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A riddle wrapped in an enigma: parasitic lice as clues to the evolutionary puzzle of Sapayoa (Aves)

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Parasites can provide powerful insights into host evolution and biogeography. The bird *Sapayoa aenigma*, the only Neotropical member of the otherwise Old World clade Eurylaimides, has long puzzled ornithologists due to its phylogenetic placement and uncertain biogeographic origin. We investigated the evolutionary origin of a chewing louse in the genus *Myrsidea* found on *Sapayoa*. Using genome-wide data from 91 *Myrsidea* specimens from oscine, suboscine and non-passerine hosts, we reconstructed a global phylogeny to evaluate hypotheses about the origin of *Sapayoa* and its parasite. Phylogenomic, molecular dating and cophylogenetic analyses support a scenario in which the *Myrsidea lineage on Sapayoa* originated outside the Neotropics and was acquired via host-switching from an Old World oscine. The parasite's divergence time (24.4–17.9 Ma) postdates the split between *Sapayoa* and other Eurylaimides, ruling out strict codivergence. Ancestral host reconstruction supports an oscine origin and Old World acquisition, and biogeographic analysis also indicates Old World origins, though with uncertainty in the exact region. These findings support a co-dispersal scenario in which *Sapayoa* acquired its parasite in the Old World and brought it to the Neotropics. Our study highlights the value of parasites as complementary tools for disentangling complex evolutionary and biogeographic histories. A Spanish translation is available in the electronic supplementary material.

1. Introduction

Parasites are not only ubiquitous components of biodiversity but also powerful markers of host evolutionary history, biogeography and ecological interactions [1–5]. Many permanent parasites form long-term, host-specific associations, making their diversity and phylogenetic relationships valuable proxies for those of their hosts, especially in lineages with complex or ambiguous phylogenies or biogeographic origins [6,7].

Among these, parasitic lice (Insecta: Phthiraptera) are particularly valuable for such evolutionary inferences. These permanent ectoparasites stand out as one of the most diverse and best-studied ectoparasitic groups. Lice exhibit high host specificity, limited dispersal ability and primarily vertical transmission [8,9]. Their mitochondrial genomes evolve up to 10 times faster than those of their hosts, and their rapid generation times make them sensitive to recent evolutionary events [10,11]. These features make lice exceptionally useful for detecting fine-scale population structure, uncovering cryptic speciation, and identifying signatures of codivergence (parallel divergence of

parasites and hosts) or host-switching (colonization of new host lineages), ultimately serving as independent markers to uncover the evolutionary history of their hosts.

Lice have been used to study host evolution across a range of temporal and spatial scales. At the population level, for instance, they have revealed genetic structure across island systems that are sometimes undetected in the hosts themselves [12]. In highly diverse regions such as Amazonia, lice have helped trace evolutionary patterns across biogeographic barriers with remarkable resolution [13]. For instance, lice in the genus *Furnaricola* on the woodcreeper *Dendrocincla fuliginosa* show deeply divergent clades on opposite banks of the Negro River, mirroring strong genetic structure in their hosts and suggesting long-term isolation and possible cryptic speciation [13]. By contrast, lice in the genera *Myrsidea* and *Tyranniphilopterus* on the manakin *Dixiphia pipra*, a species with little population structure, exhibit minimal genetic divergence across the same barrier. These contrasting Amazonian patterns demonstrate how parasite phylogeography can illuminate host evolutionary histories at fine spatial scales. At deeper timescales, phylogenies of lice and their hosts can provide reciprocal insights. For example, the discovery that grebes are closely related to flamingos led to a reinterpretation of the direction of host switching of lice between flamingos and ducks [6]. Taken together, these examples underscore the value of lice as independent and often more sensitive indicators of host diversification, biogeography and evolutionary history. Given this potential, parasite phylogenies are especially powerful in systems where host evolutionary relationships or biogeographic origins remain debated.

A striking example is *Sapayoa aenigma* (*Sapayoa*, hereafter), a Neotropical suboscine (Passeriformes) bird whose evolutionary affinities have puzzled ornithologists for decades [14–18]. As the sole representative of the family Sapayoidae [19], *Sapayoa aenigma* inhabits a restricted range in the Chocó biogeographic region of western Colombia, Panama and Ecuador [20]. Despite its exclusively Neotropical distribution, molecular analyses have consistently placed *Sapayoa* within the Old World suboscines (Eurylaimides: broadbills, pittas and asities) rather than among geographically proximate Neotropical suboscines [14,15,17,19,21,22]. This unexpected phylogenetic placement has led to two main biogeographic hypotheses: (i) the *relict hypothesis*, which proposes that *Sapayoa* is the last surviving member of an ancient lineage that diversified within the Neotropics; and (ii) the *colonizer hypothesis*, which suggests that *Sapayoa* dispersed to South America from an Old World ancestor [15,19,21]. These alternatives reflect broader hypotheses about the origin and dispersal routes of Eurylaimides (Old World suboscines), including when and how this lineage colonized or retreated from the Neotropics [21–23].

To evaluate these alternative hypotheses, we examined the evolutionary history of *Sapayoa*'s parasites, specifically the louse genus *Myrsidea* (Phthiraptera: Amblycera: Menoponidae). As the most species-rich genus of avian lice, *Myrsidea* parasitizes a wide array of passerines, as well as some non-passerines, and typically exhibits high host specificity, with most species limited to a single host species or genus [9,24]. While codivergence is common, host-switching and regional biogeographic structure are also well documented [25–27]. *Myrsidea* is currently the only louse genus reported from *Sapayoa*. Thus, its evolutionary history provides a unique opportunity to test among biogeographic scenarios proposed for *Sapayoa*, incorporating parasite-specific processes such as host associations, codivergence and host-switching into a broader framework of avian evolution.

Here, we reconstruct a global phylogenomic tree of *Myrsidea* based on genome-wide data from 92 specimens collected from oscine, suboscine and non-passerine hosts across Neotropical and non-Neotropical regions. This framework allows us to evaluate the phylogenetic placement of *Myrsidea* from *Sapayoa* and test two competing hypotheses for its origin: a Neotropical origin (*relict hypothesis*) or a non-Neotropical origin (*colonizer hypothesis*), each implying different evolutionary histories for the parasite.

