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Non-breeding season records of warblers in the *Phylloscopus reguloides* lineage from Thailand and Myanmar

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Summary.—A total of 20 individual *Phylloscopus* warblers in the 'Blyth's Leaf Warbler *P. reguloides* lineage', caught for ringing in the non-breeding season in Thailand and Myanmar, were resolved via mtDNA assay as *P. reguloides* (seven individuals), Claudia's Leaf Warbler *P. claudiae* (12) and Hartert's Leaf Warbler *P. goodsoni* (one). As expected, *P. claudiae* proved to be the most widely distributed. The occurrence of four *P. claudiae* on an island in the Thai Gulf, alongside large numbers of typically Sundaic wintering species on northbound migration during late March–early April, indicates that its non-breeding season range probably extends further south than previously recognised, into that subregion.

The *Phylloscopus* warblers formerly united as Blyth's Leaf Warbler *Phylloscopus reguloides* (*sensu lato*) have recently been shown by molecular analysis to represent three distinct lineages. *P. reguloides* is sister to the lineage comprising Claudia's Leaf Warbler *P. claudiae* and Hartert's Leaf Warbler *P. goodsoni* (Olsson *et al*. 2005, Alström *et al*. 2018). This arrangement is further supported by analyses of vocalisations (Päckert *et al*. 2009). Plumage differences among the three taxa are slight, however, all possessing a pale median crown-stripe, two wingbars and narrow white edges to the inner margins of the two or three outermost rectrices. *P. claudiae* and *P. goodsoni* differ from each other chiefly in the degree of yellow suffusion on their face and underparts, although this is difficult to judge under field conditions.

Monotypic *P. claudiae* is the most northerly distributed and widespread, its breeding range lying mostly north of the Changjiang (Yangtze) River in Gansu, Sichuan and Shaanxi, north to Hebei, in China (Cheng 1987, Dickinson & Christidis 2014). The distribution of *P. goodsoni* is more southerly, with nominate *goodsoni* in Guangxi and Guangdong, and *P. g. fokiensis* further north in Fujian, Jiangsu, Hubei and Shaanxi where it almost meets *P. claudiae* (Päckert *et al*. 2009, Dickinson & Christidis 2014). Four races of *P. reguloides* are distributed in the Himalayas from Kashmir in the west, east to north-west Vietnam and elsewhere in northern continental South-East Asia, including the montane outlier of the Langbian Plateau (Dickinson & Christidis 2014). In Thailand, breeding of *P. reguloides* [*assamensis*] was first proven on Thailand's highest mountain, Doi Inthanon, 2,565 m (Alström & Olsson 1994), and it is also apparently resident on a few other 2,000 m-plus peaks in Thailand, including Doi Pha Hom Pok (2,285 m), and probably Doi Chiang Dao, Chiang Mai Province (2,175 m), and in the west on the ornithologically little-known peak of Doi Kajela, 2,152 m, Tak Province (16º10'N, 99º02'E) based on the evidence of birds singing during April (PDR pers. obs.). Further, *P. reguloides assamensis* 'probably breeds throughout the mountains of

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Burma, and has been recorded from Mt. Victoria, North East Burma, the Shan States and Karenni' (Smythies 1986).

The winter distributions of these taxa are imperfectly known, owing to their morphological similarity. Relatively few of the specimens in museums have been subjected to DNA assay. *P. reguloides* (*sensu stricto*) is presumed to be either resident or a short-distance or elevational migrant throughout its range (e.g., Ali & Ripley 1983). The co-occurrence of *P. claudiae*, wintering alongside *P. reguloides* in parts of South-East Asia, had long been recognised, based on the slightly longer, more pointed wing of the relatively long-distance migrant *claudiae* compared to *reguloides*. Nonetheless, although Deignan (1945) acknowledged Ticehurst's (1938) identification of ten March-collected individuals from the Doi Inthanon summit (Doi Ang Ka) as *P. reguloides assamensis*, his subsequent Thailand checklist (Deignan 1963) omitted these, and unaccountably only listed *P.* [*reguloides*] *claudiae* therein: from Thailand's northern plateau (Chiang Rai, Chiang Mai and Lamphun Provinces) and the extreme north of the north-eastern plateau (Loei Province). Wintering birds presumed to be *P. claudiae* have since been found much more widely in Thailand: at lower elevations in the north-east, especially in Khao Yai National Park; in the south-eastern provinces, in the western forest complex, around Bangkok (on passage), and in the north of the peninsula south to *c*.12ºN (Lekagul & Round 1991, Treesucon & Limparungpatthanakij 2018). However, the possibility that some of these birds might have included *P. goodsoni*, the winter distribution of which is poorly known, cannot be eliminated owing to the morphological similarity of the two.

