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# Coevolutionary analysis of the *Phlopterooides* Mey, 2004 (Phthiraptera: Ischnocera) parasitizing bulbuls (Passeriformes: Pycnonotidae)

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**Introduction:** Avian head lice comprise a diverse group of distantly related genera of lice that exhibit a strongly convergent morphology. Due to their lack of free-living stages, their strong morphological adaptations to living on the host's head, and the limited opportunities for transfer between hosts during mating or nesting, the lateral transmission of head lice between non-conspecific hosts may be presumed to be restricted. Despite this, many species of head lice are ostensibly host generalists. We here examine lice of the head louse genus *Phlopterooides* Mey, 2004, from bulbuls (Passeriformes: Pycnonotidae).

**Methods:** We use two different methods, ParaFit and Jane, to get insights on the co-evolutionary history of *Phlopterooides* species and their bulbul hosts. Jane was run with a variation of event costs.

**Results:** Our phylogenetic analysis indicate that several morphologically cryptic species can be found in this group, most of which appear to be host specific. However, co-phylogenetic analyses indicate that host-switching has been common in the history of these lice, and co-speciation events have been rarer than expected. Moreover, lowest-cost co-evolutionary reconstructions under a variety of event costs are indistinguishable from random. An expanded dataset with more *Phlopterooides*-complex lice was found to be evenly balanced between host-switching and co-speciation events.

**Discussion:** The transfer of avian head lice between host species is poorly understood, but evidently fairly common. Several potential routes are discussed, but direct evidence is missing. Potentially, the presence of multiple bulbul species at fruiting trees may be an important factor in this transfer. However, such transfer routes also do not explain why *Phlopterooides* lice on bulbuls appear to be distinct from those of other hosts. Moreover, as many of the species recovered in our analysis are morphologically indistinguishable, cryptic speciation appears to be common in this group.

## KEYWORDS

coevolution, host-switching, Phthiraptera, Ischnocera, *Phlopterooides*-complex, bulbuls

## Introduction

Ischnoceran lice (Phthiraptera) of the so-called “head louse ecomorph” have a wide distribution across most groups of birds (Price et al., 2003). Head lice do not form a monophyletic group; instead, they are often more closely related to lice of other ecomorphs (Johnson et al., 2012). Presumably, similar selection pressures operate on lice living on the host’s head regardless of host identity, resulting in similar gross morphology in distantly related louse groups. For instance, the main host defense against head lice is foot scratching (Clayton and Cotgreave, 1994), which flushes lice from the head to the body feathers (Goodman et al., 2020). Convergence in morphology among different head louse groups is likely at least in part to withstand foot scratching.

The typical head louse ecomorph morphology includes (see, e.g., Gustafsson et al., 2022a,b): a proportionately large, triangular head with extensive dorsal preantennal sutures (Figure 1); large, rounded abdomens that are not dorsoventrally flattened, with medianly interrupted tergopleurites and reduced or absent sternites; proportionately short legs. Presumably, the large head and extensive sutures aid in attachment to head feathers, whereas the large abdomen may be an effect of the correlation between body size and fecundity of lice (Villa et al., 2018) made possible by the limited efficiency of foot scratching. The short legs may have evolved in response to the smaller feathers on the head; head lice are generally not slower than other ischnoceran lice when climbing on head feathers of live birds (DRG, personal observations).

The morphological characters of head lice may be disadvantageous elsewhere on the host. Birds have developed a variety of active and passive defenses against parasites (Clayton et al., 2010; Bush and Clayton, 2018), but the main method of removing lice is preening, i.e., the manipulation of feathers with the beak (Clayton et al., 1999, 2005; Vezzoli et al., 2015). On most bird groups, lice occurring on parts of the body where the bird can preen are generally more slender than head lice, presumably to allow them to hide between feather barbs. This has resulted in a correlation between the size of lice and the size of their hosts, a phenomenon known as Harrison’s rule (Johnson et al., 2005); however, this correlation is not seen in all ecomorphs of lice (Johnson et al., 2005; Harnos et al., 2017). Experimental data have shown that transfers of lice between birds of different size may severely impact the fitness of lice (Bush and Clayton, 2006). Alternatively, lice occurring on the body of their hosts may be quick enough to escape preening (Johnson et al., 2005). Large-headed and large-bodied head lice may not be able to employ either of these escape behaviors successfully and would easily be preened off by the bird. Notably, detailed studies on the positioning of eggs and adult lice on their hosts suggest that head lice spend their whole life on the head (Baum, 1968; Mey, 1982; Cicchino, 2007).

If head lice are confined to the head of their host, this would indicate that their opportunities for transmission between hosts are limited. As lice have no free-living stages, the most common modes of transmission between hosts require either the hosts to come into physical contact or the presence of hippoboscids on which the lice can hitchhike (Keirans, 1975; Harbison et al., 2009; Bartlow et al., 2016; Lee et al., 2022). Relatively few records of head lice being phoretic on hippoboscids are known (Lee et al., 2022), therefore most transmission probably occurs through physical contact between conspecific hosts, e.g., during mating or nesting (Clayton and Tompkins, 1994; Lee and Clayton, 1995; Hillgarth, 1996; Brooke,

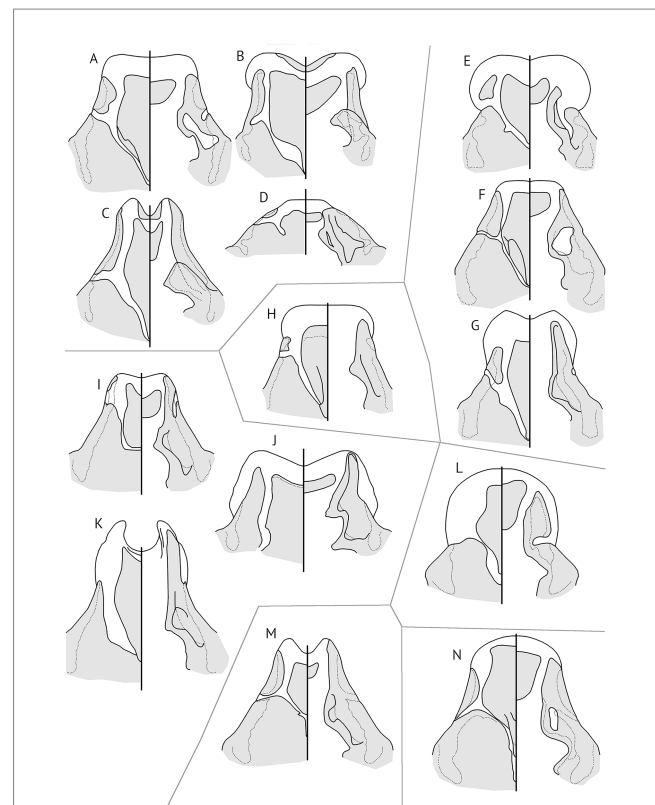


FIGURE 1

Comparison of the anterior ends of heads of lice of the head louse ecomorph from different radiations, showing the strong convergence on similar head structures in lice occupying the same niche on different groups of birds. For simplicity, setae, pulvini, and some ornamental structures have been omitted. Gray areas on each head represent sclerotized parts, whereas white areas represent hyaline regions. *Philopterus*-complex: (A) *Philopterus garruli* ex *Garrulus glandarius*; (B) *Vinceoapterus erythrocephali* ex *Harpactes erythrocephalus*; (C) *Philopteroideus haerixos* ex *Ixos mccllellandii*; (D) *Corcorides biocellata* ex *Struthidea cinerea*. Expanded *Philopterus*-complex: (E) *Alcedoecus constrictus* ex *Halcyon pileata*; (F) *Strigiphilus cursor* ex *Asio flammeus*; (G) *Craspedorrhynchus platystomus* ex *Buteo japonicus*. *Mulcticola*-complex: (H) *Galliphilopterus* sp. ex *Arborophila gingica*. *Brueelia*-complex: (I) *Sturnidoecus bannoo* ex *Acridotheres cristatellus*; (J) *Meropoecus balisong* ex *Merops americanus*; (K) *Schizosairhynchus philippinus* ex *Basilornis miranda*. *Anaticola*-complex: (L) *Anatoecus dentatus* ex *Aix galericulata*. *Penenirmus*-complex: (M) *Picophilopterus blythipici* ex *Blythipicus pyrrhotis*. *Quadriceps*-complex: (N) *Saemundssonina lari* ex *Saundersilarus saundersi*.

2010). Due to their limited dispersal capabilities, head lice present a unique opportunity to study Fahrenholz’s rule (Eichler, 1942, 1948).

Fahrenholz’s rule is the assertion that, given a lack of free-living stages and limited opportunities to spread between non-conspecific hosts, permanent parasites should be strictly host specific and co-speciate with their hosts. Over time, this should produce host and parasite phylogenies that are mirror images of each other. This proposed rule, often summarized as “parasite phylogeny mirrors host phylogeny” (e.g., Kim, 1988; Mey, 1999), has a long and complicated history (Von Kéler, 1959; Klassen, 1992; Gustafsson and Najer, 2022), and has received mixed support in co-phylogenetic analyses across a range of louse species (e.g., Sweet and Johnson, 2016, 2018; Sweet et al., 2018; Catanach et al., 2019; Johnson et al., 2021). Co-phylogenetic analyses between lice and their

hosts most often show that strict co-speciation is eroded by host-switching of lice, including between distantly related hosts. However, most co-phylogenetic analyses of lice and their hosts to date have examined either wing lice or “generalists,” groups of lice that are known to be phoretic on hippoboscids (Lee et al., 2022). Co-phylogenetic analyses of head lice are rare, but in both the genera *Penenirmus* and *Alcedoecus* host-switching, including between distantly related hosts, appears to have played a major part in the evolution of host associations (Catanach et al., 2019; Johnson et al., 2021). Other phylogenetic analyses of head lice indicate that at least some species are host generalists (e.g., Grossi et al., 2014; Yamagishi et al., 2014; Bush et al., 2016; Najer et al., 2021; Kolencik et al., 2022).

