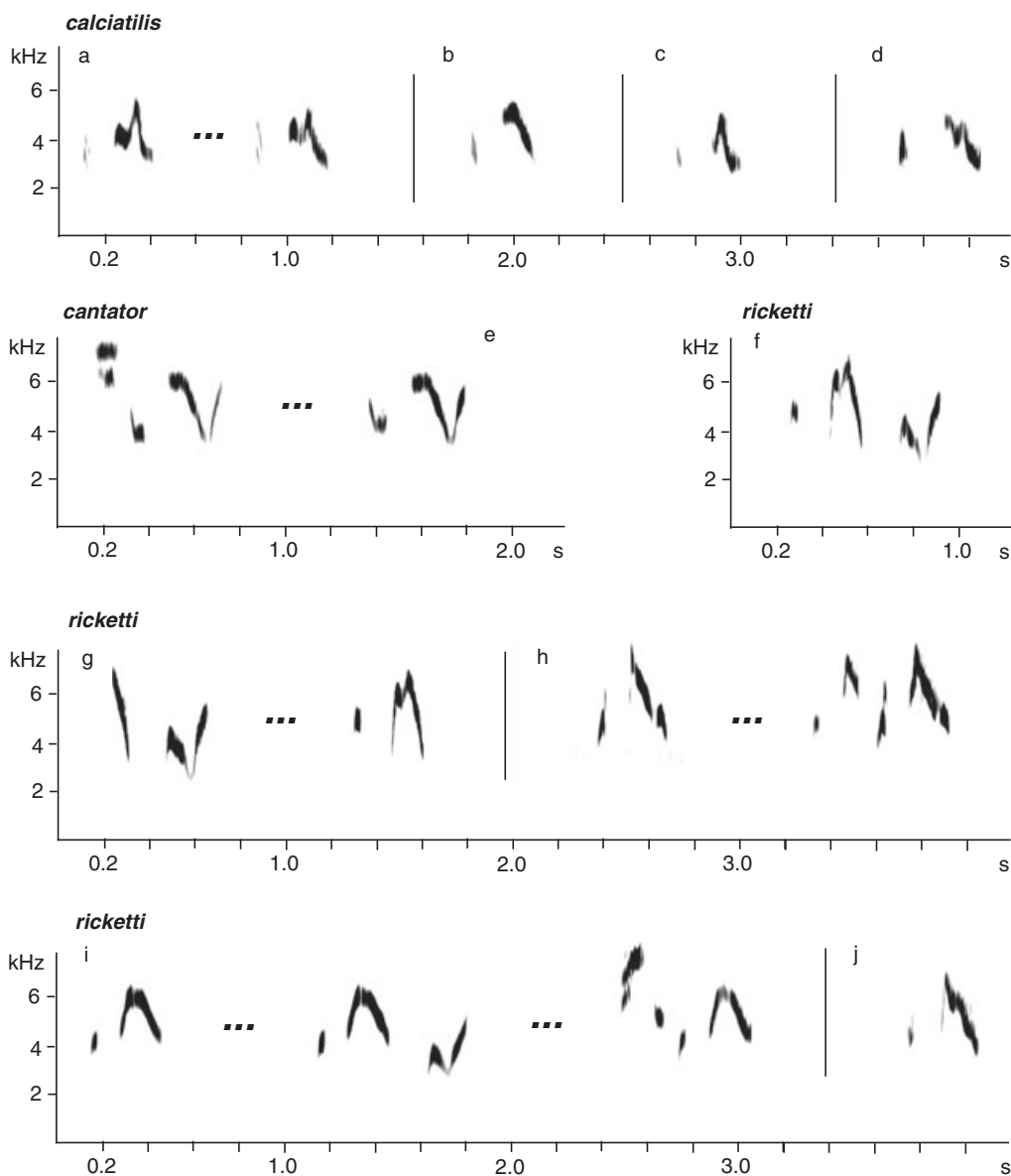


Figure 5. Calls of *Phylloscopus calciatilis*: (a–c) three different individuals at Phong Nha-Ke Bang National Park, Quang Binh Province, Vietnam, mid-April (P. Alström); (d) Hin Namno National Protected Area, mid/late February (P. Davidson); *Phylloscopus cantator cantator*, (e) Arunachal Pradesh, India, early February (P. Alström); *Phylloscopus ricketti*, (f,g,i,j) Sichuan, China, mid-May (different individuals) (P. Alström); (h) Fujian, China, mid-May (G. J. Carey). The dots between calls indicate that the pauses have been artificially shortened.

labelled *calciatilis/ricketti*), *P. ricketti* and *P. cantator*. The Limestone Leaf Warbler and *P. cantator* are inferred to be sister taxa with high MP and ML bootstrap support (92% and 87%, respectively), but with a low BI posterior probability (0.82). Previous studies including a larger number of *Phylloscopus* species (but not the Limestone Leaf Warbler) have suggested *P. ricketti* and *P. cantator* to be sister

taxa (Olsson *et al.* 2005, Johansson *et al.* 2007, Päckert *et al.* 2009). The ODC tree (Fig. 6b) has the same topology as that recovered for cytochrome-*b*, with reasonably strong BI and ML support (0.94% and 70%, respectively), but no MP support. A combined analysis of both loci (Fig. 6c) infers the same topology, with strong support (BI 1.00, ML and MP 95%) for a sister

Table 2. Divergence in the mitochondrial cytochrome-*b* (below/on diagonal, regular font; in parentheses mean \pm sd; number of pairwise comparisons) and nuclear ornithine decarboxylase (ODC) introns 6–7 (above diagonal, bold) between different haplotypes/alleles of *Phylloscopus calciatilis*, *Phylloscopus ricketti* and *Phylloscopus cantator cantator*.

	<i>Phylloscopus calciatilis</i>	<i>Phylloscopus ricketti</i>	<i>Phylloscopus cantator</i>
<i>Phylloscopus calciatilis</i>	0.09 ^a –0.37 (0.22 \pm 0.0008; 15)	0.89	2.05
<i>Phylloscopus ricketti</i>	3.35–3.90 (3.70 \pm 0.001; 30)	0.09 ^b –0.75 (0.42 \pm 0.002; 10)	1.49
<i>Phylloscopus cantator</i>	3.44–3.62 (3.54 \pm 0.0007; 6)	3.78–4.09 (3.93 \pm 0.001; 5)	–
<i>Phylloscopus calciatilis</i> / <i>Phylloscopus ricketti</i>	0.09–0.28 (0.17 \pm 0.0007; 6)	3.65–3.90 (3.79 \pm 0.0009; 5)	3.44

All values are uncorrected-p distances indicated as percentages. *Phylloscopus calciatilis*/*Phylloscopus ricketti* represents two individuals with the same haplotype from Guangxi Province, China, with uncertain identity (see Supporting Information Table S1).

