Revival of the genus *Tropicoperdix* Blyth 1859 (Phasianidae, Aves) using multilocus sequence data

DE CHEN¹, YANG LIU², GEOFFREY W. H. DAVISON³, LU DONG¹, JIANG CHANG¹, SHENGHAN GAO⁵, SHOU-HSIEN LI⁶ and ZHENGWANG ZHANG¹*

¹Key Laboratory for Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing, 100875, China
²State Key Laboratory of Biocontrol and College of Ecology and Evolution, Sun Yat-sen University, Guangzhou, 510275, China
³National Biodiversity Centre, National Parks Board, 1 Cluny Road, 259569, Singapore
⁴State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing, 100012, China
⁵Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, 100101, China
⁶Department of Life Science, National Taiwan Normal University, Taipei, 10610, Taiwan

Received 14 July 2014; revised 13 March 2015; accepted for publication 19 March 2015

Although *Tropicoperdix* has been considered to be either a full genus or a species complex within the Phasianid genus *Arborophila* (hill partridges), there is long-standing uncertainty regarding the degree of difference that warrants generic separation, including reported anatomical cranial differences. In addition, the intra-generic taxonomy remains under dispute. Most studies hypothesize that *Tropicoperdix* comprises three species, while others postulate from one to four species. However, no molecular study has been performed to clarify the systematic and taxonomic uncertainties surrounding *Tropicoperdix*. In the present study, we performed a series of molecular phylogenetic analyses of *Tropicoperdix* and *Arborophila* taxa based on two mitochondrial genes and five nuclear introns. All the results are consistent with the finding that *Tropicoperdix* and *Arborophila* are phylogenetically distinct and distant genera, although the precise phylogenetic position of *Tropicoperdix* remains undetermined. Retrospective examination of external characteristics also supports the generic separation, as well as providing evidence of remarkable multiple character convergence. We propose that *Tropicoperdix* comprises at least two full species based on mitochondrial data obtained from museum specimens by using a next-generation sequencing method.

doi: 10.1111/zoj.12273


INTRODUCTION

Recent studies in avian molecular genetics have revealed a number of disputes about traditional classification and phylogenies at various taxonomic levels (Hillis, 1987; Hackett et al., 2008). Apart from the flux of taxonomic revisions caused by upgrades of subspecies to full species, changes at higher taxonomic levels have also been reported (Fjeldså, 2013). For example, *Elachura formosa*, previously considered a babbler in the family Timaliidae, is in fact the sole member of a separate evolutionary lineage and therefore it has been proposed as being its own family, Elachuridae (Alström et al., 2014). The genus *Ptilopachus* was misplaced in Phasianidae for more than 150 years until a
molecular study showed that it should be placed in the family Odontophoridae (Cohen et al., 2012). These notable examples highlight the complexity of the avian tree of life and suggest similar unexpected relationships to be discovered in birds.

The hill partridges (Arborophila Hodgson 1837), found in Asia from the Himalayas eastwards to Taiwan and south to Java, constitute the second largest genus of Phasianidae (Johnsgard, 1988; Madge & McGowan, 2002). However, their precise systematics is somewhat confusing and controversial (del Hoyo & Collar, 2014). Nevertheless, the difference in the number of supraorbital bones appears to be a non-negligible trait because skull morphology is an important and classical characteristic in avian taxonomy and systematics (Huxley, 1807; Simonetta, 1960; Zusi, 1993) and has been highlighted in several recent studies (James et al., 2003; Chiappe & Bertelli, 2006).

Therefore, the objectives of this study were to determine the validity of Tropicoperdix and to investigate its controversial intra-generic taxonomy as a preliminary to further investigations. Phylogenetic relationships between A. chloropus and several other Arborophila species, as well as additional representative Phasianid genera, were reconstructed based on two mitochondrial (mt) genes and five nuclear introns. We also compared genetic distances based on mt genes between three conventionally considered Tropicoperdix species, namely A. charltonii, A. chloropus and A. merlini (Peters, 1934; del Hoyo, 1994; Madge & McGowan, 2002; Zheng et al., 2002; Clements et al., 2014) (see Fig. 1 for their distributions) from museum specimens by using next-generation sequencing (NGS).