Here, we examine head lice in the genus *Philopteroidea* (Mey, 2004), parasitizing bulbuls (Pycnonotidae), a bird family comprising 151 species of medium-sized frugivores distributed across Africa and tropical and subtropical Asia. Similarly, *Philopteroidea* is widely distributed throughout the Old World tropics, occurring on a wide range of host families (Valim and Palma, 2013; Gustafsson et al., 2022b: table 8). Recent genetic data indicate that the genus may not be monophyletic (Kolencik et al., 2022), with species parasitizing bulbuls forming a morphologically homogeneous and closely related group separate from the species occurring on members of other host families (Mey, 2004; Valim and Palma, 2013; Najer et al., 2016, 2021; Gustafsson et al., 2022a). The morphological homogeneity of these lice may suggest that host-switches occur regularly between different bulbul species.

Several factors of louse and host biology are expected to influence the co-phylogenetic history of *Philopteroidea* and bulbuls. First, no species of *Philopteroidea* are known to be phoretic on hippoboscids (Lee et al., 2022), suggesting that lateral transmission between host species may be limited. Moreover, *Philopteroidea* are generally common on bulbuls in Southeast Asia (Najer et al., 2012a; Chu et al., 2019), suggesting that host-switching *Philopteroidea* will only rarely encounter “empty niches” on bulbul hosts. Together, this would tend to increase the level of host specificity and degree of co-evolution between *Philopteroidea* and their bulbul hosts. Only rarely is more than one head louse species known from the same host species (e.g., some thrushes; Price et al., 2003), and it is unknown whether the cases of multiple head louse species on the same host species are influenced by geography. The number of head lice found on any species of bird is usually small (<15), suggesting that the head feather habitat is limited, and may not support more than one louse species competing for the same resource. If empty niches are relatively rare, and co-occurrence of more than one head louse species on the same host is uncommon, lateral transmission between hosts should fail more often than not, and the phylogeny of *Philopteroidea* should be similar to that of their hosts.

However, as bulbuls often form mixed flocks at, e.g., fruiting trees, this may increase the opportunities for lateral transfer between hosts. If such contact is sufficiently frequent, this may increase the chances for successful host switches, thus eroding the co-phylogenetic structure of the system. The rate of success of host switching may also increase due to the similarity in size of most bulbuls (e.g., Arlott, 2017), which would limit the influence of Harrison’s rule.

## Materials and methods

Birds were caught and fumigated for lice in several localities across South China during 2012–2021 (Figure 2) using standard mist

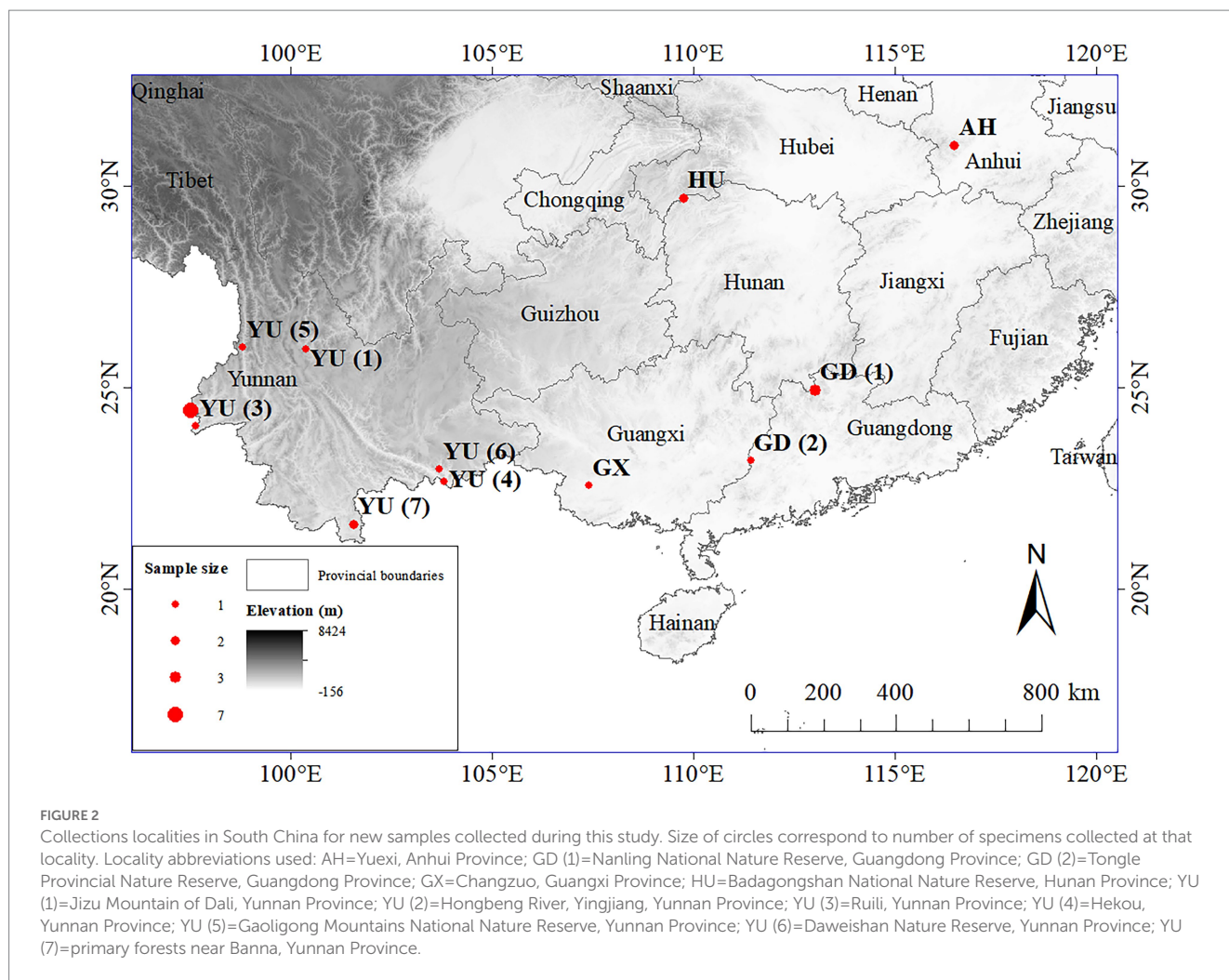
nets (net size: 2 m × 6 m; 2 m × 12 m) following the methods outlined by Gustafsson et al. (2019a). Hosts were identified using MacKinnon and Phillipps (2000) or Arlott (2017); host taxonomy has been updated to conform with Clements et al. (2021). Specimens of *Philopteroidea* were obtained from seven of the 20 species of bulbuls occurring in China, representing 5 of the 7 genera of bulbuls in this country. Additional sequence data derived from Najer et al. (2021) was obtained from GenBank (Table 1), representing another seven host species from three genera not obtained from China. Lice were stored in a –80°C freezer at the Institute of Zoology, Guangdong Academy of Sciences (IZGAS), Guangdong, China, and identified tentatively to genus level through a dissection microscope. Specimens corresponding to the morphology of *Philopteroidea* using the key of Gustafsson et al. (2019b) were selected for DNA extraction; identity of slide-mounted specimens were later confirmed using the same key.

At least 1 louse specimen (typically 1 male and 1 female, when available) identified as *Philopteroidea* from each host species was selected for DNA extraction. Selected lice (Table 1) were cut halfway through the pterothorax and extracted for DNA using the DNeasy Blood and Tissue Kit (Qiagen, Shanghai, China) following the manufacturer’s instructions except that extractions were left in 55°C water baths for 24h, and only 50 µl were used for each elution. Exoskeletons were retrieved from the extraction fluid and slide mounted in Canada balsam as vouchers, following Palma (1978). Vouchers are deposited in the collection at IZGAS.

For all specimens, we attempted to amplify three mitochondrial markers (*COI*, *12S*, *16S*) and three nuclear markers (*EF-1α*, *hyp*, *TMEDE6*), following the protocols used by Gustafsson and Olsson (2012), Sweet et al. (2014), and Tian et al. (2022). PCRs were performed using Cytiva PureTaq Ready-To-Go beads (GE Healthcare, Vienna, Austria), following the manufacturer’s instructions. Only *COI*, *TMEDE6* and *hyp* amplified consistently, and analysis proceeded based only on these genes. Samples showing satisfactory bands were sent for sequencing using the same primers as for PCR to Tianyi Huiyuan Gene Technology, Co. Ltd. (Guangzhou, China). Sequences were assembled in Seqman Pro 7.1.0 (DNASTar Inc., Madison, Wisconsin) and checked manually to rule out mismatches between forward and reverse sequencing results for each gene and each individual.