^aSame haplotypes in two individuals from Phong Nha, Central Annam, Vietnam, and in one individual from Phong Nha and one individual from Ha Giang Province, Vietnam.

^bOne individual from Sichuan Province, China, and an individual from Guangxi Province, China, have the same cytochrome-*b* haplotype.

relationship between the Limestone Leaf Warbler and *P. cantator*.

DISCUSSION

The Limestone Leaf Warbler shows significant, broadly congruent differences in morphometrics, vocalizations and DNA from its close relatives *P. ricketti* and *P. cantator*, and we name it

Phylloscopus calciatilis, sp. nov.
Limestone Leaf Warbler

Holotype

The Natural History Museum, Tring, UK, No. 1997.7.1, adult male, Phong Nha-Ke Bang National Park, Quang Binh Province, Central Annam, Vietnam (c. 17°24'N, 106°23'E), 29 June 1996, collected by Nguyen Cu, Jonathan C. Eames and Le Trong Trai.

Paratype

The Natural History Museum, Tring, UK, No. 1997.7.2, juvenile male; identical locality, date and collectors as detailed for the holotype.

Additional specimens

(1) Swedish Museum of Natural History, No. 20086510, adult male, Thai An Commune, Quan Ba district, Ha Giang Province, East Tonkin, Vietnam, 31 May 2008, collected by Ulf Johansson, Peter Nilsson, Nguyen Minh Tam, Le Manh Hung, Tran Thang Tu, Le Kim Ngan, Lenh Xuan Nien and Hang Van Minh.

(2) Field Museum of Natural History No. 79799, adult female, Pa Ham, Lai Chau Province, West Tonkin, Vietnam, 7 April 1929, collected by J. Delacour.

Diagnosis of species

Resembles *P. ricketti* (Fig. 2) and is doubtfully separable from this species in plumage. In our limited sample of males the following measurements are non-overlapping: bill-length/wing-length ratio, length of primary number 4 and length of primary number 9 relative to the other remiges (Figs 2 and 3, Table 1). However, it should be noted that larger sample sizes might reveal overlap in morphometrics, and we cannot say whether these differences hold for females. Song and call are diagnostic (Figs 4 and 5, Supporting Information Tables S2 and S4). Cytochrome-*b* sequences are diagnostic at the population level (minimum uncorrected-p divergence from *P. ricketti* and *P. cantator* 3.35% and 3.44%, respectively), although presumed introgression may render this useless at the individual level.

Description of species

As the holotype and paratype are in a rather poor state, the description is based primarily on two adult birds caught in Phong Nha-Ke Bang in 2004, which had rather fresh plumage with slightly worn wing and tail feathers (Fig. 2c). Lateral crown-stripes blackish throughout length: very marginally paler and more diffuse near bill, as a result of faint greenish infusion. Median crown-stripe well demarcated, greenish-yellow throughout length. Supercilium prominent, yellow with faint greenish

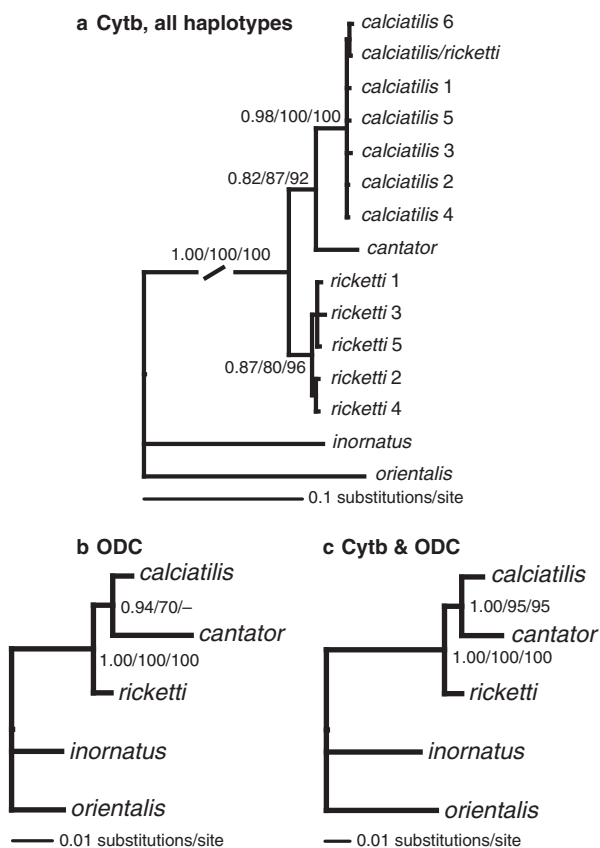


Figure 6. Relationships among *Phylloscopus calciatilis*, *Phylloscopus cantator cantator* and *Phylloscopus ricketti* (with outgroups *Phylloscopus inornatus* and *Phylloscopus orientalis*) based on (a) cytochrome-*b* sequences (1038 bp); (b) ODC introns 6–7 sequences (748 bp); and (c) concatenated cytochrome-*b* and ODC introns 6–7 sequences (in total 1.8 kbp). All trees estimated by Bayesian inference, with support values shown next to the nodes in the order (from left to right): posterior probability, maximum likelihood bootstrap and parsimony bootstrap (in (a), support only shown for the primary clades). The samples pertaining to the haplotype numbers are given in Supporting Information Table S1.

tinge (slightly less greenish-tinged than median crown-stripe). Eye-stripe on lores and upper ear-coverts well defined, blackish with faint green tinge (marginally paler than lateral crown-stripes). Rest of ear-coverts yellow, with faint greenish tinge (somewhat intermediate between median crown-stripe and supercilium), and a slightly contrasting thin yellow crescent below the eye. Mantle, scapulars, back, rump and uppertail-coverts rather bright greyish-green, slightly brighter on rump and uppertail-coverts. Throat, breast and belly bright yellow, sides of breast slightly greenish-tinged; flanks and

undertail-coverts marginally paler than rest of underparts and faintly greenish-tinged. Lesser coverts rather bright greyish-green, like upperparts. Median and greater coverts, remiges, rectrices, primary coverts and alula dark brown-grey, with rather bright greyish-green outer edges (also inner edges on median coverts); wings and tail contrast only slightly with upperparts. Five outer greater coverts have c. 1.5–2-mm-broad yellowish-white or whitish-yellow tips to outer webs, forming a narrow pale wing-bar. Two outermost median coverts have narrow, diffuse greenish-yellow tips, forming a very indistinct paler bar. Underwing-coverts pale yellow, brighter yellow along edge of hand. Inner webs of remiges have whitish edges. Inner webs of rectrices 4–6 (numbered centrifugally) have narrow whitish edges (c. 1 mm on 6th, c. 0.5 mm on 5th, even narrower on 4th). Iris, dark grey-brown. Upper mandible blackish with thin pale orange cutting edge, lower mandible pale orange. Tarsus, toes and claws pale grey-buff.