Under a codivergence scenario, *Sapayoa*'s *Myrsidea* could reflect an ancient association inherited from an ancestral host lineage. If its origin lies outside the Neotropics (*colonizer hypothesis*), we would expect the louse to cluster with Old World *Myrsidea* lineages from broadbills (suboscines) and show phylogenetic patterns consistent with deep codivergence and subsequent dispersal into the Neotropics. Alternatively, if the association arose within the Neotropics (*relict hypothesis*), we might expect the lice of *Sapayoa* to fall among a clade of lice from suboscines more broadly, potentially a relict from a more diverse Neotropical assemblage.

Under a host-switching scenario, *Myrsidea* parasite lineages could have been acquired either as a recipient (switching from another host onto *Sapayoa*) or as a donor (transferring from *Sapayoa* to other passerines), and these patterns would be reflected in the phylogenetic relationships. Such events could have taken place in the Neotropics, either after *Sapayoa*'s arrival from the Old World (*colonizer hypothesis*) or as part of a long-standing Neotropical association (*relict hypothesis*). Alternatively, a host-switching event may have occurred in the Old World prior to dispersal into the Neotropics (*colonizer hypothesis*). In either case, the phylogenetic placement of *Sapayoa*'s *Myrsidea* relative to other lice provides key evidence to distinguish among these scenarios.

2. Material and methods

(a) Taxon sampling and genome sequencing

We selected 91 *Myrsidea* and one *Apomyrsidea* specimen from the parvorder Amblycera (family Menoponidae), each from a distinct host species spanning 55 avian families for genomic sequencing (electronic supplementary material, table S1). Bird taxonomy follows AviList Checklist [28]. Sampling focused on representing major passeriform lineages (oscines and suboscines) and also included *Myrsidea* from Piciformes (non-passerines) to provide a comprehensive phylogenetic context. Seven outgroup taxa from three louse genera were included based on current knowledge of amblyceran relationships [29], and the tree was rooted on the genus *Chapinia*.

Genome sequencing, gene assembly and phylogenetic analyses were conducted following protocols outlined in [30], with the detailed coding pipeline provided in the electronic supplementary material. Louse samples were obtained either through field

collections by the authors or from genetic resources collections (95% ethanol and −80 °C). We collected *Myrsidea* specimens from *Sapayoa* in its southern range (electronic supplementary material, figure S1). Whole louse individuals were entirely consumed during the extraction process, so voucher photographs were taken prior to extraction (available at [31]).

DNA was extracted using the Qiagen QIAamp DNA Micro Kit, with a 48 h incubation at 55 °C. Libraries were prepared with the UltraLow input kit from Tecan, indexed, pooled at 48 samples per lane, and sequenced on an Illumina NovaSeq X Plus (150 bp PE reads) platform, aiming to achieve 30–60× nuclear coverage. Reads were demultiplexed with bcl2fastq v. 2.20 and deposited in NCBI SRA (electronic supplementary material, table S1).

(b) Gene assembly and phylogenomic analysis

Reads were trimmed using fastp v. 0.20.1 (Q ≥30) [32], and aTRAM v. 2.3.4 [33] was used to assemble 2395 single-copy orthologues against a reference panel of protein-coding genes from *Pediculus humanus* using tblastn and ABySS over three iterations. Exons were stitched with *Exonerate* [34]. Genes that were assembled from fewer than four samples were excluded (36 in total), leaving 2359 genes for analysis.

For each gene, DNA sequences from individual samples were concatenated using a custom R script. Nucleotide sequences were translated into amino acid sequences, then aligned using MAFFT v. 7.471 [35,36] and back-translated to nucleotides. Alignments were trimmed with trimAL (gap threshold = 0.4%) [37] and concatenated with AMAS v. 1.0 [38]. Phylogenetic reconstruction was conducted with IQ-TREE2 v. 2.1.2 [39] under a partitioned maximum likelihood model (-m TESTMERGE option, -p) [40,41] and -rclusterf 10 [42]. These parameters were then used in a search using the IQ-TREE algorithm [43]. Node support was assessed with 1000 ultrafast bootstrap (UFBoot2) [44,45]. ML gene trees were inferred with IQ-TREE2 (-m MFP), and species trees were estimated using ASTRAL-III under the multispecies coalescent model [46] which also computed local posterior probabilities for each node.

(c) Molecular dating and biogeographic analysis

Molecular dating was conducted using LSD2 (least squares dating [47]); on the maximum likelihood (ML) tree. Based on a recent dating of Amblycera [29], we incorporated two secondary calibrations within the outgroup genus *Dennyus* and used the upper bound of the 95% confidence interval (54.3 Ma) as a soft maximum for the root calibration—an approach commonly employed in louse molecular dating studies [29,30,48]. This calibration corresponds to the estimated divergence between the *Myrsidea* ingroup and the designated outgroup (*Chapinia*). Najer *et al*. [29] derived their calibrations from codivergence-based estimates in previous studies [30,49] and incorporated a minimum age of 44 Ma for the family Menoponidae, based on the only described bird louse fossil [50], to which *Myrsidea* belongs. For more recent (terminal) nodes, we incorporated codivergence events identified through our cophylogenetic analysis (see below), using host divergence times from TimeTree (timetree.org) [51]. We used the median divergence time or the 95% confidence interval, depending on availability (electronic supplementary material, table S2). A branch length constraint (u = 0.01) was applied, and 1000 bootstrap replicates were used to estimate confidence intervals.

Biogeographic analyses were performed in BioGeoBEARS v. 1.1.2 [52,53] using the ultrametric dated tree (ingroup taxa only). For the biogeographic framework, geographic regions followed Bush *et al*. [54], based on Olson *et al*. [55], with six defined zones: Neotropical, Nearctic, Afrotropical, Palaearctic, Indo-Malayan and Australasian. This scale is appropriate for a globally distributed group such as *Myrsidea* and facilitates consistency with previous avian and louse biogeographic analyses. We compared DEC, DIVALIKE and BAYAREALIKE models with and without founder-event speciation (+J), selecting the best-fit model by AIC, and then used it to infer the most likely ancestral ranges across the tree.