Two separate datasets were constructed. A smaller dataset (“Bulbul-only” dataset) was restricted to only *Philopteroidea* specimens parasitizing bulbuls derived from our own samples and Najer et al. (2021). To examine whether patterns obtained for this group were consistent across a larger diversity of head lice, a second dataset (“Expanded” dataset) was constructed using a range of different *Philopterus*-complex species derived from Najer et al. (2021). The Expanded dataset was rooted by including specimens of an undescribed species of *Sturnidoecus* Eichler, 1944, from *Geokichla citrina* (Passeriformes: Turdidae), whereas the Bulbul-only dataset was rooted on specimens from the Expanded dataset.

Sequences were aligned separately in MEGA 11 using ClustalW and MUSCLE (Edgar, 2004; Larkin et al., 2007; Kumar et al., 2018). Substitution models for each gene were evaluated in MEGA 11; the best model for *COI* was TN93 + G + I, for *hyp* was HKY + I, and for *TMEDE6* was TN93 + G. The 3 aligned and partitioned genes were imported into and concatenated by BEAST v1.10.4 (Suchard et al., 2018), with the default strict clock



prior and a Yule speciation process prior, using random starting trees, with the options of linked trees, separated clock models for each gene, 4 Gamma Categories under the strict clock, and constant size of coalescence. Markov chain Monte Carlo (MCMC) tests were run for  $1 \times 10^8$  generations and sampled every 1,000 generations. We used Tree Annotator v1.10.4 (Suchard et al., 2018) for tree integration and discarded the first 10,000,000 trees as “burnin.” The output tree from Tree Annotator was imported to FigTree v1.4.3<sup>1</sup> for figure illustration and edited in GIMP (GIMP Development Team, 2019). Louse species limits were tested using online GMYC<sup>2</sup> (Fujisawa and Barrowclough, 2013) and bPTP<sup>3</sup> (Zhang et al., 2013) analyses.

For co-phylogenetic analyses, Bayesian phylogenetic trees of the hosts were constructed online from [www.birdtree.org](http://www.birdtree.org), using the “Ericson All Species: A set of 10,000 trees with 9,993 OTUs each” as the tree source to construct 10,000 trees. TreeAnnotator V1.10.4 was used to discard the first 10% (1,000 trees), and host trees were checked in FigTree v1.4.3. A TXT matrix of the associations of the

parasites and hosts were constructed for use in R,<sup>4</sup> through the (*phytools*) *cophylo* function (Revell, 2011).

Cophylogenetic analysis was performed using the ParaFit method in *ape* (Legendre et al., 2002) in R using the consensus trees of the respective post-burnin tree distributions for the lice and hosts outlined above. Patristic distance matrices were constructed from the host and parasite trees for each dataset separately using the *cophenetic* (*stats*) function in *ape*. ParaFit was then run for 99,999 generations, using the “lingoes” correction, to test for the contributions of individual host–parasite links with ParaFitLink1 and ParaFitLink2 tests.

We also ran Jane v. 4 (Conow et al., 2010) for each of the two datasets separately, to find optimal solutions of evolutionary events. Default costs were used for different evolutionary events (cospeciation 0; Duplication 1; Duplication and Host-switching 2; Loss 1; Failure to Diverge 1). Event analysis was performed after setting the genetic algorithm (GA) parameters to 100 generations and a population size of 500 in the Stats Mode, and the same genetic algorithm was set in the Solve Mode to test the statistical significance of the best score for our data. This was

1 <http://tree.bio.ed.ac.uk/software/figtree/>

2 <https://species.h-its.org/gmyc/>

3 <https://species.h-its.org/ptp/>

4 <https://www.r-project.org/>

TABLE 1 Collection and sequence information for specimens included in this study.

Louse species	Host species	Voucher no.	Locality	COI	hyp	TMEDE6
<i>Ph. citrinellae</i>	<i>Acanthis flammea</i>	CU18816	Umeå	MG565987	MG566006	MG566025
<i>Ph. citrinellae</i>	<i>Emberiza citrinella</i>	2KS44708	Umeå	MG565992	MG566011	MG566030
<i>Ph. citrinellae</i>	<i>Emberiza schoeniclus</i>	1EV21759	Umeå	MG565989	MG566008	MG566027
<i>Ph. citrinellae</i>	<i>Fringilla coelebs</i>	1EV20889	Umeå	MG565990	MG566009	MG566028
<i>Ph. citrinellae</i>	<i>Fringilla montifringilla</i>	1EV21218	Umeå	MG565991	MG566010	MG566029
<i>Ph. citrinellae</i>	<i>Spinus spinus</i>	CT98119	Stora Fjäderäggs	MG565988	MG566007	MG566026
<i>Ph. fringillae</i>	<i>Passer domesticus</i>	LV066	Loutra Volvis	MG565985	MG566004	MG566023
<i>Ph. fringillae</i>	<i>Passer domesticus</i>	LV62	Loutra Volvis	MG565986	MG566005	MG566024
<i>Ph. gustafssoni</i>	<i>Regulus ignicapillus</i>	F48388	Klec	MG565996	MG566015	MG566034
<i>Ph. gustafssoni</i>	<i>Regulus regulus</i>	AZ14	Sete Cidades	MG565993	MG566012	MG566031
<i>Ph. gustafssoni</i>	<i>Regulus regulus</i>	F49358	Lubno	MG565994	MG566013	MG566032
<i>Ph. gustafssoni</i>	<i>Regulus regulus</i>	F49362	Janovice	MG565995	MG566014	MG566033
<i>Ph. sp. 1</i>	<i>Dicrurus bracteatus</i>	1,621	Northern Territory	MT468913	MT468934	MT468954
<i>Ph. sp. 1</i>	<i>Dicrurus bracteatus</i>	1,621–2	Northern Territory	MG565999	MG566018	MG566037
<i>Ph. sp. 2</i>	<i>Dicrurus bracteatus</i>	1,467	Oro	MG566001	MG566020	MG566039
<i>Ph. sp. 3</i>	<i>Pericrocotus solaris</i>	2,450	Guizhou	MG566000	MG566019	MG566038
<i>Ph. sp. 4</i>	<i>Euphonia hirundinacea</i>	LA50	Atlántida	MG565997	MG566016	MG566035
<i>Ph. sp. 5</i>	<i>Poecile montanus</i>	1EV21209	Umeå	MG565998	MG566017	MG566036
<i>Ph. sp. 6</i>	<i>Chaetorhynchus papuensis</i>	1,491–1	Oro	MT468916	MT468937	MT468957
<i>Ph. sp. 6</i>	<i>Chaetorhynchus papuensis</i>	1,491–2	Oro	MT468917	MT468938	MT468958
<i>Ph. sp. 7</i>	<i>Rhipidura albicollis</i>	LB028	Lang Biang	MT468918	MT468939	MT468959
<i>Pp. cucphuongensis*</i>	<i>Brachypodius melanoleucos</i>	108	YSFMA†	MT468927	MT468948	MT468968
<i>Pp. cucphuongensis</i>	<i>Calliope calliope</i>	PM58	Pù Mát	MT468926	MT468947	MT468967
<i>Pp. cucphuongensis*</i>	<i>Pycnonotus finlaysoni</i>	PM54	Pù Mát	MT468925	MT468946	MT468966
<i>Pp. cucphuongensis*</i>	<i>Pycnonotus xanthorrhous</i>	2,483	Guizhou	MT468928	MT468949	MT468969
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	J0830	GD (1)	OP476489	OP555775	OP555782
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	J0830	GD (1)	OP476484	OP555770	OP555786
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	J2606	GD (2)	OP476486	OP555772	OP555784
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	2,370	Guizhou	MT468919	MT468940	MT468960
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	2,365	Guizhou	MT468920	MT468941	MT468961
<i>Pp. flavala*</i>	<i>Hemixos castanonotus</i>	2,375	Guizhou	MT468921	MT468942	MT468962
<i>Pp. flavala*</i>	<i>Hemixos flavala</i>	J1063	YU (2)	OP476481	OP555774	OP555801
<i>Pp. flavala*</i>	<i>Hemixos flavala</i>	J1063	YU (2)	OP476483	OP555769	OP555787
<i>Pp. flavala*</i>	<i>Hemixos flavala</i>	CP142	Cúc Phương	MT468922	MT468943	MT468963
<i>Pp. flavala*</i>	<i>Iole propinqua</i>	CP56	Cúc Phương	MT468923	MT468944	MT468964
<i>Pp. flavala*</i>	<i>Ixos mccllellandii</i>	J0295	GD (1)	OP476490	OP555776	OP555781
<i>Pp. flavala*</i>	<i>Ixos mccllellandii</i>	2,750	Guizhou	MT468924	MT468945	MT468965
<i>Pp. kayanobori?*</i>	<i>Spizixos semitorques</i>	J0102	HU	OP476492	OP555778	OP555779
<i>Pp. kayanobori?*</i>	<i>Spizixos semitorques</i>	J0102	HU	OP476485	OP555771	OP555785
<i>Pp. kayanobori?*</i>	<i>Spizixos semitorques</i>	2,508	Guizhou	MT468931	MT468952	MT468972
<i>Pp. sp. 1*</i>	<i>Alophoixus bres</i>	43	YSFMA	MT468929	MT468950	MT468970
<i>Pp. sp. 2*</i>	<i>Rubigula flaviventris</i>	PM176	Khe Kèm	MT468930	MT468951	MT468971
<i>Pp. sp. 4*</i>	<i>Rubigula erythrophthalmos</i>	57	YSFMA	MT468932	MT468953	MT468973
<i>Pp. sp. 5*</i>	<i>Alophoixus flaveolus</i>	J0493	YU (2)	OP476487	OP555773	OP555783