MATERIALS AND METHODS

TAXON SAMPLING, DNA EXTRACTION AND SEQUENCING

Our taxon sampling was based on the available multilocus dataset generated by Wang et al. (2013). To ensure that no relevant taxa were overlooked, we obtained the sequence data of most Phasianid genera, except for grouse, from GenBank (supplementary Table S1 lists the accession numbers of all samples analysed). The blood sample of a single A. chloropus was obtained from Mengla County in Yunnan Province, south-west China. Toe pads of A. charltonii and A. merlini were excised from two specimens archived in the Zoological Reference Collection (ZRC) of the National University of Singapore (catalogue numbers ZRC 3.1513 for A. charltonii and ZRC 3.1478 for A. merlini) because fresh tissues are difficult to obtain from field research.

Blood sample DNA was extracted using the TIANamp blood genomic DNA extraction kit (Tiangen). We amplified two mt genes, cytochrome b (CYTB) and NADH dehydrogenase subunit 2 (ND2), as well as five nuclear introns, i.e. clathrin heavy polypeptide intron 7 (CLTC), clathrin heavy chain-like 1 intron 7 (CLTCL1), eukaryotic translation elongation factor 2 introns 5 and 6 (EEF2), ovomucoid intron G (OVOG) and ovalbumin intron C (SERPINB14), of A. chloropus using the primers listed in Table S2. A single touchdown PCR protocol was used for all loci (for details see Wang et al., 2013). Each round of PCR also included one negative control to check for contamination. Both strands of each PCR product were sequenced on a 3730 DNA sequencer (Applied Biosystems). The sequences were visually compared with the original chromatograms to avoid reading errors and were also checked against published DNA sequences. The sequences were then assembled using MEGA version 5 (Tamura et al., 2011).

The toe pads were cut into small pieces and pre-treated as described by Hung et al. (2013). The Qiagen DNeasy Tissue Kit was used to extract historical DNA (hDNA). Approximately 200 ng hDNA was used for single-end short-read sequencing (Nextera DNA Sample Preparation Kits, Illumina) according to the instructions of the manufacturer. Single-end 90-bp sequence reads were obtained from a separate lane of a HiSeq 2000 Genome Analyzer (Illumina) for each sample. Berry Genomics performed the single-end library preparation and sequencing. A total of 72 360 071 (A. charltonii)
and 85,299,440 (A. merlini) NGS reads were generated. The duplicated reads were 15,767,966 (21.79%) and 7,244,030 (8.49%), separated and then removed from the sequencing data before mapping to the reference. The PCR-based mt genes of A. chloropus were provided as references to extract the homologous mt genes from A. charltonii and A. merlini. We used Bowtie2 v2.2.4 (Langmead & Salzberg, 2012) for mapping NGS reads to the references, with the ‘local’ and ‘very-sensitive’ search strategy for better tolerance of genetic variation. Next, the mapped reads were extracted and quality-controlled (\(Q \geq 30\)) with FastQC v0.11.2 (Andrews, 2010) for NGS assembly. SOAPdenovo2 r240 (Luo et al., 2012) was then used to carry out the assemblies in continuous K-mer sizes from 17 to 63. All the contigs were combined and further assembled with CAP3 version 10/15/07 (Huang & Madan, 1999) for full-length sequences. Finally, the mt genes were re-identified using BLAST from the assembled contigs. Furthermore, to enhance the taxonomy between these three taxa, we also amplified the cytochrome oxidase I (COI) barcode (Hebert, Cywinska & Ball, 2003; Hebert

**Figure 1.** Approximate geographical distributions of Tropicoperdix species, according to del Hoyo & Collar (2014). Here, we followed the conventional taxonomy to treat three species; by contrast, del Hoyo & Collar split off the forms tonkinensis (from T. chloropus) and graydoni (from T. charltonii) as species, and treated merlini as a subspecies of T. chloropus.
et al., 2004) of A. chloropus and extracted the COI barcodes from A. charltonii and A. merlini reads. Sequences collected in this study have all been deposited in GenBank (KP968265-KP968278, Table S1).

**SEQUENCE ALIGNMENT**
We combined the sequence alignments generated by Muscle (Edgar, 2004) included in MEGA version 5 (Tamura et al., 2011) into a complete concatenated data matrix to clarify the systematic relationship between Tropicoperdix and Arborophila, and incorporated CYTB and ND2 sequences extracted from the two museum specimens into a mt-only data matrix to determine the phylogenetic relationships within Tropicoperdix.