The picture is further complicated by nomenclatural confusion, as nominate *P. g. goodsoni* was formerly placed as a subspecies of Sulphur-breasted Leaf Warbler *P. ricketti*, whilst *P. g. fokiensis* was subsumed under *P. reguloides* (Watson *et al*. 1986). There are relatively few unequivocal records remote from the Chinese breeding range of either, although *P. g. goodsoni* is known to winter on Hainan and *P. g. fokiensis* may be widespread in northern continental South-East Asia (Dickinson & Christidis 2014). The two *P. goodsoni* subspecies are thought to overwinter in roughly equal proportions in Hong Kong, where *P. claudiae* is, as yet, unrecorded (Carey & Pang in prep.).

Three individuals collected in Phongsali province, northern Laos, in March 2005, one from montane elevation (>1,000 m) and two from steep, submontane land, were determined by mtDNA assay as *P. goodsoni* (Fuchs *et al*. 2007). Although the subspecies was not determined, the specimens are deposited in Muséum national d'Histoire naturelle, Paris, and are available for scrutiny. Subsequently, a long-staying bird photographed in a Bangkok city park during 17 January to 11 February 2015 (Robson 2015, Bird Conservation Society of Thailand, unpubl.) was considered to be a nominate *P. g. goodsoni*, whilst two heard singing at. *c*.1,200 m elevation, Khao Yai National Park, on 21 February 2021 were considered to be *P. goodsoni* ssp. (Bird Conservation Society of Thailand 2021, Roddis & Loseby 2021). Sound recordings archived on Xeno-canto indicate the non-breeding presence of *P. goodsoni* in non-montane elevations of both central Myanmar (A. Lastukhin & V. Sotnikov) and Cambodia (F. Lambert; www.xeno-canto.org).

We were primarily concerned to identify the winter ranges and relative abundance of *P. claudiae* and *P. goodsoni* ssp. in Thailand and Myanmar among '*P. reguloides* lineage birds' that were captured and released during ringing activities.

Methods

We collected feathers for DNA assay from a total of 23 *P. reguloides* (*sensu lato*) mist-netted and ringed at five discrete localities (Table 1): Doi Chiang Dao and Doi Lang (the north-east ridge of Doi Pha Hom Pok), both montane, at elevations of 1,450 m and 1,900 m

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TABLE 1
Locations where *Phylloscopus* spp. were sampled

Location	Coordinates	Elevation (m)
Indawgyi Lake, Kachin State, Myanmar	25°06′N, 96°17′E and 25°14′N, 96°22′E	180 m
Doi Lang, Mae Ai District, Thailand	20°06′57″N, 99°07′44″E	1,900 m
Khun Huai Mae Kok Substation, Doi Chiang Dao Wildlife Sanctuary, Chiang Mai Province, Thailand	19°22′27″N, 98°50′05″E	1,450 m
Khao Yai National Park headquarters area, Nakhon Nayok Province, Thailand	14°24′58″N, 101°22′43″E	750 m
Ko Man Nai (Man Nai Island), Rayong Province, Thailand	12°36′44″N, 101° 41′18″E	sea level

respectively, in Chiang Mai Province; Khao Yai National Park, Nakhon Nayok Province (*c*.750 m, where exclusively winter visitors); the island of Ko Man Nai, Rayong Province (four individuals, all apparently on northbound spring migration during March–April); and Indawgyi Wildlife Sanctuary, Kachin State, northern Myanmar (three apparently wintering birds in lowland secondary forest at *c*.180 m elevation; Table 1). All were measured and weighed and basic wing formula (position of the tip of the second outermost primary, p2, numbered ascendantly, in relation to the tips of the inner primaries) was noted for most. All individuals are referred to in subsequent text by ring number.

Laboratory protocols.—We extracted DNA from feather samples using NucleoSpin tissue kit (Macherey-Nagel), with 0.1% Dithiothreitol (DTT) added to increase the DNA yield (Olsson *et al*. 2005). Partial cytochrome-*b* (Cyt b) mitochondrial gene was amplified using primers: L14841 (5′-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3′) and H15547 (5′-AATAGGAAGTATCATTCGGGTTTGATG-3′; Helbig *et al*. 1995). We performed Polymerase Chain Reaction (PCR) using AccuStart II GelTrack PCR SuperMix (Quanta BioSciences) with concentration of ingredients suggested by the manufacturer. The PCR reactions were performed using an Eppendorf Mastercycler gradient thermocycler under the following conditions: 94°C for three minutes followed by five cycles at 94°C for 30 seconds, 48°C for 30 seconds, 72°C for one minute, then 30 cycles at 94°C for 30 seconds, 51°C for 30 seconds, 72°C for one minute and a final 72°C for five minutes (Saitoh *et al*. 2015). The PCR products were visualised with 1.5% agarose gel electrophoresis and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel). The products were sent for sequence analyses using Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit protocol.