(Continued)

TABLE 1 (Continued)

Louse species	Host species	Voucher no.	Locality	COI	hyp	TMEDE6
<i>Pp. sp. 5*</i>	<i>Alophoixus flaveolus</i>	J0493	YU (2)	OP476488	OP555767	OP555789
<i>Pp. sp. 5*</i>	<i>Alophoixus pallidus</i>	J2991	GX	OP476476	OP555756	OP555790
<i>Pp. sp. 5*</i>	<i>Alophoixus pallidus</i>	J3023	YU (4)	OP476475	OP555766	OP555791
<i>Pp. sp. 5*</i>	<i>Ixos maclellandii</i>	J4155	YU (5)	OP476480	OP555755	OP555792
<i>Pp. sp. 6*</i>	<i>Hypsipetes leucocephalus</i>	J0258	YU (1)	OP476491	OP555777	OP555780
<i>Pp. sp. 6*</i>	<i>Hypsipetes leucocephalus</i>	J1195	YU (3)	OP476482	OP555768	OP555788
<i>Pp. sp. 7</i>	<i>Terpsiphone paradisi</i>	J1124	YU (7)	OP476477	OP555763	OP555795
<i>Pp. sp. 7</i>	<i>Terpsiphone paradisi</i>	J1124	YU (7)	OP476474	OP555760	OP555798
<i>Pp. sp. 8</i>	<i>Hypothymis azurea</i>	J2882	GX	OP476469	OP555757	OP555802
<i>Pp. sp. 9</i>	<i>Arachnothera magna</i>	J0507	YU (2)	OP476471	OP555759	OP555800
<i>Pp. sp. 9</i>	<i>Arachnothera magna</i>	J1040	YU (2)	OP476478	OP555764	OP555794
<i>Pp. sp. 9</i>	<i>Arachnothera magna</i>	J1040	YU (2)	OP476472	OP555761	OP555797
<i>Pp. sp. 10</i>	<i>Aethopyga saturata</i>	J0242	YU (6)	OP476479	OP555765	OP555793
<i>Ty. sp.</i>	<i>Pitangus sulphuratus</i>	LA65-1	Atlántida	MT468914	MT468935	MT468955
<i>Ty. sp.</i>	<i>Pitangus sulphuratus</i>	LA65-2	Atlántida	MT468915	MT468936	MT468956
Outgroups						
<i>St. sp.</i>	<i>Geokichla citrina</i>	J2702	AH	OP476473	OP555762	OP555796
<i>St. sp.</i>	<i>Geokichla citrina</i>	J2702	AH	OP476370	OP555758	OP555799

J-numbers refer to host individuals, abbreviations of louse genera: *Ph.* = *Philopterus*; *Pp.* = *Philopteroides*; *St.* = *Sturnidoecus*; *Ty.* *Tyranniphilopterus*. Abbreviations for localities (table 1) are given only for bulbuls: BO, Borneo; GD, Guangdong; GX, Guangxi; HU, Hunan; VI, Vietnam; YU, Yunnan. For specimens derived from Najer et al. (2021), localities are given as in that study. Louse species that could not be positively identified as any described species are denoted as "sp. #"; numbering generally follows that of Najer et al. (2021). Specimens included in the Bulbul-only dataset are marked with an \*. Numbers under the three last columns correspond to genbank accession numbers for the respective sequences.

compared to a set of randomized tip associations run 999 times to test for the statistical significance of our optimal score. For the Bulbul-only dataset, the Jane analyses were repeated with a variety of costs for Host Switching and Cospeciation; for each of these events, we varied the costs between 0 and 5, in increments of 1.

## Results

### Sampling

A total of 205 bulbuls were examined for lice, but each separate field trip normally resulted in only 1–3 bulbuls being caught; for this reason, prevalence data of *Philopteroides*, which is usually low (overall prevalence for all bulbul species examined 14.15%), cannot be calculated in a meaningful way.

### Specimen identity

All *Philopteroides* specimens from bulbuls were keyed to species using the key of Gustafsson et al. (2022a), except specimens derived from Najer et al. (2021). The identity of

*Philopteroides kayanobori* (Uchida, 1948) could not be confirmed, as the original description of this species (Uchida, 1948) includes characters that are not found in any species of *Philopteroides* (see Gustafsson et al., 2022a). As specimens from *Hemixos flava*, *Hemixos castanonotus* and the two species of *Alophoixus* are morphologically very similar, they could not be separated using this key, indicating that these may represent cryptic species. The specimens from *Hypsipetes leucocephalus* were identified as a morphologically distinct species.

### Phylogenetic reconstruction

Each of our datasets resulted in a single tree (Figures 3, 4). In the expanded dataset (Figure 3), five main clades were recovered inside the *Philopterus*-complex. *Philopteroides* was divided into two major clades, one comprising only specimens from bulbuls (PP = 1.00), corresponding to the *Philopteroides* group of Najer et al. (2021), and one comprising specimens from all other hosts (PP = 0.95), corresponding to the "Philopterus complex group 3" of Najer et al. (2021). However, neither of these two clades received high support, nor was the relationship between these two clades and *Tyranniphilopterus* clarified by our analysis.

A total of 13 species-level clades were identified in the Bulbul-only dataset (numbered 1–13 in Figure 4) by both the GMYC and

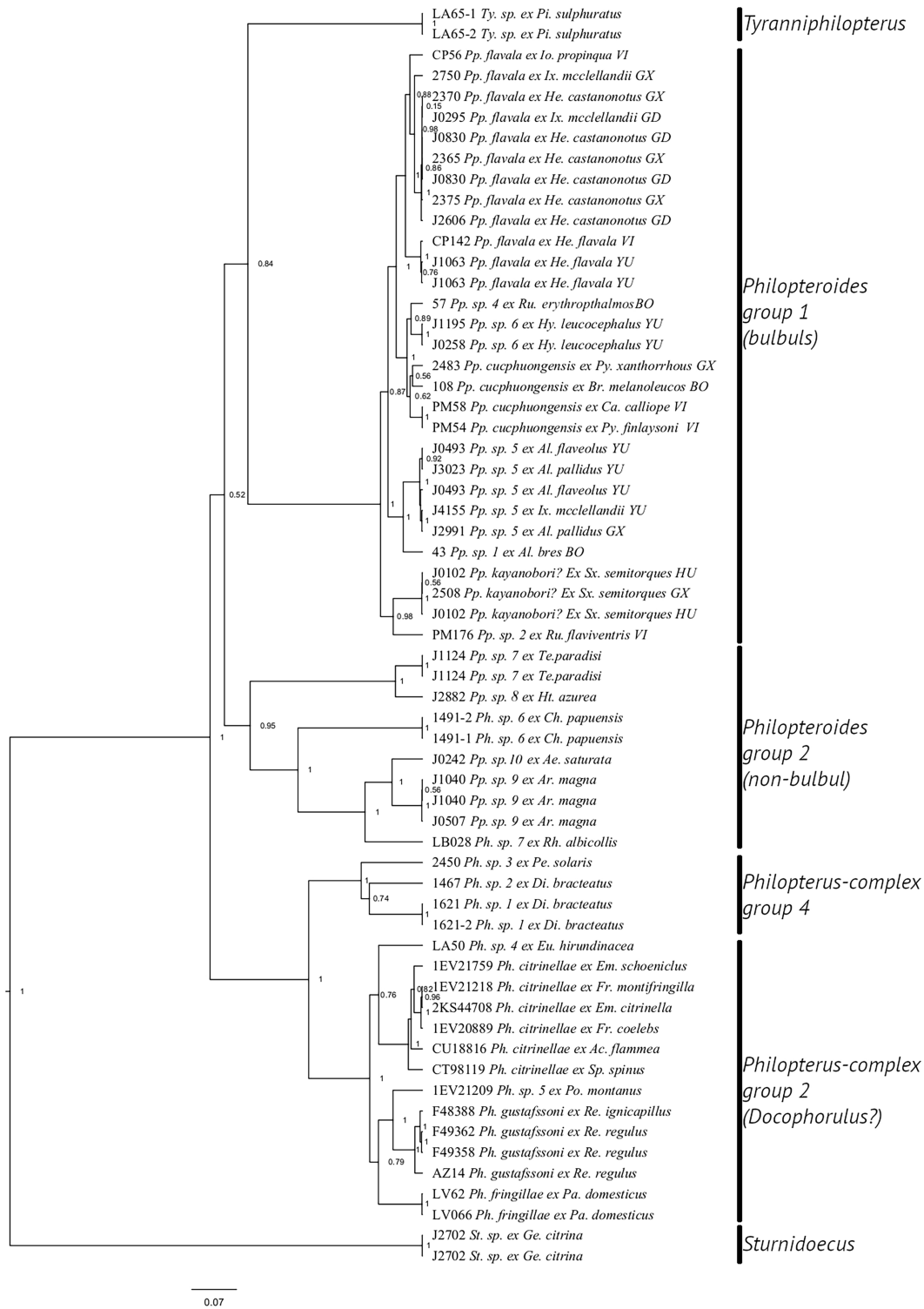


FIGURE 3

Phylogeny of the *Philopterus*-complex based on the mitochondrial *COI* and nuclear *TMEDE6* and *hyp* sequences and generated in BEAST v1.10.4.