(d) Cophylogenetic analysis and ancestral host reconstruction

Cophylogenetic reconstruction between hosts and parasites was conducted in eMPRess v. 1.0 [56] using a reconciliation analysis, which maps parasite phylogenies onto host phylogenies to infer codivergence, duplication, sorting (loss), and host-switching events under a parsimony framework. Costs were set to duplication = 1, sorting = 1 and host-switch = 2, following the standard cost scheme used in avian lice. Under this cost scheme, equal weight is given to resolving incongruence between trees under a host-switching versus a duplication/sorting scenario, because it takes both a duplication and sorting event to resolve an incongruence, whereas only a single host-switching event would be needed. Host trees were generated from 1000 BirdTree (Hackett backbone) trees (birdtree.org) [57,58] and summarized with TreeAnnotator in BEAST v. 1.10.4 [59]. The parasite tree was derived from our ML phylogeny. We compared observed MPR costs with those from 100 randomized parasite trees to test for significant codivergence. Additionally, we reconstructed ancestral host associations (oscines, suboscines, piciforms) on the dated phylogeny using maximum likelihood in R with the ape and phytools packages [60,61]. A discrete Mk model was used, and the best-fit model was selected based on AIC. Results were visualized with pie charts and stochastic character mapping.

3. Results and discussion

Our phylogenomic reconstruction, based on >2300 loci from 91 *Myrsidea* samples and using both concatenated (figure 1) and coalescent (electronic supplementary material, figure S2) approaches, yielded robust phylogenies. Bootstrap support

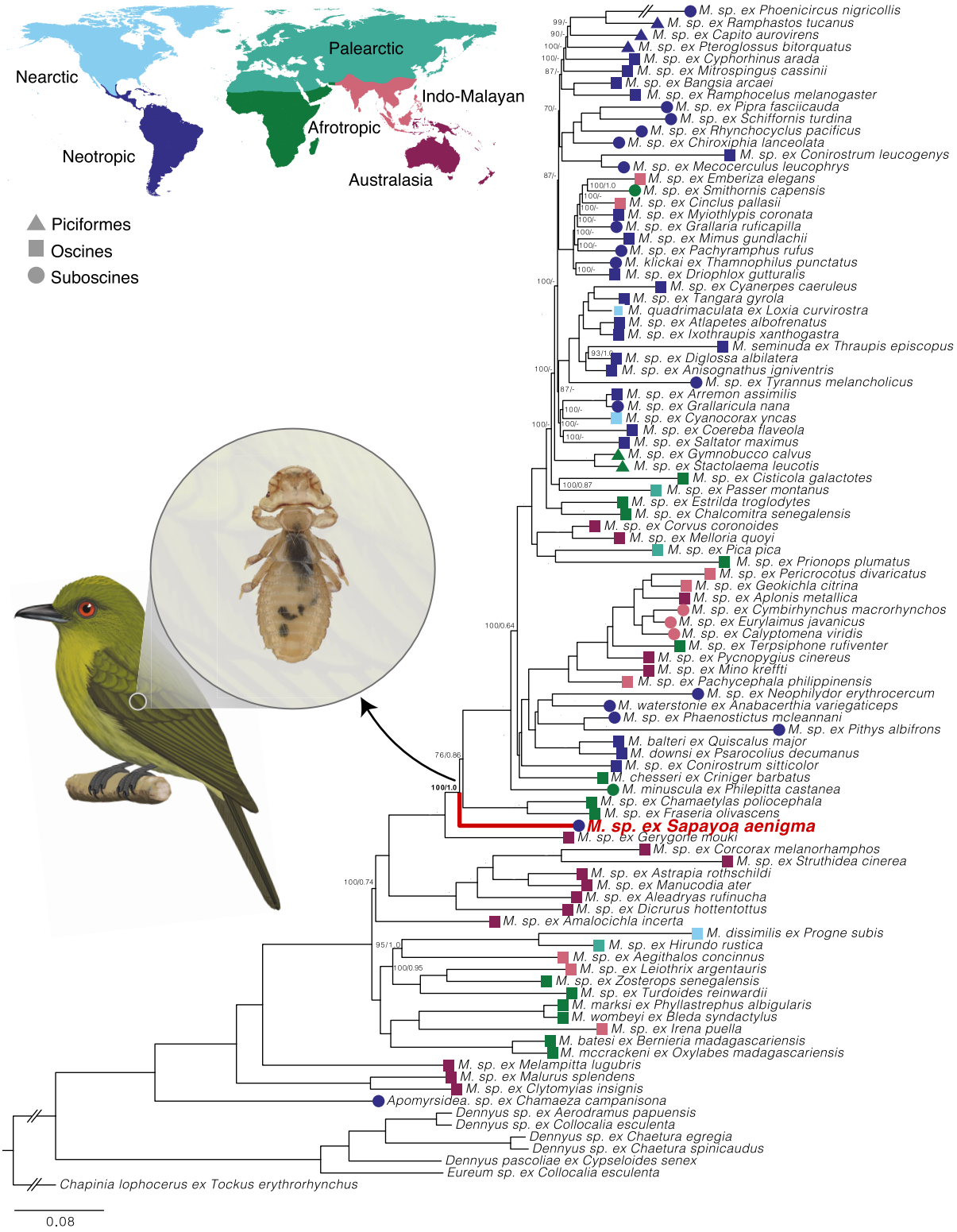

Figure 1. Phylogenomic tree of *Myrsidea* and outgroup taxa from a partitioned maximum-likelihood (ML) analysis in IQ-TREE using >2300 single-copy orthologues. Branch lengths represent substitutions per site. Node support values show ultrafast bootstraps (IQ-TREE) and local posterior probabilities (ASTRAL); when absent, support is 100/1, respectively. Dashes (–) indicate branches not recovered in the ASTRAL topology, and double lines on branches denote segments that were shortened for display purposes. Symbols at tips denote major host groups (Piciformes, suboscines, oscines) coloured by biogeographic realm, as shown on the map. *Myrsidea* names are abbreviated as *M.* followed by parasite and host species. The *Myrsidea* associated with *Sapayoa* is highlighted in red. Inset: *Sapayoa aenigma* and its *Myrsidea* sp. (not to scale). Bird illustration © Avifauna Colombiana, F. Ayerbe-Quiñones.

was generally high (100%) at deep and shallow nodes, but was lower among a few Neotropical clades, likely due to rapid diversification. Notably, the *Myrsidea* lineage from *Sapayoa* forms a distinct, well-supported (100%) clade that diverges early in the tree (figure 1, highlight in red), diverging earlier than most other Neotropical and some non-Neotropical lineages. This lineage is sister to a diverse assemblage of lice from both oscines (diverse groups) and suboscines (Eurylaimides, Tyrannides and Furnariides). Of note is that this louse does not cluster particularly near to any suboscine-associated lice from families previously proposed as *Sapayoa*'s closest relatives; rather, it falls among a grade of lice from otherwise Old World oscine