Genetic analyses.—Sequences of collected samples were deposited in GenBank (Table 2) and aligned with sequences of recognised *P. reguloides* (AY656231, AY656233, AY656238, and EU851077–EU851079), *P. claudiae* (EU851083 and MH079258), *P. goodsoni* (AY656225, EU851081, and the three individuals referred to by Fuchs *et al*. 2007) using ClustalW in MEGA7 v. 7.0.21 (Kumar *et al*. 2016). Yellow-vented Warbler *P. cantator* (AY606157) and *P. ricketti* (AY606172) served as outgroups. We used Kakusan4 (Tanabe 2007) to select the best-fit evolutionary model under the Akaike Information Criterion (AIC; Akaike 1974) and Bayesian Information Criterion (BIC; Schwarz 1978) for Maximum Likelihood (ML) and Bayesian Inference (BI) approaches, respectively. The selected model for the ML was GTR Gamma and for BI was HYK85 Gamma. The ML trees were constructed using RAxML v8.2.12 (Stamatakis 2014) on CIPRES Science Gateway V3.3 (Miller *et al*. 2010) with 1,000 bootstrapping iterations. We considered bootstrap values higher than 70% as significant support. The BI trees were performed in MrBayes v3.2.6 (Huelsenbeck & Ronquist 2001) under a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) approach, started from random tree, run twice in parallel with a four-chain analysis for

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five million generations. The trees were sampled every 100 generations and 25% of these were discarded as 'burn-in'. We then evaluated stationarity by checking Effective Sample Size (ESS > 200) using Tracer v1.7.1 (Rambaut *et al*. 2018). We considered 95% posterior probabilities or higher from the remaining trees as significant support. The ML and BI trees were visualised and edited in FigTree v1.4.3 (Rambaut 2009).

Results

The samples from 20 individuals were successfully amplified but in three further individuals, from Khao Yai, the sequences were unrecoverable, apparently due to contamination. The final alignments from collected samples and downloaded sequences revealed 670 bp (130 variable sites and 115 parsimony informative sites). As ML and BI approaches provided similar topology, only the BI tree is shown (Fig. 1).

Of these 20 birds, seven were resolved as *P. reguloides* with high statistical support (100% bootstrap value and 100% posterior probability; Table 2). The clade of *P. reguloides* was further divided into two subclades. Our three Myanmar individuals, A34517, A34516 and A34567, caught in lowlands of Indawgyi Wildlife Sanctuary, were closely related to birds from montane elevations elsewhere in the same country (AY656231 and EU851078 from Mt. Victoria (Natmataung National Park), and from Nepal (AY656238 and EU851077) with 100% bootstrap and posterior probability. The relationship of the same three to additional Myanmar birds from Mt. Victoria (AY656233 and EU851079) received lower statistical support (70% bootstrap and <90% posterior probability, Fig. 1). Four northern Thai montane-trapped birds, 1A03637 (Doi Lang), 1A23428, 1A23030 and 1A01942 (Doi Chiang Dao, Table 2) were more closely related to *P. reguloides* from Yunnan, China (EU851079) and northern Tonkin, Vietnam (AY656233) with high statistical support (100% bootstrap value and 100% posterior probability). Another Doi Chiang Dao bird, 1A01944, clustered with *P. goodsoni* from Jiangxi (EU851081) and Guangdong, China (AY656225) and with three Fuchs *et al*. (2007) *P. goodsoni* from northern Laos, OP90367, OP90368 and OP90369 (100% bootstrap value and 100% posterior probability; Fig. 1).

A further 12 individuals (two from Doi Chiang Dao, 1A01729 and 1A01730, six from Khao Yai, 1A03127, 1A03104, 1A03137, 1A03138, 1A03177 and 1A03200, and four from Man Nai Island, A35381, A35387, A35388 and A34420) nested with *P. claudiae* from Gansu (EU851083) and Shaanxi, China (MH079258), with 100% bootstrap value and posterior probability; Fig. 1, Table 2).