Abbreviations of louse genera: *Ph.*, *Philopterus*; *Pp.*, *Philopteroidea*; *St.*, *Sturnidoecus*; *Ty.*, *Tyranniphlopterus*. Abbreviations of host genera: *Ac.*, *Acanthis*; *Al.*, *Alophoixus*; *Ca.*, *Calliope*; *Ch.*, *Chaetorhynchus*; *Di.*, *Dicrurus*; *Em.*, *Emberiza*; *Eu.*, *Euphonia*; *Fr.*, *Fringilla*; *Ge.*, *Geokichla*. *He.*, *Hemixos*; *Ht.*, *Hypothymis*; *Hy.*, *Hypsipetes*; *Io.*, *Iole*; *Ix.*, *Ixos*; *Pa.*, *Passer*; *Pe.*, *Pericrocotus*; *Pi.*, *Pitangus*; *Po.*, *Poecile*; *Ps.*, *Psarisomus*; *Py.*, *Pycnonotus*; *Re.*, *Regulus*; *Rh.*, *Rhipidura*; *Sp.*, *Spinus*; *Sx.*, *Spizixos*. Abbreviations for localities are given only for bulbuls: BO, Borneo, Indonesia; GD, Guangdong, China; GX, Guangxi, China; GZ, Guizhou, China; HU, Hunan, China; VI, Vietnam; YU, Yunnan, China. Numbers before louse names correspond to voucher numbers in Table 1.

the bPTP algorithm, with high posterior probabilities in the latter analysis for all non-monotypic clades except species 1 and 6 in Figure 4. Eight of these species-level clades were monotypic, and of

the remaining 5 clades, 3 comprised only specimens derived from the same host species; all non-monotypic species-level clades received good support (PP = 1.00). With the exception of lice from

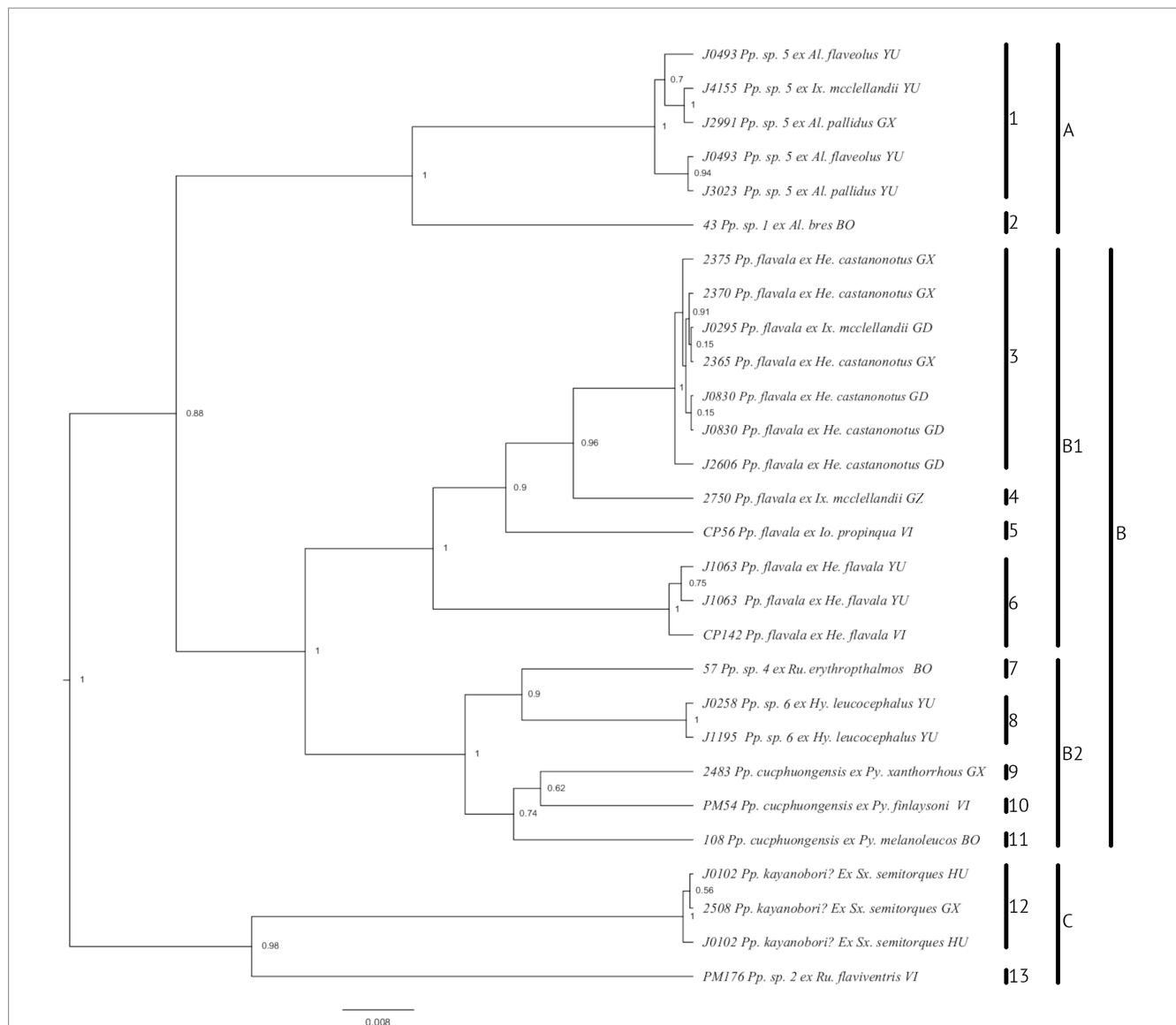


FIGURE 4

Phylogeny of *Philopteroides* (Mey, 2004), parasitic on bulbuls (Pycnonotidae) based on the mitochondrial *COI* and nuclear *TMEDE6* and *hyp* sequences and generated in BEAST v1.10.4. Vertical bars to the right correspond to clades discussed in the text. Species-level identifications follow the key of Gustafsson et al. (2022a); note that *Philopteroides kayanobori* cannot be adequately identified, as original description shows characters that are not found in any *Philopteroides*, and specimens from *Sx. semitorques* are identified tentatively. Abbreviations of genus names: *Al.*, *Alophoixus*; *He.*, *Hemixos*; *Hy.*, *Hypsipetes*; *Io.*, *Iole*; *Ix.*, *Ixos*; *Pp.*, *Philopteroides*; *Py.*, *Pycnonotus*; *Sx.*, *Spizixos*. Abbreviations for localities: BO, Borneo, Indonesia; GD, Guangdong, China; GX, Guangxi, China; GZ, Guizhou, China; HU, Hunan, China; VI, Vietnam; YU, Yunnan, China. Numbers before louse names correspond to voucher numbers in Table 1.

*Ixos mccllellandii* which were divided into three clades, all lice from the same host grouped together in the same clade. In one case (clade A in Figure 4), specimens from two different species of *Alophoixus* were mixed together with a single specimen from *Ix. mccllellandii*. These 13 species were divided into three main groups, all with good support (clades A–C in Figure 4; PP = 0.98–1.00).

## Cophylogenetic analyses

Our ParaFit analyses showed strong congruence between the host and louse trees for both datasets (ParaFitGlobal 179828.70, value of  $p$ -value 0.000001 for Expanded dataset; ParaFitGlobal 170.82,  $p$ -value 0.00548 for the Bulbul-only dataset). Independence of the host and parasite trees can thus be rejected for both datasets.

In contrast, the Jane analyses yielded different results for the two datasets (Table 2). Each dataset resulted in >15,000 solutions. In the Expanded dataset, these could be compressed to two isomorphic solutions of equal cost. The observed cost (44) in the Expanded dataset was well below that of random tip associations (60–70;  $p < 0.01$ ). Each of the two compressed solutions had equal numbers of inferred Cospeciations and Host Switches (18), with smaller numbers of Duplications (2) and Losses (6).

The Bulbul-only dataset compressed into a single set of isomorphic solutions, in which the cost of the optimal solution (21) fell within the range of random associations (18–25) and was thus not significant ( $p > 0.05$ ). This solution had equal numbers of Cospeciations and Host Switches (7), but also many Losses (6). Changing statistical or algorithmic parameters or event costs (Host Switching 0–5,



TABLE 2 Summary of the Jane v.4 results for both the Expanded and Bulbul-only dataset analyses.

Solution	#Solutions	#Cospeciations	#Duplications	#Duplications and host switches	#Losses	#Failures to diverge	Total cost	Cost range of random associations	Value of <i>p</i>
Expanded dataset (1)	9,250	18	2	18	6	0	44	60–70	<0.01
Expanded dataset (2)	6,096	18	2	18	6	0	44	60–70	<0.01
Bulbul-only	16,272	7	1	7	6	0	21	18–25	>0.05
Rerun analysis									
B1	2,886	4	0	9	0	0	18	15–23	>0.05
B2	2,871	4	0	9	0	0	18	15–23	>0.05
B3	1,742	4	0	9	0	0	18	15–23	>0.05
B4	3,109	5	0	8	2	0	18	15–23	>0.05
B5	1,254	5	0	8	2	0	18	15–23	>0.05
B6	5,272	7	0	6	6	0	18	15–23	>0.05

Each dataset yielded multiple solutions (>15,000), which could be compressed into 6 isomorphic solutions with the same costs. Under “Rerun analysis”, we list the six isomorphic solutions (B1–B6) obtained from a reduced bulbul dataset in which samples J0295 and J4155 were removed, under the assumption that these may represent stragglers that influence the cophylogenetic analysis unduly.