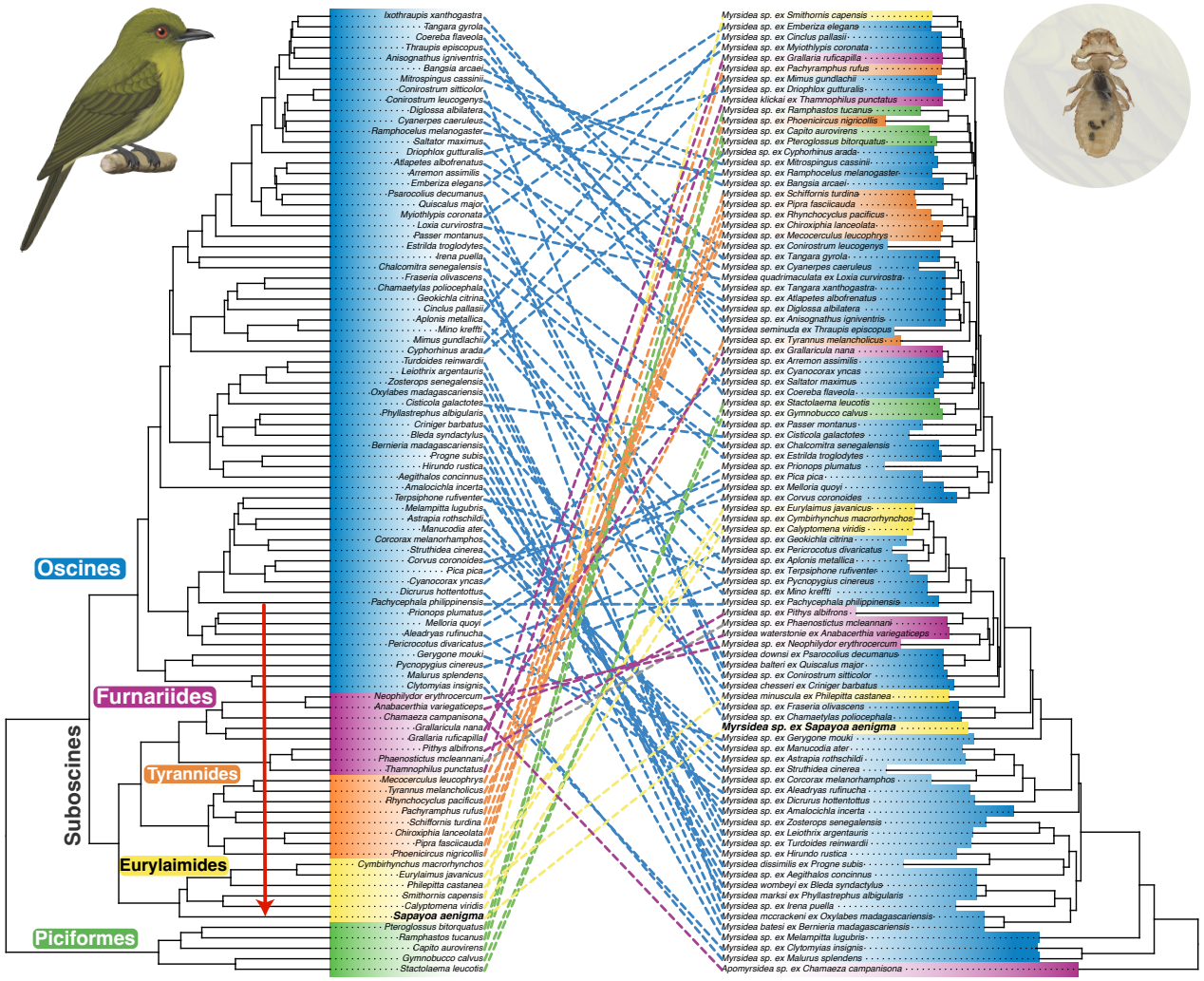

Figure 2. Tanglegram showing the cophylogenetic relationships between *Myrsidea* louse (right) and their avian hosts (left). Colours denote major host bird groups and are used for both tip labels and the connecting lines, highlighting cophylogenetic structure across lineages; trees were rotated to minimize line crossings. The red arrow marks the direction of the host switch inferred from the reconciliation analysis in eMPRess, representing the event that gave rise to the *Myrsidea* lineage currently found on *Sapayoa aenigma*. Bird illustration: © Avifauna Colombiana, F. Ayerbe-Quiñones.

passerines. The phylogenetic placement of *Sapayoa* itself has long been debated, with proposed affinities to the eurylaimid broadbills and asities [17], the pittas [21], the calyptomenid broadbills [19] or even as sister to all other Old World suboscines [62]. However, our parasite phylogeny does not support a particularly close association with lice from any of these clades, highlighting the unique evolutionary trajectory of the *Myrsidea* lineage on *Sapayoa* (figure 1).

Molecular dating places the origin of the *Myrsidea* lineage found on *Sapayoa* between 24.4 and 17.9 Ma, during the Miocene (electronic supplementary material, figure S3). This timing postdates the estimated divergence between *Sapayoa* and its Old World relatives Eurylaimides, which has been inferred to have occurred between 52.5 and 28 Ma [17,21–23]. These earlier estimates suggest that *Sapayoa* had already diverged as a distinct lineage before acquiring its *Myrsidea* lice. Despite the broad range of host divergence estimates, our results consistently indicate that the *Myrsidea* lineage on *Sapayoa* arose after the bird had already split from other Eurylaimides.

This temporal disconnect points to a host-switching event rather than codivergence, a pattern further supported by our cophylogenetic analyses. The parasite and host phylogenies are topologically incongruent; that is, the *Myrsidea* lineage found on *Sapayoa* does not occupy a phylogenetic position that mirrors the placement of *Sapayoa* within the avian host tree (figure 2). Instead, reconciliation analyses suggest that the *Myrsidea* lineage on *Sapayoa* originated through a host-switching event from an oscine lineage. Specifically, the most likely donor is a lineage of *Myrsidea* parasitizing an ancestor of the lineage leading to *Pachycephala philippinensis* (yellow-bellied whistler) (figure 2), an oscine species distributed in the Indo-Malayan region.