The juvenile resembles the adults, but has looser, fluffier plumage (as is generally the case in passerines) and, possibly as a result of this, slightly less intense yellow underparts. It has marginally whiter and more clear-cut pale tips to the greater coverts, and probably has slightly more green admixed on the anterior part of the lateral crown-stripes (difficult to judge with certainty due to the imperfect state of the specimen).

Measurements and wing formula of types: *holotype*: wing-length 52 mm, bill-length 13.8 mm (other measurements and wing formula not possible to take); *paratype*: wing-length 54.5 mm, tail-length 37.0, bill-length 13.9 mm, bill-width 2.7 mm; wing point P6 = P7, P9 –8.5 mm (\approx secondaries), P10 –25.5 mm (9 mm > primary coverts), P8 –1 mm, P5 –1 mm, P4 –3 mm, P3 –5.5 mm, P2 –7 mm, P1 –7.5 mm. Emarginations on P5–P8.

Etymology

The name *calciatilis* means ‘dwelling on limestone’, referring to the species’ habitat.

Taxonomy

The taxonomic rank of *calciatilis* is debatable, as it is essentially allopatric with its closest relatives, *ricketti* and *cantator* (including *pernotus* in the following discussion) (Fig. 1). Under the biological

species concept (Mayr 1942) the treatments of *ricketti* and *cantator* are open to discussion, as their breeding ranges are non-overlapping. The two latter taxa are usually treated as specifically different (e.g. Ticehurst 1938, Williamson 1967, Watson *et al.* 1986, Sibley & Monroe 1990, Dickinson 2003, Bairlein *et al.* 2006), although Cheng (1987) treats them as conspecific (without giving any reasons). We have taken an integrative approach to this question, analysing morphology, vocalizations, mitochondrial and nuclear DNA, and geographical distributions of all three taxa.

In plumage, *calciatilis* and *ricketti* are not safely distinguishable, whereas *cantator* is markedly different. Measurements and wing formula separate with very little ambiguity male *calciatilis* in Laos and Vietnam (sampled during the breeding season over a range of c. 600 km) from male *ricketti* from the Chinese breeding grounds (over a range of at least 1400 km). Although there are specimens from Thailand and Indochina matching Chinese *ricketti*, their dates all fit those of migrants or winter visitors (Supporting Information Table S1). Three of our four studied females also fit this pattern. However, one of these (KUNHM 96954) agrees with *ricketti* in measurements and wing formula, but has a *calciatilis* cytochrome-*b* haplotype (see below). The fourth female (KUNHM 93362) is somewhat intermediate between *calciatilis* and *ricketti*. Its bill-length/wing-length ratio matches male *ricketti*, but differs without overlap (smaller) from male *calciatilis*. This ratio can be assumed to be fairly similar in males and females of the same taxon, although on average larger in females of both taxa, given that in *Phylloscopus* species in general, females have marginally longer bills in relation to wings (Alström & Olsson 1992, Cramp 1992). The wing- and tail-length of this specimen are the same as the mean values for male *calciatilis*, which is larger than expected for a female *calciatilis* and more in agreement with what would be expected for female *ricketti*, as females are usually smaller overall than males in *Phylloscopus* (Ticehurst 1938, Williamson 1967, Cramp 1992). In contrast, in wing formula (P4, P5, relative length of P9) KUNHM 93362 matches male *calciatilis*, and it also has a *calciatilis* cytochrome-*b* haplotype (see below). Both these specimens are from the Diding Headwater Nature Reserve, Guangxi Province, China, where the ranges of *calciatilis* and *ricketti* might meet, and it seems possible that they are hybrids (see below). *Phylloscopus cantator* is

somewhat intermediate between *calciatilis* and *ricketti* in size, whereas its wing formula is more similar to that of *calciatilis*.

Wing-length and, especially, wing formula may have shortcomings as taxonomically informative characters, due to the potential correlation with the degree of seasonal movement; sedentary birds generally have shorter and rounder wings than seasonal migrants (Ticehurst 1938, Cramp 1992, Marchetti *et al.* 1995). Thus, the differences noted could potentially reflect an adaptation to different lifestyles, and a larger sample size (especially from south China) might reveal the variation in morphology to be clinal.

The vocalizations of *ricketti* are consistent among all localities sampled within its breeding range, spanning a distance of over 1400 km, and are congruent with the morphometrics from the same areas (and DNA, see below). Songs of *calciatilis* appear to be slightly more variable, with small differences between localities, but are still consistently and diagnosably different from those of *ricketti*, across a range of at least 500 km. In the Phong Nha–Hin Namno area, there is perfect correspondence between vocalizations and morphometrics (and DNA, see below). However, from northern Vietnam, two areas in central Laos, and southern China, congruence between vocalizations and morphometrics can only be indirectly inferred. From three areas (Nadi/Sayphou Loyang, Cuc Phuong and Nong Gang) we have sound recordings matching *calciatilis* but no morphological (or DNA) data, and from two other localities (Pa Ham and Ha Giang) we have morphological data (and DNA from the second locality) matching *calciatilis*, but no sound recordings. From the Diding Headwater in Guangxi Province, where we have some evidence that *calciatilis* might be present, we have no sound recordings. Our only recording of the song of the isolated population of *cantator* from northern Laos (*pernotus*) differs slightly from our recordings from the Himalayas, although they are essentially similar, but distinct from those of both *calciatilis* and *ricketti*. The vocalizations of *calciatilis* are the most divergent of the three taxa analysed. Subjectively, the differences in song, especially between *calciatilis* and the two others, are at least as pronounced as between some sympatric species of *Phylloscopus* and *Seicercus* warblers (e.g. Western Crowned Warbler *Phylloscopus occipitalis* vs. Blyth's Leaf Warbler *Phylloscopus reguloides*; Blyth's Leaf Warbler vs. Davison's Leaf Warbler *Phylloscopus davisoni*;

Martens's Warbler *Seicercus omeiensis* vs. Grey-crowned Warbler *Seicercus tephrocephalus*; Päckert et al. 2004, 2009, Rasmussen & Anderton 2005).