Cospeciation 0–5, increments of 1) did not significantly change the overall result, and the observed cost was always within the range of random associations (data not shown). Only when Cospeciation costs were set to 0 and Host Switching costs set to 4 was the observed cost (32) lower than the cost distribution of random tip associations (33–47), but this result was not significant; increasing the cost of Host Switching to 5 placed the observed cost within the range of random costs again. The co-speciation events inferred by our Jane analysis were scattered rather evenly throughout the phylogeny (Figure 5).

As one host species (*Ixos mclellandii*) was parasitized by three different lineages of lice, two of which were shared with lice from other hosts, we reran the Jane analysis with standard settings, removing the samples J0295 and J4155, to test whether these two potential contaminations or stragglers (specimens incidentally associated with a host at the time of collection, but not representative of an established population) disrupted co-phylogenetic patterns. This analysis resulted in 17,134 solutions, which could be compressed into six isomorphic solutions (B1–B6 in Table 2). These varied in their inferred number of co-evolutionary events, but in most solutions the numbers of Host Switches (8–9) were inferred to be greater than the number of Cospeciations (4–5). In all these solutions, the observed cost fell within the range of costs for random associations, and were thus not significant.

## Discussion

The transfer of head lice between hosts is poorly understood. Their position on the head is far away from the main points of contact between different birds during mating and nesting, and their gross morphology suggest that they would be easily preened off when leaving the head. These disadvantages for transferring between hosts are presumably even greater when hosts of different species are concerned, as direct contact between the heads of two different birds may be limited. As such, head lice should be prime candidates for adherence to Fahrenholz’s rule: head lice would be expected to be host specific and to have co-specified with their hosts.

The ParaFit analysis of the Expanded dataset, including a variety of *Philoferus*-complex genera, indicated that host and louse trees were overall congruent. The Jane reconciliation of this dataset also indicated that the two trees are consistent with each other (Table 3). The number of inferred Cospeciation events in this dataset was the same as the number of inferred Duplications and Host-switches (Table 2). In contrast, while the ParaFit analysis of the Bulbul-only dataset showed that the trees were significantly congruent, the Jane Analysis did not result in significant results for any combination of costs. Under standard cost settings, the Jane analysis of the Bulbul-only dataset resulted in a solution that included as many Cospeciation as Host Switching events, and almost as many Losses (Table 2). Removing potential stragglers of louse species 1 and 3 from *Ixos mclellandii* did not alter the results (Table 2).

In general, congruence between the two organism groups is thus high regardless of dataset, and in both datasets host-switching is inferred to have been as common as cospeciation. Collectively, the *Philoferus*-complex and its close relatives (e.g., *Alcedoecus*, *Craspedorrhynchus*, *Cuculoecus*, *Strigiphilus*) parasitize a range of distantly related host groups (Supplementary Table 1), suggesting that host-switching has been prevalent throughout their evolutionary history (De Moya et al., 2019). This expanded *Philoferus*-complex

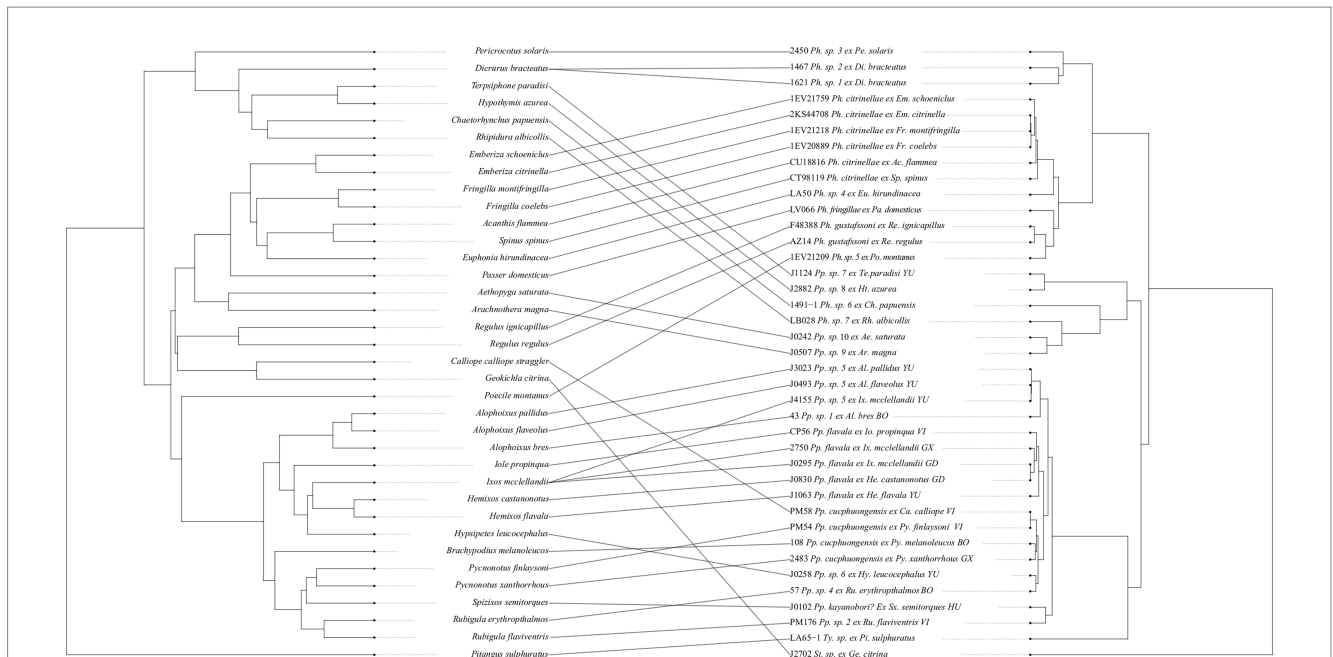


FIGURE 5

Tanglegram showing the relationships between bulbul hosts (left) and *Philopteroides* lice (right). Host phylogeny was obtained from [www.birdtree.org](http://www.birdtree.org) and louse phylogeny generated in BEAST v1.10.4 from mitochondrial *COI* and nuclear *TMEDE6* and *hyp* sequences. Both louse and host trees are cladograms of the trees resulting from the BEAST analysis or the online consensus tree, respectively. Black circles denote co-speciation events inferred by the Jane v.4 analysis, with numbers on each tree corresponding to the same event. Note that the solution containing these inferred co-speciation events was found not to be different from random in our analyses, and they are included here for completeness. Abbreviation of louse genus: *Pp.*, *Philopteroides*. Abbreviations of host genera: *Al.*, *Alophoixus*; *Ca.*, *Calliope*; *He.*, *Hemixos*; *Hy.*, *Hypsipetes*; *Io.*, *Iole*; *Ix.*, *Ixos*; *Py.*, *Pycnonotus*; *Sx.*, *Spizixos*. Abbreviations for localities: BO, Borneo, Indonesia; GD, Guangdong, China; GX, Guangxi, China; GZ, Guizhou, China; HU, Hunan, China; VI, Vietnam; YU, Yunnan, China. Numbers before louse names correspond to voucher numbers in Table 1.

notably comprises only head lice, whereas most other head louse groups (e.g., *Saemundsonia*, *Anatoecus*, *Echinophilopterus*) belong to radiations that include a variety of louse ecomorphs (Johnson et al., 2012). More data from different genera within the *Philopterus*-complex and its close relatives are needed to establish whether deeper nodes in this group are dominated by cospeciation or host-switching.

The Jane analysis could not separate the observed host-louse associations from random, but even accepting the inferred events at face value, there is no strong signal of louse-host cospeciation among the *Philopteroides* on bulbuls. The bulbul-*Philopteroides* dataset results are thus in line with previous data from head louse studies (Table 3). Several species of head lice occur on multiple closely related host species (Weckstein, 2004; Grossi et al., 2014; Yamagishi et al., 2014), and formal co-phylogenetic analyses typically result in at best partial congruence between host and louse trees (e.g., Johnson et al., 2021). Moreover, head lice are as common on first-year cuckoos, a known brood-parasite in which parents never come into contact with their chicks in the nest, as on older cuckoos (Brooke and Nakamura, 1998), indicating that transfer of lice between older and younger birds may happen outside the mating season.

## Transfer of head lice

In our two datasets, there are either as many inferred host-switches as cospeciation events (Expanded dataset; Figure 6), or the host-louse association patterns are not significantly different from random (Bulbul-only dataset; Figure 5), indicating no strong signal of

co-speciation. One interpretation of this data is that successful host-switches have been common within the *Philopteroides*-bulbul group, as perhaps indicated by the presence of three different louse species on *Ixos maclellandii* (species 1, 3 and 4; Figure 4) and the apparent lack of species limits between lice on *Alophoixus flaveolus* and *A. pallidus* (species 1; Figure 4). As all bulbuls belong to the same family, and many of our bulbul hosts are congeneric, this may indicate that successful host-switches are more likely between closely related hosts, and that cophylogenetic analyses within a head louse genus will typically result in mixtures of host-switching and cospeciation. Over time, host-switches between distantly related hosts would be expected to be less and less frequent, perhaps even impossible, resulting in more congruence between trees that take longer time spans into account. However, even in the larger dataset, there are cases of distantly related hosts being parasitized by closely related lice, for instance the *Philopteroides* from *Arachnothera magna* and *Aethopyga saturata* (Passeriformes: Nectariniidae) being closely related to lice from hosts in the Corvidae radiation (*Philopteroides* group 2; Figure 3). Host-switching thus appears to be an ongoing process within head louse evolution, both between distantly and closely related hosts.