**CONCATENATED DATA ANALYSES**
Phylogenetic analyses were performed using Bayesian inference (BI) and maximum-likelihood (ML) analyses. BI was performed in BEAST v1.8.0 (Drummond & Rambaut, 2007) with a random starting tree and a Yule tree prior. The best-fitting nucleotide substitution model for each of the five nuclear introns was selected using the Akaike information criterion (AIC) by jmodeltest v2.1.4 (Darriba et al., 2012) and mt genes were partitioned by codon position (Yang, 1996). The log-likelihood values and effective sample size were checked using Tracer v1.5 (Rambaut & Drummond, 2009) to confirm the stationarity of the chains. The final analysis was run for 500 million generations with trees sampled every 1000 generations. TreeAnnotator v1.8.0 was then used to discard the first 20% of trees and to generate a consensus tree with Bayesian posterior probability (PP). ML analysis was performed in RaxML v8.0.26 (Stamatakis, 2014) using the same strategy as BI. Bootstrap values (BS) were calculated using 1000 replicates.

**SPECIES TREE ANALYSIS**
We constructed a coalescent-based species tree using *BEAST (Heled & Drummond, 2010) within BEAST v1.8.0 (Drummond & Rambaut, 2007). The species tree analysis was carried out using all six partitions (five nuclear introns and one linked mt partition) and all species (without A. charltonii and A. merlini) with a UPGMA starting tree and a Yule tree prior. The final analysis was run for 500 million generations with trees sampled every 1000 generations, of which the first 20% was discarded as burn-in.

**GENETIC DISTANCES WITHIN TROPICOPERDIX**
We calculated pairwise distances based on the mt genes CYTB and ND2 between each pair of the three Tropicoperdix taxa, and compared these with the distances among the Arborophila species. The pairwise distances and standard errors (SE) were calculated using a bootstrap method with 1000 replicates under the Tamura–Nei model using the program MEGA version 5 (Tamura et al., 2011). We also compared the COI divergences (in %) between each pair of the three Tropicoperdix taxa.

**EXTERNAL CHARACTERISTICS**
Qualitative comparisons were made based on external morphologies and plumage characteristics obtained from colour photographs of live birds representing the majority of Arborophila and Tropicoperdix taxa collected on the website http://www.orientalbirdimages.org, including multiple images of the adults of most taxa and the juveniles from a few taxa. Additional images of A. orientalis were obtained from http://www.i.b cynxeds.com and A. campbelli images were supplied by Con Foley (pers. comm.). Quantitative comparisons were made based on the ratio of the length between tarsus, toe and claw of the various taxa of Arborophila and Tropicoperdix from specimens in the Natural History Museum (NHM) at Tring, UK, and (for A. diversa) data in Riley (1930). Measurements were taken with sliding vernier callipers, to the nearest 1 mm for tarsus and toes and the nearest 0.5 mm for hind toe and claws. Toe lengths consisted of the sum from measurements of each phalanx in their respective digits, beginning from the base of the lowest tarsal scute to the base of the claw. Claw measurements were taken as the straight-line chord from the tip to the base where it joins the last dorsal scute of the toe. No test for repeatability of measurement was carried out.

**RESULTS**
**ANALYSES FROM MOLECULAR DATA**
Average sequence coverage was 437 (COI), 491 (CYTB) and 481 (ND2) for A. charltonii and 76 (COI), 82 (CYTB) and 59 (ND2) for A. merlini, suggesting these sequences were correct. The complete concatenated data matrix aligned by Muscle was 5234 bp in length, including 2135 bp of the mt genes and 3099 bp from the nuclear introns. The concatenated BI and ML analyses from all data matrices indicate that Tropicoperdix forms a separate clade away from Arborophila (Figs 2, 3). This is further supported by our species tree analysis (Fig. S1). We located Tropicoperdix within the ‘Chickens and allies’ clade, whereas other Arborophila were monophyletic and placed in the ‘Arborophilinae’ clade (Fig. 2). However, the Chickens and allies clade was only well supported in the complete concatenated BI tree (PP = 0.99, Fig. 2); other analyses showed extremely low support or even failed to reveal this clade.
Furthermore, our results indicated a *Tropicoperdix* cluster formed as a separate clade within the Chickens and allies clade (Fig. 3), but the relationships between the *Tropicoperdix* clade and four other well-supported clades remain controversial. Although the *Tropicoperdix* clade is shown to be a sister group to the peafowl clade (*Pavo*, *Afropavo* and *Argusianus*) in the complete concatenated BI tree (PP = 0.96, Fig. 2), other analyses showed low support or even failed to uncover this relationship (Figs 2, 3, Fig. S1).