The within-taxon cytochrome-*b* divergences are very slight, and in *calciatilis* one haplotype is shared between Ha Giang and Phong Nha, c. 600 km apart. In contrast, the divergences among the taxa are on average more than an order of magnitude higher. This is concordant with a study of intraspecific vs. interspecific differences in 260 species of North American birds (Hebert et al. 2004). The differences between *P. calciatilis*, *P. cantator* and *P. ricketti* are fairly equal in all pairwise comparisons. The values are at the low end of differences between other *Phylloscopus* and *Seicercus* taxa that are currently classified as separate species, but exceed those between taxa nowadays generally regarded as subspecies (Helbig et al. 1995, 1996, Hansson et al. 2000, Martens et al. 2004, Olsson et al. 2004, 2005, Päckert et al. 2004, 2009). The cytochrome-*b* data are congruent with morphology in all samples except one female specimen from Guangxi Province (KUNHM 96954), which has a *calciatilis* cytochrome-*b* haplotype, but in meristic data is closer to *ricketti* (see above) and another female from the same locality (KUNHM 93362) with ambiguous morphometrics and a *calciatilis* cytochrome-*b* haplotype. However, our sample is geographically limited, and does not include the type locality of *ricketti*. Both the mitochondrial and the nuclear data suggest that *calciatilis* is more closely related to *cantator* than to *ricketti*.

Our DNA samples from the presumed breeding ranges of *calciatilis* and *ricketti* leave a hiatus of more than 600 km in southern China, for which we have only late September samples from a single locality, namely the Diding Headwater Nature Reserve in Guangxi Province. At this locality we found two specimens with different *ricketti* cytochrome-*b* haplotypes and two others with the same *calciatilis* haplotype. As noted above, one of the latter specimens matches *ricketti* in measurements and wing formula, whereas the other individual appears to be intermediate between *calciatilis* and *ricketti* in morphology. These observations could have several causes: (1) the two specimens that appear to exhibit conflict between molecular markers and morphometrics are indeed *calciatilis* (in which case the characteristics suggested to distinguish males of *calciatilis* from *ricketti* do not hold for females); (2) introgression of mitochondrial DNA from *calciatilis* to *ricketti* has

occurred as a consequence of hybridization; or (3) incomplete sorting of ancestral polymorphisms. Based on our present knowledge, we cannot eliminate the possibility that they are in fact *calciatilis*. Although we do not know whether these specimens represent a local breeding population or migrants, the observations of birds matching *calciatilis* in morphology and DNA at Ha Giang c. 90 km to the southwest and recordings of songs that match *calciatilis* from Nong Gang c. 120 km to the southeast suggest that at least *calciatilis* might breed at Diding Headwater. Further fieldwork is required.

Similarly, we cannot eliminate the possibility of introgressive hybridization. Price and Bouvier (2002) concluded that complete loss of F1 fertility in birds generally takes millions of years. Hybridization has been proven once and suspected several times between the sympatric Wood Warbler *Phylloscopus sibilatrix* and Western Bonelli's Warbler *Phylloscopus bonelli* (Dietzen et al. 2007 and references therein), which are genetically much more divergent (8.2–8.5%, uncorrected-p; Helbig et al. 1995). Moreover, introgressive hybridization (albeit male-biased) has been shown to occur regularly in a hybrid zone between the parapatric Common Chiffchaff *Phylloscopus collybita* and Iberian Chiffchaff *Phylloscopus ibericus* (now generally treated as separate species, but previously considered conspecific, the latter under the name *Phylloscopus collybita brehmii*). Accordingly, it would not be surprising if *calciatilis* and *ricketti* (and *cantator*) are not yet intrinsically reproductively isolated. If their ranges indeed meet in southern China, it would not be surprising to find a hybrid zone there. Hybridization between *calciatilis* and *cantator* might also occur in the limited region in northern Indochina where these two taxa potentially meet, although there seems to be greater ecological separation between these taxa in habitat usage (see below).

The third explanation for the observed pattern, namely incomplete lineage sorting, is least likely. In theory, any one of *calciatilis*, *ricketti* or *cantator* could retain shared ancestral haplotypes with any of the other species (Pamilo & Nei 1988, Wu 1991, Hudson 1992, Moore 1995). However, the *calciatilis* haplotype in question is very similar to the other *calciatilis* haplotypes, which is not to be expected for an ancestral polymorphism, in view of the 3.35–3.90% difference between *calciatilis* and *ricketti*.

On balance, we favour treatment of *calciatilis*, *cantator* (including *pernotus*) and *ricketti* as separate species based on the congruent differences between them in multiple independent characteristics, and their apparent long independent evolutionary histories. In particular, we consider the vocal differences between *calciatilis* and the other two taxa to be too pronounced to treat *calciatilis* as a subspecies. If *calciatilis* were to be given a subspecific rank, according to our mitochondrial and nuclear DNA data, it would be as a subspecies of the dissimilar (in plumage) *P. cantator* rather than of the very similar *P. ricketti*. Alternatively, both *calciatilis* and *ricketti* could be treated as subspecies of *P. cantator*.

Distribution and status

The known distribution of *P. calciatilis* is detailed in Figure 1. In the Phong Nha sector of the Phong Nha-Ke Bang National Park in Quang Binh Province, Central Annam, Vietnam, it is common in suitable habitat, at least between mid-February and late June, and breeding has been confirmed there both in 1996 and again in 2004. In addition, one bird was seen in a mixed species flock at Thuong Hoa Commune, Minh Hoa District, Quang Binh Province (17°35'N, 105°58'E), on 25 June 1996, and two birds in a mixed species flock at the same location the following day. In the Thai An Commune, Quan Ba district, Ha Giang Province, East Tonkin, Vietnam, four adult birds were caught in late May and early June 2008 (U. Johansson & P. Nilsson unpubl. data). The song of one male was recorded in Cuc Phuong National Park, Ninh Binh Province, East Tonkin, Vietnam, in mid-March 2003. One female specimen with measurements matching *P. calciatilis* was collected at Pa Ham, Lai Chau Province, West Tonkin, Vietnam, on 7 April 1929 (FMNH 79799), and it seems plausible that the species breeds there.