This raises the question: how do head lice on different host groups overcome the apparent limitations to spread from one host to another, especially when the hosts are of different species? Not only do lice lack free-living stages, but head lice are uniquely disadvantaged by having a gross morphology that is adapted to the head of the bird, and are limited in their habitat to areas of the birds that do not easily get into contact with birds of other species. Presumably, transfer of head lice

TABLE 3 Summary of findings in phylogenetic or co-phylogenetic studies on head lice.

Louse genus	Host group	Result	Source
<i>Alcedoecus</i>	Coraciiformes: Alcedinidae	No evidence of cospeciation	Catanach et al. (2019)
<i>Anatoecus</i>	Anseriformes: Anatidae	Samples of one species conspecific across six host species, but other species possibly more restricted	Grossi et al. (2014)
<i>Austrophilopterus</i>	Piciformes: Ramphastidae	No evidence of cospeciation	Weckstein (2004)
<i>Brueelia</i> -complex	Coraciiformes, Cuculiformes, Passeriformes, Piciformes: various families	"head louse ecomorph was significantly associated with incongruent associations"	Sweet et al. (2018)
<i>Penenirmus s. lat.</i>	Passeriformes & Piciformes: various families	Host switches as common as cospeciation events	Johnson et al. (2021)
<i>Philopteroides</i>	Passeriformes: Pycnonotidae	Host switches more common than or as common as cospeciation events for both datasets	This study
<i>Philopterus</i> -complex	Passeriformes: various families	Mixture of host generalists and host specialists	Najer et al. (2021)
	Coraciiformes: Momotidae; Galbuliformes: Bucconidae, Galbulidae; Passeriformes: various families; Trogoniformes: Trogonidae	No evidence of co-speciation	Kolencik et al. (2022)
<i>Saemundssonina</i>	Charadriiformes: Laridae	Samples conspecific on six host species	Yamagishi et al. (2014)

between host species depends primarily on two main groups of routes: (1) routes that involve direct physical contact between the head of one host species and any feathered part of the body of another, and: (2) routes that do not involve physical contact between two hosts.

The first group of transmission routes involve a diverse array of host interactions. Interspecific allopreening has been observed for many different bird species (e.g., Selander et al., 1961; Harrison, 1965; Verbeek et al., 1981; Mo, 2016) and could conceivably allow head lice to transfer between species. In some species pairs, interspecific allopreening may be relatively common (e.g., caracaras and New World vultures; Ng and Jaspersen, 1984; Lopes Palmeira, 2008; Souto et al., 2009), although in this case the two host groups do not share any head louse genera (Price et al., 2003), and the New World vultures have nude heads and thus cannot support head louse populations. Interspecific aggression or defense that includes pecking, biting or other attacks with the head may be a possible route. This may include cases of nest site defense (e.g., Cordero and Senar, 1990), defense against nest predation (e.g., Ehrlich and McLaughlin, 1988), or aggression at feeding sites (e.g., Minock, 1972). Host kleptoparasitism may also be a possible route (Hopkins, 1942). Finally, interspecific feeding may be a possible route to transmission of head lice (Muszalski Shy, 1982; Jiang et al., 2016; Harmackova, 2021).

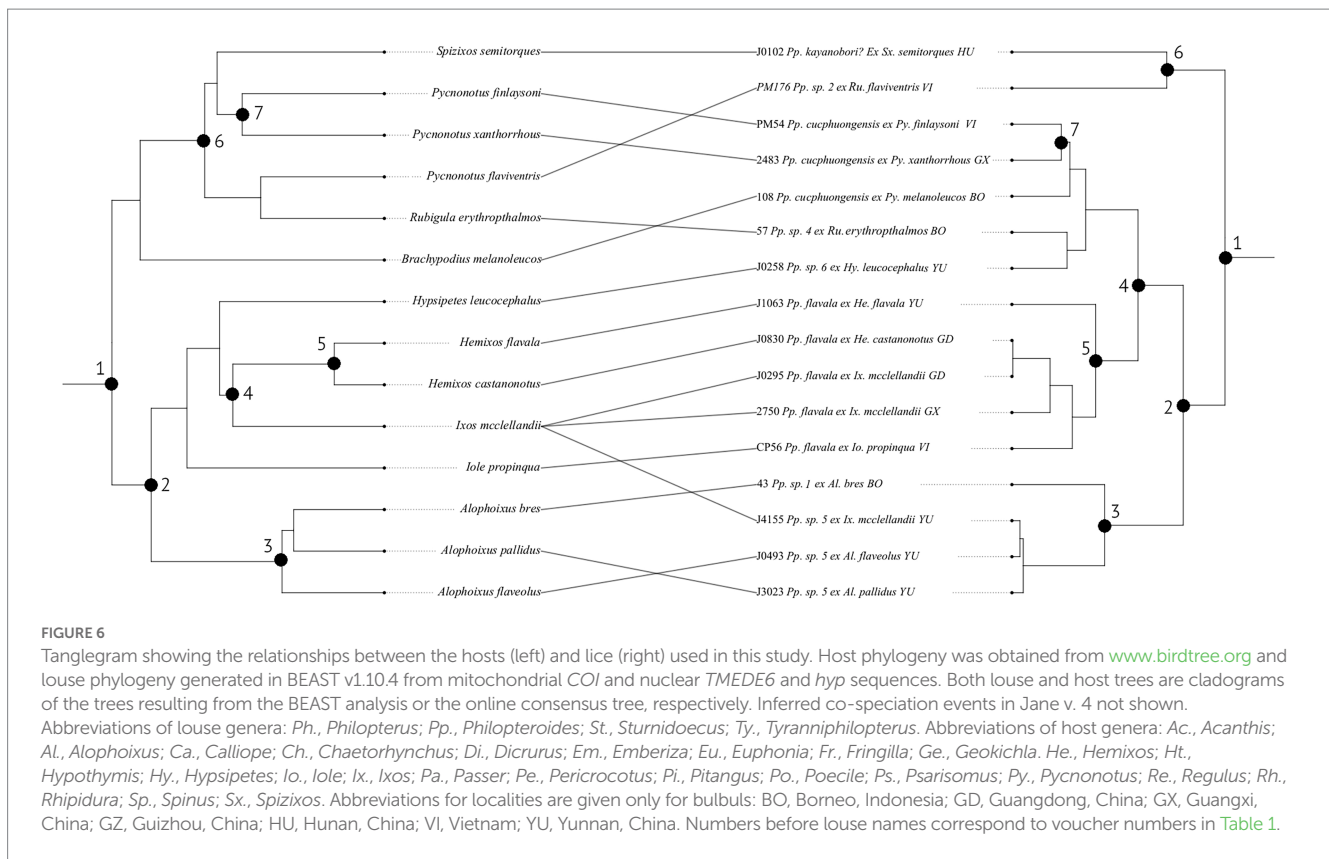
The second group of possible transmission routes may be more limited, as head lice or their eggs need to remain on feathers left behind by birds. Transmission through sequentially shared nests has repeatedly been suggested as a possible transmission route (Harrison, 1915; Eveleigh and Threlfall, 1976; Clayton, 1990; Johnson et al., 2002; Weckstein, 2004). However, as the lifespan of lice and eggs are short (Price et al., 2003), this relies on short time frames between one bird moving out and the next moving in. Nest usurpation, in which one bird moves into an active nest and evicts the previous birds, is relatively common (Lindell, 1996), and may be more important than sequential nest use. Notably, both sequential nest use and nest usurpation has been observed in a range of different birds, often including interspecific interactions, and may result in mixed broods (e.g., Pulliainen and Saari, 1991; Samplonius and Both, 2013; Kubelka et al., 2014; Chedad et al., 2022). Theft of nest material may be another route of transmission falling into this category (Jones et al., 2007; Thompson et al., 2017), especially in cases where nest theft results in

defense behavior (Tortosa and Redondo, 1992), but this would likely be limited to cases where feathers are stolen.

Presumably, different mechanisms are more important to different groups of head lice. Moreover, the importance of phoresy on hippoboscid flies for transmission of head lice requires further study. Of the 135 records of phoretic lice that were identified to at least genus level listed by Lee et al. (2022), 25 records (18.5%) refer to head lice. However, 18 of those 25 records (72%) refer to a single genus of lice, *Sturnidoecus*, and another 6 records (24%) refer to lice in the genus *Philopterus s. lat.* The only remaining record belongs to the genus *Strigiphilus* on an owl. No records of phoretic lice in speciose head louse genera such as *Saemundssonina*, *Ibidoecus*, *Penenirmus*, or *Anatoecus* have been published, although hippoboscid flies are known from the hosts of at least some of these head louse genera (Maa, 1969). Possibly, phoretic head lice have been overlooked.