Six of the seven *P. reguloides* were tentatively identified as that taxon before release based on wing formula (p2 falling between p9 and the tips of the secondaries, Table 2). Seven of the 12 *P. claudiae* were likewise correctly attributed to taxon on the basis of their longer p2 (= p7, one individual; = p8, four individuals; and = p8/9, two individuals; primaries numbered ascendantly). A further *P. claudiae* could not be reliably assigned on wing formula (p2 = 9/10), and wing formula was not noted for the four remaining birds (Table 2). While *P. goodsoni* could not be excluded for any of these *claudiae* based on wing formula, the relative weakness of any yellow suffusion on the supercilium and throat observed at time of capture was then judged to more likely indicate *P. claudiae*. Neither plumage details nor wing formula were specifically noted for the single *P. goodsoni* that was sampled.

Discussion

Although *P. reguloides* (*sensu stricto*) is known as an elevational migrant that winters in the Himalayan foothills and plains of northern India (Ali & Ripley 1983, Rasmussen &

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TABLE 2
Biometrics, wing formula, body mass, ring numbers and accession numbers of sampled *Phylloscopus* (wp = wing point; nr = not recorded). Primaries are numbered ascendantly.

Ring no	Date	Location	mtDNA determination	wing	tail	bill	tarsus	wp	p2	mass (g)	GenBank no.
A34516	29 Jan 18	Indawgyi,	*reguloides*	61	44	13.5	18.2	p5 (p4)	=p10	7.3	MT921120
A34517	29 Jan 18	Indawgyi,	*reguloides*	62	46	12.8	19.0	p5 (p4)	=p10	7.0	MT921122
A34567	06 Feb 18	Indawgyi,	*reguloides*	57	43	12.5	17.7	p4=p5	=p9/ss	nr	MT921121
1A03637	28 Mar 08	Doi Lang	*reguloides*	56	42	12.0	13.9	p4=p5	=ss	7.3	MT921119
1A01942	3 Dec 09	Doi Chiang Dao	*reguloides*	61	47	13.0	17.8	nr	nr	7.6	OP381448
1A23030	24 Jan 15	Doi Chiang Dao	*reguloides*	58	45	13.5	18.2	p4=p5	=p9	10.1	MT921131
1A23428	16 Dec 15	Doi Chiang Dao	*reguloides*	61.5	47	13.7	19.9	p4=p5	=10/ss	7.2	MT921123
1A01944	3 Dec 09	Doi Chiang Dao	*goodsoni*	60	46	13.7	16.8	nr	nr	7.4	MT921116
1A01729	30 Jan 09	Doi Chiang Dao	*claudiae*	63	47	13.6	nr	nr	nr	8.1	MT921118
1A01730	30 Jan 09	Doi Chiang Dao	*claudiae*	61.5	46	13.5	17.4	nr	nr	7.5	MT921117
1A03104	19 Nov 06	Khao Yai	*claudiae*	62	46	nr	nr	nr	nr	7.3	MT921130
1A03127	28 Oct 07	Khao Yai	*claudiae*	61	46	nr	nr	p4=p5	=p7	7.0	MT921129
1A03137	10 Nov 07	Khao Yai	*claudiae*	61	45	nr	nr	p4=p5	=p8	7.3	MT921128
1A03138	11 Nov 07	Khao Yai	*claudiae*	62	43	nr	nr	p4=p5	=p9/10	7.6	MT921127
1A03177	20 Jan 08	Khao Yai	*claudiae*	60	45	nr	nr	p4=p5	=p8	7.2	MT921126
1A03200	5 Mar 08	Khao Yai	*claudiae*	58	44	nr	nr	nr	nr		MT921125
A35381	19 Mar 21	Ko Man Nai	*claudiae*	62	45	13.0	16.7	p4	=p8	8.3	MZ404594
A35387	20 Mar 21	Ko Man Nai	*claudiae*	57	41	12.3	16.8	p4 (p5)	=p8/p9	7.4	MZ404595
A35388	20 Mar 21	Ko Man Nai	*claudiae*	61	44	13.9	17.6	p5 (p4)	=p8/p9	6.8	MZ404596
A35420	6 Apr 21	Ko Man Nai	*claudiae*	60	42	14.3	16.5	p4	=p8	9.3	MZ404597

Anderton 2005) our three Indawgyi individuals may be the first confirmed records from comparable lowland elevations in Myanmar or South-East Asia.