At the Nadi limestone and Sayphou Loyang, Bolikhamxai Province, north/central Laos, *P. calciatilis* was common based on identification of songs (a minimum of nine singing birds per day at the former, heard and seen seven times, over both days in the latter area) during 4–9 May and 12–13 May 1995, respectively (R.J.T.; Duckworth *et al.* 1998). At Hin Namno National Protected Area, Khammouan Province, Laos, *P. calciatilis* was recorded on 7 days in February 1998 during a 20-day survey period, of which 13 were spent in

suitable habitat; on three dates up to at least 12 birds were recorded (P.D.; Walston & Vinton 1999).

Large parts of these karsts are almost inaccessible due to very steep terrain and a lack of trails, so the total population must be much higher than the actual counts, even in the small sampling sites, which represent only a small fraction of available (largely unsurveyed) habitat. There are large areas of karst within central and northern Laos and Vietnam, which have not been well surveyed to determine whether the species is present; in some of these karsts, within the latitudinal and longitudinal range of sites known to hold *P. calciatilis*, there are eight records of indeterminate *P. calciatilis/P. ricketti*.

Two birds with songs similar (but not identical) to Indochinese *P. calciatilis* were recorded on 26 May 1998 in the Longshan section of the Nong Gang Nature Reserve, Guangxi Province, China (G. J. Carey unpubl. data). This, in combination with the *P. calciatilis* cytochrome-*b* haplotypes found at nearby Diding Headwater Nature Reserve, Guangxi, China, strongly suggests, as would be expected, that *P. calciatilis* breeds in the extensive karst of Guangxi, although the species' northern and eastward limits still require further work to define.

Although four specimens of *P. ricketti* were verified from Laos, Vietnam and Thailand (Supporting Information Table S1), all verified summer and spring records of *P. ricketti* are from China (Fig. 1, Supporting Information Table S1). The southernmost confirmed records of *P. ricketti* from China are three September records from the Diding Headwater Nature Reserve, Guangxi Province. Two females of indeterminate identity, with *P. calciatilis* cytochrome-*b* haplotypes, have also been collected at the same locality and time (see above). Based on our present knowledge, we cannot say whether these three *P. ricketti* were on migration from breeding grounds further north, or whether they represent local breeders. As Diding Headwater is only *c.* 90 km from Ha Giang and 120 km from Nong Gang, where *P. calciatilis* almost certainly breeds, and karst formations are largely contiguous between these three areas, it seems very plausible that *P. calciatilis* also breeds at Diding. The southernmost breeding season record of definite *P. ricketti* is from Nanling (Babao Shan; *c.* 24°42'N, 113.03'E), where two singing birds were recorded on 1 May 2007 (Van Lu) and five singing birds in late April 2009 (G. J. Carey unpubl. data). Clearly, further research is needed

to establish the distributions of both species in southern China and to establish the nature of the boundary between them.

It seems likely that *P. calciatilis* is resident. Consistent with resident status, unidentified (silent) *P. calciatilis*/*P. ricketti* were found commonly in the karsts of Hin Namno National Protected Area in December 1995 to January 1996 and in Phong Nha-Ke Bang National Park in September–October 1998 (Timmins & Khounbolin 1996, Timmins *et al.* 1999). Adding these to the confirmed identifications, there are therefore plausible records of *P. calciatilis* from the Hin Namno-Phong Nha karsts in all months except March, May, August and November (when there have been no suitable surveys). However, determining the extent of dispersal outside the breeding range will be difficult because of the presumed widespread presence in these parts of Southeast Asia of *P. ricketti* (e.g. Robson 2000, Bairlein *et al.* 2006, confirmed in the present study). However, in Indochina, indeterminate *P. calciatilis*/*P. ricketti* have not often been found away from limestone karst areas in autumn or winter, despite extensive surveys of non-limestone areas. We have studied four individuals matching *P. ricketti* in morphometrics and wing formula from Thailand, Vietnam and Laos between early October and late March (Supporting Information Table S1). As a consequence of the recognition of *P. calciatilis* as a distinct taxon, the records published as *P. ricketti* from throughout that species' formerly assumed breeding and non-breeding range need to be re-evaluated.

Habitat

Phylloscopus calciatilis occurs in broadleaved evergreen and semi-evergreen forest associated with limestone karst mountains. At Phong Nha-Ke Bang it was only found in the forest on the mountains or, uncommonly, in valleys between karsts, frequently on steep slopes, between 80 m and at least 200 m; it was never encountered in the forest in the plains away from the hills. At Hin Namno National Protected Area *P. calciatilis* was found in old-growth and mature secondary semi-evergreen forest on limestone karst, between 280 and 460 m. Most records came from taller forest (15–20-m-tall trees) that was generally restricted to valley floors in limestone karst. However, this may have been an artefact of trails in limestone karst areas frequently following valley floors, the 'paths of

least resistance' in such habitat. In the Nadi and Sayphou Loyang areas, Laos, it was found in dry evergreen and semi-evergreen forest and secondary growth on limestone over 600–1000 m. In the Thai An Commune, Ha Giang, East Tonkin, it was observed in tall secondary scrub on a well-vegetated slope within a karst landscape at approximately 1200 m.

The two males with songs matching *P. calciatilis* in the Nong Gang Nature Reserve, Guangxi Province, China, were observed in an area of dense secondary evergreen broadleaved forest with rocky limestone cliffs at an altitude of 250 m (G. J. Carey unpubl. data).

Due to the difficulty of interpretation of previous data relating to *P. ricketti*, we can only be certain of its habitat usage in the breeding areas of Emei Shan, Wuyi Shan and Qinling Shan, where *P. ricketti* occurs in broadleaved forest at rather low elevation in mountains (mainly *c.* 700–1200 m). At least locally, it occurs on karst limestone (for example, the type locality in Wuyi Shan in Fujian Province, China, is renowned for its karst), and all verified localities (Fig. 1, Supporting Information Table S1) within the breeding range of *P. ricketti* appear to have topography typical of limestone terrain, at least nearby, although other terrain types are also present (determined from remote imagery available on Google Earth, NASA Shuttle Radar Topography Mission elevation datasets, and geological maps from the China Geological Survey). *Phylloscopus cantator* also breeds in broadleaved forest in the Indian subcontinent at 300–2000 m (Rasmussen & Anderton 2005, Bairlein *et al.* 2006) and in northern Laos at 550–1000 m (Tizard *et al.* 1997, Davidson 1998). We are not aware of *P. cantator* breeding in karst limestone, and at least the Lao sites are remote from such habitat.