Within *Tropicoperdix*, the mt trees showed that *T. chloropus* and *T. merlini* group together and are sister to *T. charltonii* with full support from both the BI and the ML analyses (Fig. 3). In addition, the pairwise distances of the concatenated mt genes CYTB and ND2 between *T. charltonii* and *T. chloropus* was 0.061 (± 0.005 SE), which was double that between *A. ardens* and *A. crudigularis* at 0.031 (± 0.004 SE). However, the pairwise genetic distance between *T. chloropus* and *T. merlini* was only 0.014 (± 0.003 SE). The COI divergence between *T. charltonii* and *T. chloropus* is 7.70%, but only 1.33% between *T. chloropus* and *T. merlini*.

**DATA ANALYSIS ON EXTERNAL CHARACTERISTICS**

In addition to previously published distinctions for the colours of axillary feathers and legs, additional ...
characteristics such as the plumage patterns of greater secondary coverts (Fig. S2) and the colours of other bare parts, including the bill, claw and orbital ring, show recognizable differences between *Arborophila* and *Tropicoperdix* (overall, bill: black versus yellow, claw: red versus yellow, orbital ring: red versus grey, see Table S3 for details).

In 13 *Arborophila* species, the ratio of the length of inner toe (digit II) to tarsus ranges from 0.47 to 0.62, whereas in *Tropicoperdix* this is slightly shorter, i.e. from 0.40 to 0.44 (Table 1). The ratio of the length of the middle toe (digit III) to tarsus is 0.63–0.70 in *Tropicoperdix*, also slightly shorter than that in *Arborophila*, from 0.71 to 0.90 (Table 1). In addition, in 13 *Arborophila* species, the ratio of hind claw to hind toe (digit I) length is 0.39–0.75, whereas in *Tropicoperdix* this is a little longer, from 0.72 to 0.83 (Table 1).

**DISCUSSION**

**SYSTEMATIC STATUS OF THE TROPICOPERDIX GROUP**

In the present study, we performed a series of molecular phylogenetic analyses to determine the systematic position of *Tropicoperdix*. Together with qualitative and quantitative morphological comparisons between *Tropicoperdix* and other *Arborophila* species, our results contradict the prevailing view that...
places Tropicoperdix within Arborophila (Davison, 1982; Johnsgard, 1988; Madge & McGowan, 2002; Zheng et al., 2002; Dickinson & Remsen, 2013; Clements et al., 2014; del Hoyo & Collar, 2014; Gill & Donsker, 2014), but support the revival of Tropicoperdix as a separate genus (Blyth, 1859; Ogilvie-Grant, 1895; Morony et al., 1975).

More interestingly, our analyses indicate that Tropicoperdix and Arborophila are distinct and distantly related taxa, residing in two different major Phasianidae clades, the Chickens and allies clade and the Arborophila clade (Fig. 2).

While the Chickens and allies clade, to which Tropicoperdix belongs, received mixed support in our analyses as well as in numerous earlier studies (reviewed by Wang et al., 2013; Kimball & Braun, 2014), there is strong support for Tropicoperdix being within the clade that includes all Phasianidae minus Arborophila (Figs 2, 3, Fig. S1). The uncertainty about the Chickens and allies clade was eventually supported by the use of large amounts of ultraconserved element (UCE) data (Sun et al., 2014). Furthermore, four monophyletic clades have been identified within the Chickens and allies clade (Wang et al., 2013; Kimball & Braun, 2014; Sun et al., 2014). Our results suggest strongly that Tropicoperdix should not be included in any of these four clades but forms another separate monophyletic clade (Fig. 3). Similar to the uncertainty surrounding the Chickens and allies clade, our smaller dataset failed to resolve the precise phylogenetic position of the Tropicoperdix clade. Additional nuclear loci, such as UCEs, are likely to resolve the exact position of Tropicoperdix in the Phasianidae phylogeny (Kimball & Braun, 2014; Sun et al., 2014).