P. claudiae was confirmed, as expected, to be a widespread winter visitor in forested habitats of both lowland and montane elevations in Thailand. While the *P. claudiae* from Doi Chiang Dao and Khao Yai were apparently over-wintering birds, four others from the island of Ko Man Nai during late March–early April were almost certainly northbound passage migrants. The island, which lies 5.5 km off the eastern Thai coast, is a noted hotspot for migration during March to May (Round *et al*. 2015, Round & Dymond 2022). Almost all migrants caught on the island during those months were judged to be Sundaic winterers, probably making landfall following a *c*.600 km sea-crossing from Pattani Province, southern Thailand, which is *c*.600 km due south, on the extreme northern edge of the Peninsular Malayan bulge. Our captures might suggest, therefore, that small numbers of *P. claudiae* winter, as yet undetected, in the Sunda subregion, perhaps alongside the relatively common wintering Eastern Crowned Leaf Warbler *P. coronatus*.

The status of *P. goodsoni* remains to be further elucidated, however. Judged on the few Thai and other South-East Asian records, it is either scarce or overlooked, and is possibly

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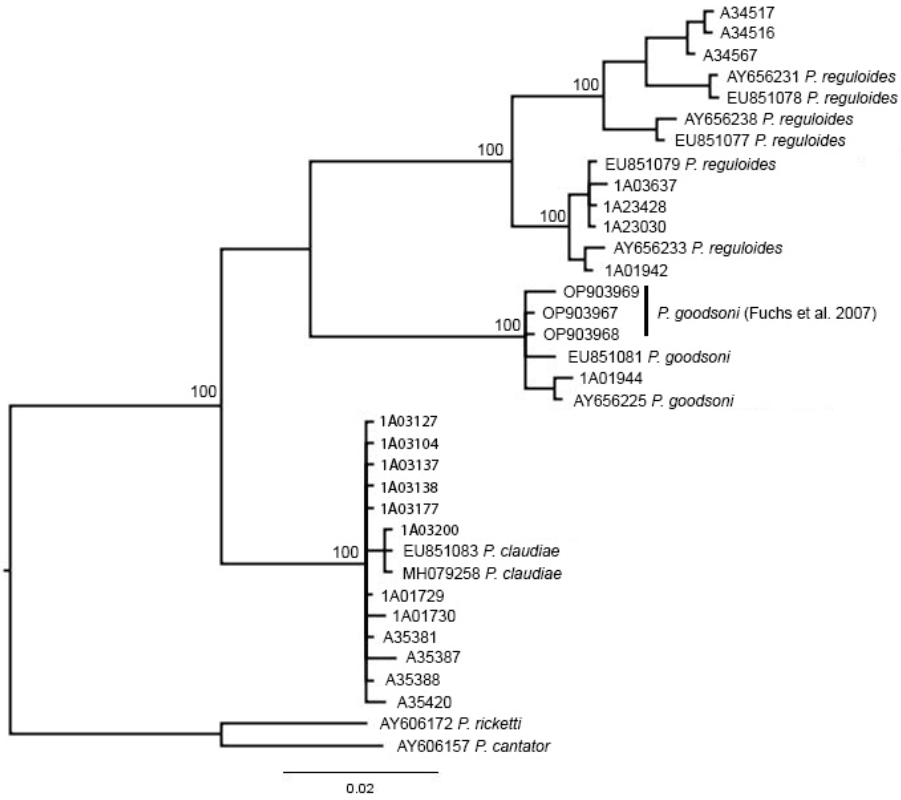

Figure 1. Bayesian Inference phylogenetic tree based on partial Cyt-*b* (B) gene of collected samples and those downloaded from GenBank, with Sulphur-breasted Warbler *P. ricketti* and Yellow-vented Warbler *P. cantator* serving as outgroups. Numbers on each node represent posterior probabilities. (All samples collected in this study are represented by ring numbers with either A or 1A preceding the five-digit number.)

more frequent in the east of the region. Separation of *P. goodsoni* and *P. claudiae* in field observations is problematical, and their similarity extends to behavioural traits. Nuthatch-like clambering on tree trunks and larger branches is frequently observed in birds identified both as *P. claudiae* (Treesucon & Limparungpatthanakij 2018) and *P. goodsoni* (Carey & Pang in prep.) but so far may not have been recorded in *P. reguloides*. Further sampling, whether by mist-netting and examination in the hand, or from sound-recordings of winter-singing birds, across a range of habitats and elevations in mainland South-East Asia, may improve our knowledge of the non-breeding season distribution and status of *P. claudiae* and *P. goodsoni* alongside *P. reguloides*.

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