In summary, *P. calciatilis* and *P. ricketti* have at least partly similar habitat preferences, whereas *P. cantator* appears to differ to a greater extent in habitat preference.

Breeding

At Hin Namno National Protected Area, birds were holding territory in mid-February, the time when the surveys commenced, generally singing from the mid-canopy or canopy of taller trees. Most territorial individuals responded rather vigorously to tape playback of song of *P. calciatilis*. In mid-April 2004 at Phong Nha-Ke Bang, males

were singing, although the song activity was considered to be lower than would be expected at the beginning of the breeding season. When exposed to playback of song of *P. calciatilis*, they always responded strongly. One male was seen carrying food on 15 April, and thus presumably had young. The fledged juvenile paratype was collected on 29 June. At the Nadi and Sayphou Loyang Limestone areas, during 4–13 May 1995 the birds were probably holding breeding territories: they were seen on successive days in the same patches of forest and were calling and singing frequently. *Phylloscopus ricketti* sings on the breeding grounds from at least the last week of April, although there are specimens from the type locality in Fujian Province from early April onwards.

Behaviours

When agitated by playback, males raised their crown-feathers and flicked their wings slightly, giving slightly accelerated song strophes and frequent bouts of persistent calling.

Moult

The holotype, which is an adult, collected on 29 June, is in active body and wing moult, with the inner primaries being replaced; the tail is probably in moult (only two old feathers left). The paratype collected at the same locality on the same day, which is a juvenile, has not yet commenced the post-juvenile moult. The two birds caught in Phong Nha-Ke Bang in mid-April had rather fresh head and body plumage and slightly more worn wings and tail, indicating that they had gone through a partial pre-breeding moult earlier in the year. The four birds caught in late May–early June at Ha Giang had moderately to rather heavily worn plumages (based on photos and one specimen). At least one bird had commenced the moult of the innermost two primaries.

Conservation

The conservation status of *P. calciatilis* depends largely on the status of its habitat, because it is too small to be hunted specifically, and does not behave in a way that is likely to make bycatch (through hunting of other species) a significant problem. There are large areas of karst within its known range: in the known areas, birds are clearly

at reasonably high densities, and accordingly a large total population size seems likely. The terrain is unfavourable for industrial agriculture in any form or for any other type of landscape-level conversion. However, in marginal and some internal parts of each karst block, piecemeal clearance for subsistence agriculture and collection of wood for fire and timber is reducing the extent and 'quality' of forest patches. If the species is dependent upon the stands of taller forest in karst, its population may be declining. Without study of habitat use it is impossible to say. Even if it is dependent upon such forest, which in some areas is experiencing intense local pressure, a flight over much of the karst in central Laos (but not including Hin Namno National Protected Area) in November 2008 by J.W.D. revealed many large areas of reasonably tall forest remaining within the limestone karst formations.

P.A. is most grateful to Le Van Bao for his invaluable assistance during P.A.'s visit to Phong Nha Ke Bang. We are most grateful to Mark Adams, Robert Prys-Jones and the Natural History Museum, Tring, UK, for granting us permission to study specimens and take footpad samples from the type specimens of *P. calciatilis*, and for lending us specimens of *P. ricketti*; to Mark Adams and Nigel Cleere for rechecking some characteristics on the type specimens of *calciatilis*; to John Bates, David Willard and The Field Museum, Chicago, USA for granting access to the collections; to Paul Sweet, Peter Capainolo and the American Museum of Natural History, New York, USA for providing details of holdings, including measurements; James Dean and the Smithsonian National Museum of Natural History, Washington, DC, USA for providing details of holdings, including measurements; to Eric Pasquet and the Muséum National d'Histoire Naturelle, Paris, for loan of specimens of *P. ricketti*; to Peter Nilsson, Ulf Johansson, Per Ericson and the Swedish Museum of Natural History, Stockholm, for tissue and blood samples of *P. calciatilis* and *P. ricketti*; to Norman David for advice on formation of the scientific name; to Mark Robbins, Townsend Peterson and the University of Kansas Natural History Museum for loan of tissue samples and specimens of *P. ricketti*; to Jochen Martens for a tissue sample of *P. cantator*; to Geoff J. Carey, Mike Crosby, Hannu Jännes, Van Lu, Pratap Singh, Tim Woodward and Richard Ranft/British Library National Sound Archive for recordings of *P. ricketti* and *P. cantator*; to Hannu Jännes and Mika Bruun for measurements and photos of one *P. ricketti*; to Ulf Johansson/Swedish Museum of Natural History for photos of *P. calciatilis*; to Dave Farrow for digitizing some of the sound recordings; to Paul J. Leader for photos of *P. goodsoni*; to René Pop for brushing up P.A.'s video cuts to usable photos; to Christopher Reisborg for brushing up

U.O.'s scanned photos of *P. ricketti*; to Yang Liu for distributional data from China and help with obtaining additional sound recordings; to Hanna Göransson and Anders Isaksson, Department of Medical Sciences, Uppsala University, for help with the PCA; and to George Sangster for comments on the manuscript. The surveys in Laos during which the species was found were possible only with the help of numerous people and institutions, as acknowledged in Timmins and Khounboline (1996), Duckworth *et al.* (1998) and Walston and Vinton (1999). P.A. also gratefully acknowledges the Riksmusei Vänners Linnaeus award, which has allowed him to take time off to work on this paper.