Clues to the distinctiveness of Tropicoperdix can be found in the morphological comparisons with Arborophila, although these differences are subtle. The colours of the bare parts (bill, orbital ring, legs and claws) (Table S3) show recognizable differences between Tropicoperdix and Arborophila. These bare-part colour differences are shown to be associated with interspecific differences in their life histories and habitat use (Olson & Owens, 2005). Tarsus, toe and claw measurements also indicate subtle differences, with proportionately longer inner and mid toes in Arborophila than in Tropicoperdix, and a proportionately shorter claw on the hallux of Arborophila than of Tropicoperdix (Table 1).

Such differences between the hind limb structures may also indicate different foraging techniques and habitat use (Keast & Saunders, 1991; Carrascal, Moreno & Valido, 1994; Einoder, Richardson & Briskie, 2007). However, Tropicoperdix and Arborophila are sympatric and both inhabit evergreen forests in tropical southeast Asia (Davison, 1982), masking their possible habitat difference. Furthermore, Tropicoperdix and Arborophila are all small, round-bodied partridges with dark brown cryptic plumage, skulking behaviour, with long, sharp and nearly straight claws, performing ventriloquial and antiphonal duets, and building domed nests of dead leaves on the forest floor (Davison, 1982; Johnsgard, 1988). Individually each of these features is exhibited by at least one other genus, for example domed nests in Rollulus and Caloperdix, antiphonal duets in Rhizothera and Odontophorus, and long sharp claws in Odontophorus and Dactyloryctes. These seem convergent in tropical forests but not all are shared by all such genera, and the examples cut across

---

**Table 1.** Ratios derived from comparative measurements of Arborophila and Tropicoperdix taxa based on specimens in NHM Tring and (for A. diversa) data in Riley (1930) (see Table S4 for details)

<table>
<thead>
<tr>
<th>Taxon (and sample size)</th>
<th>Inner toe/tarsus</th>
<th>Mid toe/tarsus</th>
<th>Hind toe/tarsus</th>
<th>Mid claw/mid toe</th>
<th>Hind claw/hind toe</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. atrogularis (1)</td>
<td>0.500</td>
<td>0.761</td>
<td>0.190</td>
<td>0.406</td>
<td>0.437</td>
</tr>
<tr>
<td>A. brunnepectus (9)</td>
<td>0.534</td>
<td>0.837</td>
<td>0.209</td>
<td>0.361</td>
<td>0.555</td>
</tr>
<tr>
<td>A. cambodiana (1)</td>
<td>0.547</td>
<td>0.785</td>
<td>0.226</td>
<td>0.424</td>
<td>0.631</td>
</tr>
<tr>
<td>A. crudigularis (2)</td>
<td>0.609</td>
<td>0.878</td>
<td>0.219</td>
<td>0.416</td>
<td>0.611</td>
</tr>
<tr>
<td>A. diversa (1)</td>
<td>–</td>
<td>0.845</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A. gingica (5)</td>
<td>0.534</td>
<td>0.767</td>
<td>0.197</td>
<td>0.424</td>
<td>0.588</td>
</tr>
<tr>
<td>A. hyperythra (6)</td>
<td>0.511</td>
<td>0.777</td>
<td>0.177</td>
<td>0.342</td>
<td>0.625</td>
</tr>
<tr>
<td>A. javanica (5)</td>
<td>0.466</td>
<td>0.711</td>
<td>0.177</td>
<td>0.453</td>
<td>0.625</td>
</tr>
<tr>
<td>A. mandelli (3)</td>
<td>0.619</td>
<td>0.904</td>
<td>0.214</td>
<td>0.342</td>
<td>0.555</td>
</tr>
<tr>
<td>A. orientalis (5)</td>
<td>0.478</td>
<td>0.739</td>
<td>0.195</td>
<td>0.411</td>
<td>0.722</td>
</tr>
<tr>
<td>A. rubrirostris (2)</td>
<td>0.477</td>
<td>0.772</td>
<td>0.181</td>
<td>0.382</td>
<td>0.750</td>
</tr>
<tr>
<td>A. rufpectus (2)</td>
<td>0.533</td>
<td>0.755</td>
<td>0.200</td>
<td>0.411</td>
<td>0.388</td>
</tr>
<tr>
<td>A. Rufugularis (7)</td>
<td>0.575</td>
<td>0.825</td>
<td>0.200</td>
<td>0.393</td>
<td>0.687</td>
</tr>
<tr>
<td>A. torqueola (12)</td>
<td>0.511</td>
<td>0.800</td>
<td>0.200</td>
<td>0.361</td>
<td>0.555</td>
</tr>
<tr>
<td>T. charltonii (6)</td>
<td>0.395</td>
<td>0.627</td>
<td>0.209</td>
<td>0.425</td>
<td>0.833</td>
</tr>
<tr>
<td>T. chloropus (4)</td>
<td>0.441</td>
<td>0.697</td>
<td>0.209</td>
<td>0.400</td>
<td>0.722</td>
</tr>
</tbody>
</table>

