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

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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.

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, Bailein 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 (BMNH), where we compared them with two specimens, one adult male and one juvenile male, in Phong Nha-Ke Bang National Park, c. 17°24′N, 106°23′E. 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

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

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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.
Figure 1. (a) Distribution of verified breeding season samples of *Phylloscopus calciatilis* and *Phylloscopus ricketti*, and breeding record samples of *Phylloscopus cantator pernottus* (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 Nam-no 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, 651133; 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 Louey 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, Rodriguez 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; Gù 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 + \(\Gamma\) 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 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>
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<th>Table 1. Measurements and wing formula of <em>Phylloscopus calciatilis</em>, <em>Phylloscopus ricketti</em> and <em>Phylloscopus cantator cantator</em> (in mm).</th>
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<td>Relative length P9</td>
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Values given are: minimum—maximum (mean; sd; sample size). Samples of *Phylloscopus ricketti* males are from Fujian (17), Guangxi (1), Guizhou (1), Guangdong (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.
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 Cúc 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. Jäannes); (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. Jäannes); 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).
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,
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

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Figure 4. Continued

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These elicited mutually similar curious responses, Limestone Leaf Warbler at Phong Nha in 2004. P. cantator and even higher-pitched than in stone Leaf Warbler, though it is less stereotyped reminiscent of that of P. ricketti (Table S2 and Supporting Information Table S3) is more of notes is repeated. In all strophes of Fig. 4b(i)). Occasionally, a 'block' in pitch and noticeably emphasized (e.g. element E resembles the pair succeeding it, but falling deeper marked in bold in Fig. 4b and Supporting Information Table S3); (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 strophe 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-tsiu 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
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
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.

**Holotype**


**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**


(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-stripses 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
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 (≈ 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 \textit{ricketti} and \textit{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, \textit{calciatilis} and \textit{ricketti} are not safely distinguishable, whereas \textit{cantator} is markedly different. Measurements and wing formula separate with very little ambiguity male \textit{calciatilis} in Laos and Vietnam (sampled during the breeding season over a range of c. 600 km) from male \textit{ricketti} from the Chinese breeding grounds (over a range of at least 1400 km). Although there are specimens from Thailand and Indochina matching Chinese \textit{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 \textit{ricketti} in measurements and wing formula, but has a \textit{calciatilis} cytochrome-b haplotype (see below). The fourth female (KUNHM 93362) is somewhat intermediate between \textit{calciatilis} and \textit{ricketti}. Its bill-length/wing-length ratio matches male \textit{ricketti}, but differs without overlap (smaller) from male \textit{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 \textit{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 \textit{calciatilis}, which is larger than expected for a female \textit{calciatilis} and more in agreement with what would be expected for female \textit{ricketti}, as females are usually smaller overall than males in \textit{Phylloscopus} (Ticehurst 1938, Williamson 1967, Cramp 1992). In contrast, in wing formula (P4, P5, relative length of P9) KUNHM 93362 matches male \textit{calciatilis}, and it also has a \textit{calciatilis} cytochrome-b haplotype (see below). Both these specimens are from the Diding Headwater Nature Reserve, Guangxi Province, China, where the ranges of \textit{calciatilis} and \textit{ricketti} might meet, and it seems possible that they are hybrids (see below). \textit{Phylloscopus cantator} is somewhat intermediate between \textit{calciatilis} and \textit{ricketti} in size, whereas its wing formula is more similar to that of \textit{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 \textit{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 \textit{calciatilis} appear to be slightly more variable, with small differences between localities, but are still consistently and diagnosably different from those of \textit{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 \textit{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 \textit{calciatilis}, but no sound recordings. From the Diding Headwater in Guangxi Province, where we have some evidence that \textit{calciatilis} might be present, we have no sound recordings. Our only recording of the song of the isolated population of \textit{cantator} from northern Laos (\textit{pernotus}) differs slightly from our recordings from the Himalayas, although they are essentially similar, but distinct from those of both \textit{calciatilis} and \textit{ricketti}. The vocalizations of \textit{calciatilis} are the most divergent of the three taxa analysed. Subjectively, the differences in song, especially between \textit{calciatilis} and the two others, are at least as pronounced as between some sympatric species of \textit{Phylloscopus} and \textit{Seicercus} warblers (e.g. Western Crowned Warbler \textit{Phylloscopus occipitalis} vs. Blyth’s Leaf Warbler \textit{Phylloscopus reguloides}; Blyth’s Leaf Warbler vs. Davison’s Leaf Warbler \textit{Phylloscopus davisoni};

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 936954), 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 932632) 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 brehmi*). Accordingly, it would not be surprising if *calciatilis* and *ricketti* (and *cantator*) are not yet intrinsically reproductively isolated. If their ranges instead 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*. 

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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, Bolikhamsai 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 sight 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 & Khounboline 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 Namo National Protected Area) in November 2008 by J.W.D. revealed many large areas of reasonably tall forest remaining within the limestone karst formations.

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REFERENCES


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**SUPPORTING INFORMATION**

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


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 calciatilis* (stars), *Phylloscopus ricketti* (dots) and *Phylloscopus cantator* (squares) for which we have measurements of wing-, tail- and bill-length, and bill-width.

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