REFERENCES

- Akaike, H.** 1973. Information theory as an extension of the maximum likelihood principle. In Petrov, B.N. & Csaki, F. (eds) *Second International Symposium on Information Theory*: 267–281. Budapest: Akademiai Kiado.
- Allen, E.S. & Omland, K.E.** 2003. Novel intron phylogeny supports plumage convergence in orioles (*Icterus*). *Auk* **120**: 961–969.
- Alström, P. & Olsson, U.** 1990. Taxonomy of the *Phylloscopus proregulus* complex. *Bull. Brit. Orn. Club* **110**: 38–43.
- Alström, P. & Olsson, U.** 1992. On the taxonomic status of *Phylloscopus affinis* and *Phylloscopus subaffinis*. *Bull. Brit. Orn. Club* **112**: 111–125.
- Alström, P. & Olsson, U.** 1995. A new species of *Phylloscopus* warbler from Sichuan Province, China. *Ibis* **137**: 459–468.
- Alström, P., Olsson, U. & Colston, P.R.** 1992. A new species of *Phylloscopus* warbler from central China. *Ibis* **134**: 329–334.
- Alström, P., Olsson, U. & Colston, P.** 1997. Re-evaluation of the taxonomic status of *Phylloscopus proregulus kansuensis* Meise. *Bull. Brit. Orn. Club* **117**: 177–193.
- Bairlein, F., Alström, P., Aymí, R., Clement, P., Dyrce, A., Gargallo, G., Hawkins, F., Madge, S., Pearson, D. & Svensson, L.** 2006. Family Sylviidae (Warblers). In del Hoyo, J., Elliott, A. & Christie, D.A. (eds) *Handbook of the Birds of the World*, Vol. 11: 492–709. Barcelona: Lynx Edicions.
- Charif, R.A., Clark, C.W. & Fristrup, K.M.** 2003. *Raven 1.1*. Ithaca, NY: Cornell Laboratory of Ornithology.
- Cheng, T.-H.** 1987. *A Synopsis of the Avifauna of China*. Beijing: Paul Parey.
- Connop, S.** 1995. *Birdsongs of the Himalayas*. Toronto: Turaco Nature Services.
- Cramp, S. (ed.)** 1992. *The Birds of the Western Palearctic*, Vol. 6. Oxford: Oxford University Press.
- Davidson, P. (ed.)** 1998. *A wildlife and habitat survey of Nam Et and Phou Loey NBCAs, Houaphanh Province, Lao PDR*. Vientiane: CPAWM/WCS.
- Dickinson, E.C.** 2003. *The Howard and Moore Complete Checklist of the Birds of the World*. London: Christopher Helm.
- Dietzen, C., Hackenberg, C., Heyne, K.-H., Sauer-Gürth, H., Staudter, H. & Wink, M.** 2007. Genetically confirmed interbreeding between Western Bonelli's Warbler (*Phylloscopus bonelli*) and Wood Warbler (*P. sibilatrix*). *J. Ornithol.* **148**: 85–90.
- Duckworth, J.W., Evans, T.D., Robichaud, W.G., Thewlis, R.M., Timmins, R.J. & Tizard, R.J.** 1998. Bird records from Laos, October 1994–August 1995. *Forktail* **13**: 33–68 (including errata sheet distributed with *Forktail* 14).
- Fuchs, J., Cibois, A., Duckworth, J.W., Eve, R., Robichaud, W.G., Tizard, T. & Van Gansberghe, D.** 2007. Birds of Phongsaly province and the Nam Ou river, Laos. *Forktail* **23**: 22–86.
- Gill, F., Wright, M. & Donsker, D.** 2009. *IOC World Bird Names (version 2.2)*. Available at <http://www.worldbirdnames.org/>.
- Gu, X., Fu, Y.-X. & Li, W.-H.** 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Mol. Biol. Evol.* **12**: 546–557.
- Hansson, M.C., Bensch, S. & Brännström, O.** 2000. Range expansion and the possibility of an emerging contact zone between two subspecies of Chiffchaff *Phylloscopus collybita* ssp. *J. Avian Biol.* **31**: 548–558.
- Hasegawa, M., Kishino, H. & Yano, T.** 1985. Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**: 160–174.
- Hebert, P.D.N., Stoeckle, M.Y., Zemiak, T.S. & Francis, C.M.** 2004. Identification of birds through DNA barcodes. *PLoS Biol.* **2**: 1657–1663.
- Helbig, A.J., Seibold, I., Martens, J. & Wink, M.** 1995. Genetic differentiation and phylogenetic relationships of Bonelli's Warbler *Phylloscopus bonelli* and Green Warbler *Phylloscopus nitidus*. *J. Avian Biol.* **26**: 139–153.
- Helbig, A.J., Martens, J., Seibold, I., Henning, F., Schottler, B. & Wink, M.** 1996. Phylogeny and species limits in the Palearctic chiffchaff *Phylloscopus collybita* complex: mitochondrial genetic differentiation and bioacoustic evidence. *Ibis* **138**: 650–666.
- Hudson, R.R.** 1992. Gene trees, species trees, and the segregation of ancestral alleles. *Genetics* **131**: 509–512.
- Irwin, D., Alström, P., Olsson, U. & Benowitz-Fredericks, Z.M.** 2001. Cryptic species in the genus *Phylloscopus* (Old World leaf warblers). *Ibis* **143**: 233–247.
- Jobb, G.** 2008. *Treefinder*. Munich. Distributed by the author at <http://www.treefinder.de> [version June 2008].
- Jobb, G., von Haeseler, A. & Strimmer, K.** 2004. Treefinder: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol. Biol.* **4**: 18.
- Johansson, U., Alström, P., Olsson, U., Ericson, P.G.P., Sundberg, P. & 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.
- Lanave, C., Preparata, C., Saccone, C. & Serio, G.** 1984. A new method for calculating evolutionary substitution rates. *J. Mol. Evol.* **20**: 86–93.
- Marchetti, K., Price, T.D. & Richman, A.D.** 1995. Correlates of wing morphology with foraging behaviour and migration distance in the genus *Phylloscopus*. *J. Avian Biol.* **26**: 177–181.
- Martens, J., Tietze, D.T., Eck, S. & Veith, M.** 2004. Radiation and species limits in the Asian Pallas's warbler complex (*Phylloscopus proregulus* s.l.). *J. Ornithol.* **145**: 206–222.
- Martens, J., Sun, Y.-H. & Päckert, M.** 2008. Intraspecific differentiation of Sino-Himalayan bush-dwelling *Phylloscopus* leaf warblers, with description of two new taxa (*P. fuscatus*, *P. fulgiventis*, *P. affinis*, *P. armandii*, *P. subaffinis*). *Vert. Zool.* **58**: 233–265.
- Mayr, E.** 1942. *Systematics and the Origin of Species*. Cambridge, MA: Harvard University Press.