This finding is further supported by ancestral host reconstruction, which provides another line of evidence for an oscine origin of the *Myrsidea* lineage on *Sapayoa*. Despite its suboscine host, this lineage is nested within an oscine-associated clade, suggesting that its ancestors also parasitized oscines (figure 2, electronic supplementary material, figure S4). Moreover, this analysis indicates that the most recent common ancestor of *Myrsidea* parasitized oscines is a pattern consistently retained across the deepest nodes of the phylogeny. Together, these results support a scenario in which *Myrsidea* originated in oscines and later colonized suboscines and piciforms through multiple, independent host-switching events, including the acquisition of the *Sapayoa* lineage via a host switch from an Old World oscine.

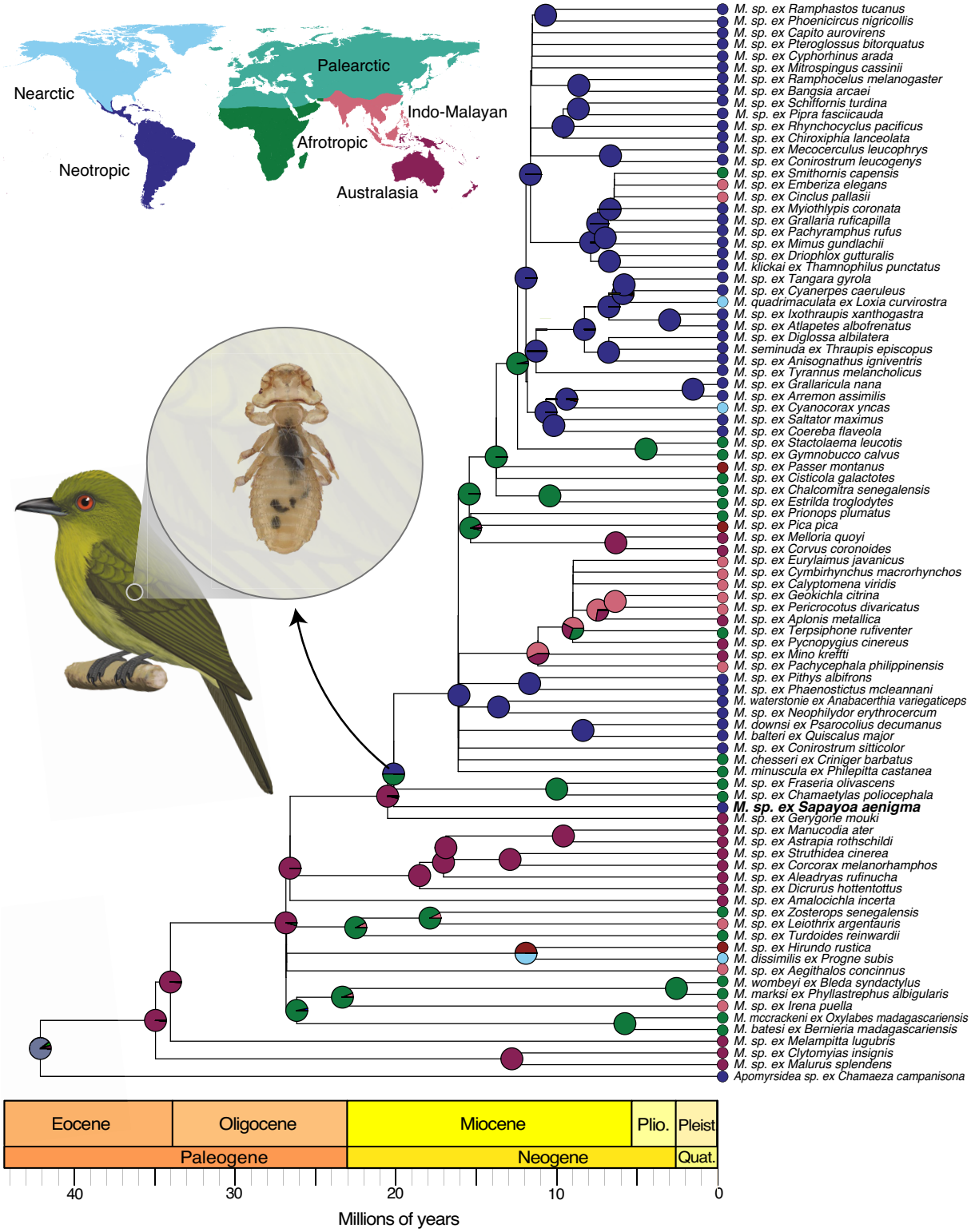

Figure 3. Ultrametric tree of *Myrsidea* lice from least-squares dating (LSD2), with ancestral range reconstruction in BioGeoBEARS under the DIVALIKE+J model. Tip circles show current distributions across six biogeographic regions. Node pie charts represent relative likelihoods of ancestral ranges. *Myrsidea* names are abbreviated as *M.* followed by the parasite species and the host species name. The arrow marks the *Myrsidea* lineage from *Sapayoa aenigma*; illustrations not to scale. *Sapayoa* illustration: © Avifauna Colombiana, F. Ayerbe-Quiñones. Geological timescale shown at bottom.

In addition to host-association patterns, biogeographic reconstruction provides insights into the history of the *Myrsidea* lineage on *Sapayoa* (figure 3). Under the best-fit model (DIVALIKE+J) (electronic supplementary material, table S3), the ancestral range at the node uniting this lineage with its sister clade is ambiguous, with probabilities divided almost equally between Neotropical and Afrotropical origins. However, the prior node is strongly supported as Australasian, providing support for ultimate origin in the Old World. Across the phylogeny, *Myrsidea* lineages generally exhibit strong geographic structure, with clades corresponding to distinct regions. Given the placement of the *Sapayoa* lineage among Old World oscine-associated lineages and its divergence time, the most plausible scenario is that it was acquired in the Old World from oscine passerine and co-dispersed with *Sapayoa* during colonization of the Neotropics.