© 2015 The Linnean Society of London, Zoological Journal of the Linnean Society, 2015, 175, 429–438
subfamilies. All these features come together in Tropicoperdix and Arborophila, and although not unique to that pair, would encourage the traditional view that they are congeneric. Overall, our molecular phylogenetic results indicate that Tropicoperdix is genetically different from Arborophila, although their appearance, vocalization and habitat selection are so similar that this similarity has misled many ornithologists into thinking that they are closely related.

**TAXONOMY WITHIN THE GENUS TROPICOPERDIX**

Contrary to the view that all Tropicoperdix are in a single species T. charltonii (Johnsgard, 1988; Dickinson & Remsen, 2013), T. charltonii and T. chloropus should be considered as two separate species. Their genetic distance is much greater than that between sister species of Arborophila, and COI divergence between T. charltonii and T. chloropus is 7.70%, far higher than the 3% threshold of COI sequence divergence typically used to characterize different species (Hebert et al., 2003). By contrast, the genetic distance between T. chloropus and T. merlini is only 0.014, approximately half the distance between sister species of Arborophila; their COI divergence is only 1.33%, much less than the 3% threshold. Morphological differences between T. chloropus and T. merlini are also very subtle, in which T. merlini has more distinct scaling on its flanks and yellowish legs (Delacour & Jabouille, 1924; Madge & McGowan, 2002). However, we were unable to confirm the grouping of T. merlini within T. chloropus, given that we only evaluated one individual of each taxon; therefore, we could not compare the sequence divergences within species to those between species (Hebert et al., 2004). Yet, T. chloropus has several other geographically isolated and taxonomic uncertain subspecies (Fig. 1) (del Hoyo & Collar, 2014). A firm conclusion on the status of T. merlini therefore requires more sampling.

**CONCLUSIONS**

Our phylogenetic analyses support the conclusion that the A. chloropus complex (T. charltonii and T. chloropus) is not the member of the hill partridges (genus Arborophila). We suggest the genus Tropicoperdix should be revived. This lineage represents a distinct lineage within the Chickens and allies clade of the family Phasianidae.

**ACKNOWLEDGEMENTS**

This research was supported by the National Natural Science Foundation of China (No. 31272296) and National Basic Research Program of China (2011BAZ03186). We thank Prof. Yanyun Zhang and Dr Limin Feng for their help in sample collection, Mr Kevin Lim's permission to collect tissues from the Lee Kong Chian Natural History Museum at the National University of Singapore, and the Natural History Museum at Tring (UK) for access to specimens for measurement. We also thank the editors and anonymous reviewers for their comments on the manuscript.

**REFERENCES**


TROPICOPERDIX IS A SEPARATE GENUS RATHER THAN A SPECIES COMPLEX 437


Huxley TH. 1897. On the classification of birds: and on the taxonomic value of the modifications of certain of the cranial bones observed in that class. London: The Society.


Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Figure S1.** Species tree analysis from the complete concatenated data matrix. The taxa representing the clade of interest are highlighted in different colours. Numbers above nodes represent Bayesian posterior probability (PP).

**Figure S2.** Sketches of the exposed tips of the greater secondary coverts (left wing) of *Arborophila* and *Tropicoperdix*, from photographs. Upper row from left to right: *A. brunneopectus*, *A. rufogularis*, *A. crudigularis* and *A. torqueola* (reversed from right wing). Lower row from left to right: *T. charltonii*, *T. chloropus* and *T. merlini*.

**Table S1.** GenBank accession number for each sequence used in this study.

**Table S2.** Names, location and primer sequences of the eight regions.

**Table S3.** Colours of bare parts between the *Arborophila* and *Tropicoperdix* taxa.

**Table S4.** Measurements (mm) of various taxa of *Arborophila* and *Tropicoperdix* based on specimens in NHM Tring and (for *A. diversa*) data in Riley (1930).