- Moore, W.S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49**: 718–726.
- Nylander, J.A.A. 2004. MrModeltest. Version 2.0. Available at <http://www.ebc.uu.se/systzoo/staff/nylander.html>
- Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* **53**: 47–67.
- Olsson, U., Alström, P. & Colston, P.R. 1993. A new species of *Phylloscopus* warbler from Hainan Island, China. *Ibis* **135**: 2–7.
- Olsson, U., Alström, P. & Sundberg, P. 2004. Non-monophyly of the avian genus *Seicercus* (Aves: Sylviidae) revealed by mitochondrial DNA. *Zool. Scr.* **33**: 501–510.
- Olsson, U., Alström, P., Ericson, P.G.P. & Sundberg, P. 2005. Non-monophyletic taxa and cryptic species – evidence from a molecular phylogeny of leaf-warblers (*Phylloscopus*, Aves). *Mol. Phylogenet. Evol.* **36**: 261–276.
- Päckert, M., Martens, J., Sun, Y.-H. & Veith, M. 2004. The radiation of the *Seicercus burkii* complex and its congeners (Aves: Sylviidae): molecular genetics and bioacoustics. *Org. Div. Evol.* **4**: 341–364.
- Päckert, M., Blume, C., Sun, Y.-H., Wei, L. & Martens, J. 2009. Acoustic differentiation reflects mitochondrial lineages in Blyth's leaf warbler and white-tailed leaf warbler complexes (Aves: *Phylloscopus reguloides*, *Phylloscopus davisoni*). *Biol. J. Linn. Soc.* **96**: 584–600.
- Pamilo, P. & Nei, M. 1988. Relationships between gene trees and species trees. *Mol. Biol. Evol.* **5**: 568–583.
- Price, T.D. & Bouvier, M.M. 2002. The evolution of F1 postzygotic incompatibilities in birds. *Evolution* **56**: 2083–2089.
- Rasmussen, P.C. & Anderton, J.C. 2005. *Birds of South Asia: The Ripley guide*. Barcelona: Lynx Edicions.
- Robson, C. 2000. *A Field Guide to the Birds of South-East Asia*. London: New Holland.
- Rodríguez, J., Oliver, L., Marín, A. & Medina, R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* **142**: 485–501.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Saitoh, T., Nishiumi, I., Alström, P., Olsson, U. & Ueda, K. 2006. Deep phylogeographical divergences among far eastern populations of the widespread Arctic Warbler. *J. Ornithol.* **147**: 242.
- Sibley, C.G. & Monroe, B.L. Jr 1990. *Distribution and Taxonomy of Birds of the World*. New Haven: Yale University Press.
- Sorensen, M.D. & Quinn, T.W. 1998. Numts: a challenge for avian systematics and population biology. *Auk* **115**: 214–221.
- Swofford, D.L. 2002. *PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods)*. Version 4.0b10. Sunderland, MA: Sinauer Associates.
- Tavaré, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. *Lect. Math. Life Sci.* **17**: 57–86.
- Thewis, R.M., Duckworth, J.W., Evans, T.D. & Timmins, R.J. 1998. The conservation status of birds in Laos: a review of key species. *Bird Conserv. Int.* **8**(Suppl.): 1–159.
- Ticehurst, C.B. 1938. *A Systematic Review of the Genus Phylloscopus*. London: British Museum.
- Timmins, R.J. & Khounboline, K. 1996. *A Preliminary Wildlife and Habitat Survey of Hin Namno National Biodiversity Conservation Area, Khammouan Province, Lao PDR*. Vientiane: Centre for Protected Areas and Watershed Management/Wildlife Conservation Society.
- Timmins, R.J., Do, T., Trinh, V.C. & Hendrichsen, D.K. 1999. *A Preliminary Assessment of the Conservation Importance and Conservation Priorities of the Phong Nha-Ke Bang Proposed National Park, Quang Binh Province, Vietnam*. Hanoi: Fauna & Flora International.
- Tizard, R.J., Davidson, P., Khounboline, K. & Salivong, K. 1997. *A Wildlife and Habitat Survey of Nam Ha and Nam Kong Protected Areas, Luang Namtha Province, Lao PDR*. Vientiane: CPAWM/WCS.
- Walston, J.L. & Vinton, M.D. (eds) 1999. *A Wildlife and Habitat Survey of Hin Namno National Biodiversity Conservation Area and Adjacent Areas, Khammouane Province, Lao PDR*. Vientiane: WWF Lao Project Office and Wildlife Conservation Society Lao Programme.
- Watson, G.E., Traylor, M.A. Jr & Mayr, E. 1986. Family Sylviidae. In Mayr, E. & Cottrell, G.W. (eds) *Checklist of Birds of the World*, Vol. 11: 3–294. Cambridge, MA: Museum of Comparative Zoology.
- Williamson, K. 1967. *Identification for Ringers 2. The Genus Phylloscopus*, 2nd edn. Tring: British Trust for Ornithology.
- Wu, C.-I. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. *Genetics* **127**: 429–435.
- Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* **39**: 306–314.

Received 23 June 2009;
revision accepted 23 October 2009.

SUPPORTING INFORMATION

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

Audio file S1. *Phylloscopus calciatilis* Call No. 1 Phong Nha-Ke Bang, Vietnam, April 2004.

Audio file S2. *Phylloscopus calciatilis* Call No. 2 Phong Nha-Ke Bang, Vietnam, April 2004.

Audio file S3. *Phylloscopus calciatilis* Song No. 1 Hin Namno, Laos, Feb 1998.

Audio file S4. *Phylloscopus calciatilis* Song No. 1 Phong Nha-Ke Bang, Vietnam, April 2004.

Audio file S5. *Phylloscopus calciatilis* Song No. 8 Phong Nha-Ke Bang, Vietnam, April 2004.

Table S1. Material studied (including samples used for the molecular study and their GenBank accession numbers).

Table S2. Characteristics of the songs of *Phylloscopus calciatilis*, *Phylloscopus ricketti* and *Phylloscopus cantator*.

Table S3. Syntax of songs of *Phylloscopus calciatilis*, *Phylloscopus ricketti* and *Phylloscopus cantator*.

Table S4. Characteristics of calls of *Phylloscopus calciatilis*, *Phylloscopus ricketti* and *Phylloscopus cantator cantator*.

Figure S1. Principal component analysis including all males of *Phylloscopus colciatilis* (stars), *Phylloscopus ricketti* (dots) and *Phylloscopus cantator* (squares) for which we have measurements of wing-, tail- and bill-length, and bill-width.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.