In the specific case of *Philopteroides* from bulbuls, no records of phoretic lice are known, and host behavioral data of bulbuls that could be used to ascertain whether the other mechanisms discussed above are valid transmission routes are limited. Bulbuls are frequent members of mixed-species flocks (e.g., McClure, 1967; Partridge and Ashcroft, 1976; Hino, 2002; Kotagama and Goodale, 2004; Zou et al., 2011; Srinivasan et al., 2012; Sridhar and Shanker, 2014; Chandran and Vishnudas, 2018), and intraspecific kleptoparasitism has been observed in some bulbuls (Partridge and Ashcroft, 1976). Significantly, heterospecific bulbul assemblages may feed at the same fig trees, and interspecific aggression at these feeding sites have been reported repeatedly (e.g., Sanitjan and Chen, 2009; Wydhayagarn et al., 2009; Sreekar et al., 2010; Kamtaeja et al., 2012; Shermila and Wikramsinghe, 2013; Balakrishnan, 2014). In at least one case, mid-air fights between feeding bulbuls have been reported, in which birds grasped each other and fell to the ground (Sreekar et al., 2010). Bulbuls have also been reported to fight with or be attacked by other birds (e.g., Clunie, 1976; Blanvillain et al., 2003), and at least one case of nest material theft from a bulbul nest has been published (Mahesh et al., 2010). However, the potential of these interactions as transmission routes of *Philopteroides* lice between bulbul hosts is unknown and would need to be tested by targeted collection from bulbuls in mixed flocks.

Nevertheless, such transmission evidently occurs. Many species of *Philopteroides* from bulbuls included in our dataset occur on more than



one host species, and lice from *Ixos maclellandii* were divided into more than one species (Figure 4). The morphospecies *Philoateroides cucphuongensis* occurs on three different bulbul species from Borneo (*Brachypodius melanoleucos*), Vietnam (*Pycnonotus finlaysoni*), and South China (*Pycnonotus xanthorrhous*). This diversity of hosts for many species of bulbul *Philoateroides* was previously suggested by, e.g., Gustafsson et al. (2022a) on a morphological basis, although that study underestimated the diversity of *Philoateroides* lice on bulbuls somewhat. Our genetic data suggests that several undescribed species of *Philoateroides* may occur on bulbuls, including a species largely confined to hosts in the genus *Alophoixus*, specimens of which were considered conspecific with *Philoateroides flavala* by Gustafsson et al. (2022a). More detailed examinations of the morphology of these specimens are needed before they can be described formally.

## Relationships within *Philoateroides*

Our analysis confirmed the results of Najer et al. (2021), which indicated that the *Philoateroides* parasitizing bulbuls form a closely related group of species. Kolencik et al. (2022) also recovered a clade of African *Philoateroides* from bulbuls. However, differences in gene selection between our study and that of Kolencik et al. (2022) prevented us from testing whether these two clades are closely related. Most African bulbuls form a separate radiation from the Asian species, but the Asian bulbul species have colonized Africa several times (Shakya and Sheldon, 2017).

Notably, both Najer et al. (2021) and Kolencik et al. (2022) found a second group of *Philoateroides*. Najer et al. (2021) called this group “*Philoaterus* complex group 3,” whereas Kolencik et al. (2022) called this group “*mitsusui* species-group.” We also recovered a second group of *Philoateroides* in our analysis. This group includes specimens from monarch flycatchers, sunbirds, and some other hosts (Figure 3):

“*Philoateroides* group 2 (non-bulbul).” This group includes some of the samples published by Najer et al. (2021), and some of the samples in the “*mitsusui* species-group” clade of Kolencik et al. (2022) are from monarch flycatchers, which may be closely related to those included in our analysis. The relationship between the bulbul-group *Philoateroides* group and the second group of *Philoateroides* is not resolved in either of these three studies.

Morphologically, specimens from the two *Philoateroides* groups we have examined are not generically separable. Mey (2004) included *Philoateroides* in two different morphotypes in his outline of the structure of the preantennal area (Mey, 2004; Figure 4), but the differences between morphotypes D1 and D2 appear to be gradual and may be species-level differences only. Moreover, neither of these groups in our data represent the “*beckeri*” species group as described by Valim and Palma (2013), contra Kolencik et al. (2022). The morphology of the dorsal anterior plate may be different between the “bulbul” and “monarch flycatcher” groups (cf. Najer et al., 2012b; Gustafsson et al., 2022a), but few species of either group are known. As noted by Gustafsson et al. (2022b) for *Philoaterus*, somatic and setal characters may not be very useful for taxon delimitation in *Philoateroides*, and more detailed studies of the male genitalia may be necessary. For the present, no taxonomic recommendations about the status of these two groups, and the *beckeri*-species group, can be made.

Both groups of *Philoateroides* in our dataset include undescribed species. Preliminary examinations of the specimens from *Alophoixus* spp. (species 1; Figure 4) indicate that this species appears to be morphologically indistinguishable from *Philoateroides flavala*. Similarly, the specimens from *Hemixos flavala* (species 6; Figure 4) and *Hemixos castanonotus* (species 3; Figure 4) appear morphologically similar and were treated as conspecific by Gustafsson et al. (2022a). In both cases, specimens from non-type

hosts of *Pp. flavala* may be cryptic species impossible to separate morphologically, but detailed examinations of the male genitalia need to be performed before this can be ascertained. The deep branches separating the species identified as *Philopteroides cucphuongensis* (species 9–11; Figure 4) may also indicate that this is a complex of cryptic species; we have not seen these specimens, and cannot ascertain whether differences in, e.g., male genitalia can be used to separate these populations into different species. Finally, a new species from *Hypsipetes leucocephalus* (species 8; Figure 4) may be close to *Philopteroides holosternus* (not included in analyses). These species will be described in a separate manuscript.

Three different species of lice were recovered from *Ixos mccllellandii* (species 1, 3, 4; Figure 4). One of these species is otherwise known from *He. castanonotus*, one from *Alophoixus* spp., whereas the specimen from Najer et al. (2021) is a singleton. Neither of the specimens we examined correspond to *Philopterus haerixos* described from this host by Gustafsson et al. (2022a), but the specimen derived from Najer et al. (2021) was not seen, and may represent this species. No other species of bulbuls were caught on the days our samples were collected, indicating that these specimens are not contaminations. Potentially, these records indicate recent host-switches to *I. mccllellandii* from other host species. Removing these two specimens under the assumption that they represent fortuitous collections of stragglers did not significantly affect the cophylogenetic analysis (Table 2). This suggests that the overall lack of significant co-speciation between *Philopteroides* and its bulbul hosts may be genuine, and not affected by the presence of stragglers in the dataset.

Asian bulbuls are divided into two major clades (Oliveros and Moyle, 2010; Shakya and Sheldon, 2017; Jha et al., 2021). Our dataset includes louse samples from both of these radiations: the host genera *Alophoixus*, *Hemixos*, *Hypsipetes*, *Iole* and *Ixos* from Clade 2 (*sensu* Jha et al., 2021), and *Brachypodius*, *Pycnonotus*, *Rubigula* and *Spizixos* from Clade 1 (*sensu* Jha et al., 2021). However, the lice do not form two clades that correspond to those of the hosts (Figure 4). The deepest supported division of bulbul *Philopteroides* in our dataset is between three different clades (Figure 4), one of which is divided into two major subclades. *Philopteroides* Clades A and B1 include only specimens from hosts in bulbul Clade 2, *Philopteroides* Clade C includes only specimens from hosts in bulbul Clade 1, and *Philopteroides* Clade B2 includes a mixture of specimens from bulbul Clades 1 and 2 hosts. The basal split within our bulbul *Philopteroides* phylogeny was inferred by Jane to be a co-speciation event (event 1 in Figure 5), suggesting that the early history of these bulbul lice may have been characterized by cospeciation, but that such patterns have later been eroded due to host switching.

More samples from a larger variety of bulbuls are needed before a thorough analysis of host association patterns can be performed. However, even in this limited dataset there are some interesting patterns. For instance, the relationships among lice from *Alophoixus* spp. closely mirror that of their hosts, with lice from the Bornean population of *Al. bres* well separated from those from mainland hosts. The lice from *Al. flaveolus* and *Al. pallidus* are closely related, which may be connected to the fact that their hosts comprise a ring species with gene flow between *Al. flaveolus* and *Al. pallidus* (Fuchs et al., 2015).

## Conclusion

In conclusion, our data from *Philopteroides* head lice of Chinese bulbuls indicate that a mixture of host-switching and

co-speciation with their hosts has probably shaped host association patterns in this group. Conceivably, the sociality of bulbuls, which may include heterospecific birds in the same feeding flocks, may contribute toward the lack of evidence of cospeciation in this group. However, more direct observations of lice from bulbuls would be desirable before the influence of mixed species feeding flocks can be established. The presence of three different species of *Philopteroides* on three different *Ixos mccllellandii* sampled at different localities may suggest that host switching is frequent in these lice, even if the mechanisms are currently unknown.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/genbank/>, OP476469–OP476492; OP555755–OP555801.

## Ethics statement

The studies involving animals were reviewed and approved by the Animal Care and Welfare Committee, Institute of Zoology, Guangdong Academy of Sciences [then: Guangdong Institute of Applied Biological Resources], and the lice studied for this paper were collected in strict accordance with the Regulation for the Administration of Laboratory Animals (Decree No. 2, State Science and Technology Commission of the People's Republic of China). A collection permit was previously obtained from the Panel on Laboratory Animal Care of the Institute of Zoology, Guangdong Academy of Sciences (then Guangdong Institute of Applied Biological Resources). In addition, specific collecting permits were obtained from forestry bureaus in the respective provinces (no number on permits; copies available on request).

## Author contributions

MR performed fieldwork, extracted DNA, and performed analyses. DG performed fieldwork, planned and wrote the manuscript. CT performed analyses. AG wrote the manuscript. ZL helped with fieldwork. FZ helped editing the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2023.1053820/full#supplementary-material>

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