This co-dispersal hypothesis offers an independent line of evidence that informs debates about the biogeographic origins of *Sapayoa* and the broader Eurylaimides group. Three main scenarios have been proposed under the relict and colonizer hypotheses. The first scenario (i) suggests a South American origin of Eurylaimides, in which the entire group arose in South America (approx. 37 Ma) [21]. The ancestor of the core Eurylaimides dispersed northward across the Caribbean, through North America and into Asia via Beringia. In this scenario, *Sapayoa* represents a relict lineage that remained in the Neotropics after the early divergence of other Eurylaimides. A second scenario (ii) posits an African origin of core Eurylaimides, in which suboscines, including the ancestor of *Sapayoa*, originated in South America, followed by an early divergence (approx. 28 Ma) that left *Sapayoa* isolated in the Neotropics [23]. The remaining Eurylaimides dispersed from South America to Africa via island chains (Atlantogea) and subsequently expanded into Asia. The third scenario (iii) suggests a Eurasian origin of Eurylaimides, in which the group originated in Eurasia and dispersed rapidly across continents during the Late Oligocene warming (approx. 28–23 Ma). Favourable climatic conditions and habitat corridors at the time likely facilitated intercontinental movement, enabling lineages (including *Sapayoa*) to reach the Americas via Africa or directly through connected landmasses [22]. However, *Sapayoa* may represent the only surviving lineage from such dispersal events. Although early passerine fossils are rare, a recently described early Oligocene specimen from Europe suggests that Tyrannida—a lineage now restricted to the New World—may have been distributed in the Old World in the past before disappearing. The authors note that this biogeographic pattern echoes that of *Sapayoa*, with both cases involving Oligocene transatlantic movement driven by similar dispersal dynamics. This reverse pattern provides independent evidence for the possibility of early transatlantic dispersal in suboscines, paralleling—though in the opposite direction—the scenario proposed for *Sapayoa* [63].

The integration of parasite genomic data with host evolutionary history reveals a compelling case in which host-switching and long-distance intercontinental co-dispersal jointly shaped the origin of a unique host–parasite association. Rather than mirroring *Sapayoa*'s own phylogenetic trajectory, the *Myrsidea* lineage found on this bird reflects a distinctive evolutionary and biogeographic history, shaped by an ancestral louse on oscines and subsequent colonization of a suboscine host. Crucially, this parasite-based perspective offers an independent line of evidence in the long-standing debate over *Sapayoa*'s origin (whether it arose within the Neotropics or arrived there from the Old World). Among the three main scenarios proposed for *Sapayoa*'s biogeographic history, our findings most closely align with the Eurasian origin scenario [22]. The divergence between the *Myrsidea* on *Sapayoa* and its common ancestor is estimated between 24.4 and 17.9 Ma, which falls within the timeframe of the Late Oligocene warming, further supporting this scenario. Under this model, *Sapayoa* and its parasite could have dispersed together from the Old World into the Neotropics, providing a rare case of host–parasite co-dispersal across biogeographic realms and supporting an extra-Neotropical origin for this enigmatic bird.

Our study highlights the power of host–parasite systems to illuminate patterns of diversification, dispersal, and host association beyond what is possible through host data alone. In the case of *S. aenigma*, an example of a bird species with an unresolved biogeographic history, its lice provide a window into past evolutionary events, supporting a likely scenario of Old World origin and host-switching prior to colonization of the Neotropic. More broadly, our findings underscore the value of parasite phylogenies for testing hypotheses about host evolution and movement, particularly in taxa with ambiguous or unresolved histories (such as those classified as *incertae sedis*), where traditional host-based phylogenies have struggled to clarify evolutionary origins. As parasite genomic resources continue to grow, future studies will be increasingly equipped to uncover similar coevolutionary dynamics across avian radiations and major biogeographic transitions.

Ethics. Research on animals was conducted according to University of Illinois IACUC protocols 10119, 13121 and 15212. *Sapayoa aenigma* louse sample was collected under permits granted by the Colombian Ministry of Environment to Instituto Alexander von Humboldt (decree 1376). Specimens were deposited in the Colección Nacional de Aves, Instituto de Ciencias Naturales, Universidad Nacional de Colombia (registry RNC no. 5). Colombian sample export to the USA was authorized by the Agencia Nacional de Licencias Ambientales (ANLA export permit no. 3470).

Data accessibility. Data reported in this paper are deposited in NCBI SRA (see electronic supplemenntarysupplementary material, Table S1). Concatenated data matrix, gene alignments, gene trees, and all tree files are available from the Figshare Repository [64]. A Spanish translation of the manuscript is provided in the Supplementary material.

Supplementary material is available online [65].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. J.S.-P.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing—original draft; J.D.: formal analysis, methodology, visualization, writing - review and editing; K.P.J.: conceptualization, funding acquisition, investigation, methodology, resources, supervision, writing - review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Nieberding CM, Olivieri I. 2007 Parasites: proxies for host genealogy and ecology? *Trends Ecol. Evol*. **22**, 156–165. (doi:10.1016/j.tree.2006.11.012)
2. Page RDM. 2003 Introduction. In *Tangled trees: phylogeny, cospeciation and coevolution* (ed. RDM Page), pp. 1–21. Chicago, IL: The University of Chicago Press.
3. Štefka J, Hoeck PE, Keller LF, Smith VS. 2011 A hitchhikers guide to the galápagos: co-phylogeography of galápagos mockingbirds and their parasites. *BMC Evol. Biol*. **11**, 284. (doi:10.1186/1471-2148-11-284)
4. Thomas F, Verneau O, de Meeûs T, Renaud F. 1996 Parasites as to host evolutionary prints: Insights into host evolution from parasitological data. *Int. J. Parasitol*. **26**, 677–686. (doi:10.1016/0020-7519(96)00023-9)
5. Whiteman NK, Parker PG. 2005 Using parasites to infer host population history: a new rationale for parasite conservation. *Anim. Conserv*. **8**, 175–181. (doi:10.1017/s1367943005001915)
6. Johnson KP, Kennedy M, McCracken KG. 2006 Reinterpreting the origins of flamingo lice: cospeciation or host-switching? *Biol. Lett*. **2**, 275–278. (doi:10.1098/rsbl.2005.0427)
7. Klimov PB, Mironov SV, OConnor BM. 2017 Detecting ancient codispersals and host shifts by double dating of host and parasite phylogenies: application in proctophyllodid feather mites associated with passerine birds. *Evolution* **71**, 2381–2397. (doi:10.1111/evo.13309)
8. Clayton DH, Bush SE, Johnson KP. 2016 *Coevolution of life on hosts: integrating ecology and history*. Chicago, IL: The University of Chicago Press. (doi:10.7208/chicago/9780226302300.001.0001)
9. Price RD, Hellenthal RA, Palma RL, Johnson KPC. 2003 *The chewing lice: world checklist of chewing lice with host associations and keys to families and genera*. Champaign, IL: Illinois Natural History Survey. (doi:10.5962/bhl.title.154191)
10. Clayton DH, Johnson KP. 2003 Linking coevolutionary history to ecological process: doves and lice. *Evol. Int. J. Org. Evol*. **57**, 2335–2341. (doi:10.1111/j.0014-3820.2003.tb00245.x)
11. Page RDM, Lee PLM, Becher SA, Griffiths R, Clayton DH. 1998 A different tempo of mitochondrial DNA evolution in birds and their parasitic lice. *Mol. Phylogenetics Evol*. **9**, 276–293. (doi:10.1006/mpev.1997.0458)
12. Whiteman NK, Santiago-Alarcon D, Johnson KP, Parker PG. 2004 Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylogenetic patterns. *Int. J. Parasitol*. **34**, 1113–1119. (doi:10.1016/j.ijpara.2004.06.003)
13. Lima MA, Weckstein JD, Batista R, Ribas CC. 2023 Do parasitic lice exhibit endemism in parallel with their avian hosts? A comparison across northern Amazonian areas of endemism. *J. Parasitol.* **109**, 506–513. (doi:10.1645/18-135)
14. Chesser RT. 2004 Molecular systematics of New World suboscine birds. *Mol. Phylogenetics Evol*. **32**, 11–24. (doi:10.1016/j.ympev.2003.11.015)
15. Fjeldså J, Zuccon D, Irestedt M, Johansson US, Ericson PGP. 2003 *Sapayoa aenigma*: a new world representative of 'Old World suboscines'. *Proc. R. Soc. Lond. B* **270 (Suppl. 2)**, S238–41. (doi:10.1098/rsbl.2003.0075)
16. Lanyon SM. 1985 Molecular perspective on higher-level relationships in the Tyrannoidea (Aves). *Syst. Biol*. **34**, 404–418. (doi:10.1093/sysbio/34.4.404)
17. Moyle RG, Chesser RT, Prum RO, Schikler P, Cracraft J. 2006 Phylogeny and evolutionary history of Old World suboscine birds (Aves: Eurylaimides). *Am. Museum Novitates* **3544**, 1. (doi:10.1206/0003-0082(2006)3544[1:PAEHOO]2.0.CO;2)
18. Sibley CG, Ahlquist JE. 1985 Phylogeny and classification of new world suboscine passerine birds (Passeriformes: Oligomyodi: Tyrannides). *Ornithol. Monogr*. 396–428. (doi:10.2307/40168296)
19. Irestedt M, Ohlson JI, Zuccon D, Källersjö M, Ericson PGP. 2006 Nuclear DNA from old collections of avian study skins reveals the evolutionary history of the old world suboscines (Aves, Passeriformes). *Zool. Scr*. **35**, 567–580. (doi:10.1111/j.1463-6409.2006.00249.x)
20. Snow D. 2020 Sapayoa *Sapayoa aenigma*. In Birds of the World (eds SM Billerman, BK Keeney, PG Rodewald, TS Schulenberg). See https://birdsoftheworld.org/bow/species/sapayo1/1.0/introduction.
21. Claramunt S, Cracraft J. 2015 A new time tree reveals Earth history's imprint on the evolution of modern birds. *Sci. Adv*. **1**, e1501005. (doi:10.1126/sciadv.1501005)
22. Oliveros CH *et al*. 2019 Earth history and the passerine superradiation. *Proc. Natl Acad. Sci. USA* **116**, 7916–7925. (doi:10.1073/pnas.1813206116)
23. Selvatti AP, Galvão A, Pereira AG, Pedreira Gonzaga L, Russo C de M. 2016 An african origin of the eurylaimides (Passeriformes) and the successful diversification of the ground-foraging pittas (Pittidae). *Mol. Biol. Evol.* **34**, 483–499. (doi:10.1093/molbev/msw250)
24. Kolencik S, Sychra O, Johnson KP, Weckstein JD, Sallam MF, Allen JM. 2024 The parasitic louse genus *Myrsidea* (Amblycera: Menoponidae): a comprehensive review and world checklist. *Insect Syst. Divers* **8**, 1–60. (doi:10.1093/isd/ixae007)
25. Gajdošová M, Sychra O, Kreisinger J, Sedláček O, Nana ED, Albrecht T, Munclinger P. 2020 Patterns of host–parasite associations in tropical lice and their passerine hosts in Cameroon. *Ecol. Evol*. **10**, 6512–6524. (doi:10.1002/ece3.6386)
26. Kolencik S, Cacioppo JA, Johnson KP, Allen JM, Sychra O, Weckstein JD. 2022 Phylogenetics and host-specificity of the mega-diverse louse genus *Myrsidea* (Amblycera: Menoponidae). *Syst. Entomol*. **47**, 390–401. (doi:10.1111/syen.12536)
27. Madrid RS *et al*. 2020 Diversity and host associations of *Myrsidea* chewing lice (Phthiraptera: Menoponidae) in the tropical rainforest of Malaysian Borneo. *Int. J. Parasitol. Parasites Wildl.* **13**, 231–247. (doi:10.1016/j.ijppaw.2020.10.011)
28. AviList Core Team. 2025 *The Checklist v2025—AviList: The Global Avian Checklist*. See https://www.avilist.org/checklist/v2025/.
29. Najer T, Doña J, Buček A, Sweet AD, Sychra O, Johnson KP. 2025 Phylogenomics reveals the timescale of diversification in Amblycera. *Syst. Entomol*. **50**, 540–553. (doi:10.1111/syen.12668)
30. Johnson KP, Weckstein JD, Virrueta Herrera S, Doña J. 2021 The interplay between host biogeography and phylogeny in structuring diversification of the feather louse genus *Penenirmus*. *Mol. Phylogenetics Evol*. **165**, 107297. (doi:10.1016/j.ympev.2021.107297)
31. Soto-Patiño J, Doña J, Johnson KP. 2025 Voucher photos lice *Sapayoa myrsidea*. Figshare. (doi:10.6084/m9.figshare.30069154)
32. Chen S, Zhou Y, Chen Y, Gu J. 2018 fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890. (doi:10.1093/bioinformatics/bty560)
33. Allen JM, LaFrance R, Folk RA, Johnson KP, Guralnick RP. 2018 aTRAM 2.0: an improved, flexible locus assembler for NGS data. *Evol. Bioinform. Online* **14**, 1176934318774546. (doi:10.1177/1176934318774546)
34. Slater GSC, Birney E. 2005 Automated generation of heuristics for biological sequence comparison. *BMC Bioinform* **6**, 31. (doi:10.1186/1471-2105-6-31)
35. Katoh K. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. *Nucleic Acids Res*. **30**, 3059–3066. (doi:10.1093/nar/gkf436)

36. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol*. **30**, 772–780. (doi:10.1093/molbev/mst010)
37. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009 trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973. (doi:10.1093/bioinformatics/btp348)
38. Borowiec ML. 2016 AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ* **4**, e1660. (doi:10.7717/peerj.1660)
39. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020 IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol*. **37**, 1530–1534. (doi:10.1093/molbev/msaa015)
40. Chernomor O, von Haeseler A, Minh BQ. 2016 Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol*. **65**, 997–1008. (doi:10.1093/sysbio/syw037)
41. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589. (doi:10.1038/nmeth.4285)
42. Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2016 PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **34**, msw260. (doi:10.1093/molbev/msw260)
43. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015 IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol*. **32**, 268–274. (doi:10.1093/molbev/msu300)
44. Hoang DT, Chernomor O, Haeseler A von, Minh BQ, Vinh LS. 2018 UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **35**, 518–522. (doi:10.1093/molbev/msx281)
45. Minh BQ, Nguyen MAT, von Haeseler A. 2013 Ultrafast approximation for phylogenetic bootstrap. *Mol. Biol. Evol*. **30**, 1188–1195. (doi:10.1093/molbev/mst024)
46. Zhang C, Rabiee M, Sayyari E, Mirarab S. 2018 ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**, 153. (doi:10.1186/s12859-018-2129-y)
47. To TH, Jung M, Lycett S, Gascuel O. 2016 Fast dating using least-squares criteria and algorithms. *Syst. Biol*. **65**, 82–97. (doi:10.1093/sysbio/syv068)
48. Sweet AD, Doña J, Johnson KP. 2025 Biogeographic history of pigeons and doves drives the origin and diversification of their parasitic body lice. *Syst. Biol.* **74**, 198–214. (doi:10.1093/sysbio/syae038)
49. Johnson KP, Matthee C, Doña J. 2022 Phylogenomics reveals the origin of mammal lice out of Afrotheria. *Nat. Ecol. Evol*. **6**, 1205–1210. (doi:10.1038/s41559-022-01803-1)
50. Wappler T, Smith VS, Dalgleish RC. 2004 Scratching an ancient itch: an eocene bird louse fossil. *Proc. R. Soc. Lond. B* **271**. (doi:10.1098/rsbl.2003.0158)
51. Kumar S, Suleski M, Craig JM, Kasprowicz AE, Sanderford M, Li M, Stecher G, Hedges SB. 2022 TimeTree 5: an expanded resource for species divergence times. *Mol. Biol. Evol.* **39**, msac174. (doi:10.1093/molbev/msac174)
52. Matzke NJ. 2013 Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Front. Biogeogr.* **5**, F55419694. (doi:10.21425/f55419694)
53. Matzke NJ. 2014 Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst. Biol*. **63**, 951–970. (doi:10.1093/sysbio/syu056)
54. Bush SE, Weckstein JD, Gustafsson DR, Allen J, DiBlasi E, Shreve SM, Boldt R, Skeen HR, Johnson KP. 2016 Unlocking the black box of feather louse diversity: a molecular phylogeny of the hyper-diverse genus *Brueelia*. *Mol. Phylogenetics Evol*. **94**, 737–751. (doi:10.1016/j.ympev.2015.09.015)
55. Olson DM *et al*. 2001 Terrestrial ecoregions of the world: a new map of life on Earth. *Bioscience* **51**, 933. (doi:10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2)
56. Santichaivekin S, Yang Q, Liu J, Mawhorter R, Jiang J, Wesley T, Wu YC, Libeskind-Hadas R. 2021 eMPRess: a systematic cophylogeny reconciliation tool. *Bioinformatics* **37**, 2481–2482. (doi:10.1093/bioinformatics/btaa978)
57. Jetz W, Thomas GH, Joy JB, Hartmann K, Mooers AO. 2012 The global diversity of birds in space and time. *Nature* **491**, 444–448. (doi:10.1038/nature11631)
58. Jetz W, Thomas GH, Joy JB, Redding DW, Hartmann K, Mooers AO. 2014 Global distribution and conservation of evolutionary distinctness in birds. *Curr. Biol*. **24**, 919–930. (doi:10.1016/j.cub.2014.03.011)
59. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018 Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol*. **4**, y016. (doi:10.1093/ve/vey016)
60. Paradis E, Claude J, Strimmer K. 2004 APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290. (doi:10.1093/bioinformatics/btg412)
61. Revell LJ. 2024 phytools 2.0: An updated R ecosystem for phylogenetic comparative methods (and other things). *PeerJ* **12**, e16505. (doi:10.7717/peerj.16505)
62. Ericson PGP, Klopfstein S, Irestedt M, Nguyen JMT, Nylander JAA. 2014 Dating the diversification of the major lineages of Passeriformes (Aves). *BMC Evol. Biol.* **14**, 8. (doi:10.1186/1471-2148-14-8)
63. Riamon S, Tourment N, Louchart A. 2020 The earliest Tyrannida (Aves, Passeriformes), from the Oligocene of France. *Sci. Rep.* **10**, 9776. (doi:10.1038/s41598-020-66149-9)
64. Soto-Patiño J, Doña J, Johnson KP. 2025 Data availability. Figshare. (doi:10.6084/m9.figshare.30069151)
65. Soto-Patiño J, Doña J, Johnson K. 2025 Supplementary material from: A riddle wrapped in an enigma: Parasitic lice as clues to the evolutionary puzzle of Sapayoa (Aves). Figshare. (doi:10.6084/m9.figshare.c.8186441)