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Phylogenetics and host-specificity of the mega-diverse louse genus *Myrsidea* (Amblycera: Menoponidae)

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Abstract

Myrsidea Waterston is the most diverse genus of chewing lice, primarily parasitizing perching birds (Passeriformes), which is the most speciose avian order. *Myrsidea* also parasitize several hosts from non-passerine groups, including toucans, barbets, woodpeckers (Piciformes) and hummingbirds (Apodiformes). To examine host specificity, host switching and generic limits, we reconstructed a phylogeny of the avian feather louse genus *Myrsidea* using DNA sequence data from two fragments of the mitochondrial COI gene and a fragment of the nuclear EF-1α gene for 152 *Myrsidea* specimens collected from 23 avian host families. Unlike other highly diverse louse genera, only a small proportion of *Myrsidea* species parasitize more than one host species. We found that host family has significant phylogenetic signal on the *Myrsidea* phylogeny. These results suggest that *Myrsidea* is generally highly host-specific, with some exceptions where host switching is important. We found that there are two separate groups of *Myrsidea* that parasitize toucans, and that both are nested within *Myrsidea* found on perching birds, suggesting that these toucan ectoparasites may have arisen from two independent host switching events. Lastly, representatives of the genus *Ramphasticola* Carriker, which was originally described as a distinct genus due to a suite of morphologically unique characters, falls in with a strongly supported clade of *Myrsidea* parasitizing *Ramphastos* toucans, and therefore we definitively place *Ramphasticola* as a synonym of *Myrsidea*.

KEYWORDS
chewing lice, DNA, parasites, Phthiraptera, phylogeny, *Ramphasticola*, toucans

INTRODUCTION

Birds are among the most well-studied taxonomic groups on the planet, yet their ectoparasite communities remain relatively unknown. For example, the avian feather louse genus *Myrsidea* (Waterston) (Phthiraptera: Menoponidae) mainly parasitizes birds from the order Passeriformes (Price et al., 2003; Sychra, 2010), which includes over 6000 described bird species. However, only ~380 species of *Myrsidea* have been described to date (Kolencik et al., 2018). Yet, even this level of known diversity makes *Myrsidea* the most speciose louse genus, with many more species thought to be undiscovered (Valim & Weckstein, 2013). For example, Valim and Weckstein (2013) estimated that there may be over 900 species of *Myrsidea* yet to be described from Brazil alone.

This massive diversity of the genus *Myrsidea* is thought to be due, in part, to the extreme host specificity of species in this group, with estimates of more than 80%–88% of species parasitizing only a single host species (Price et al., 2003; Sychra, 2010). However, an analysis of host specificity in the context of a phylogeny for the genus *Myrsidea* has never been conducted. Clay (1966) suggested that the genital sclerites of males could be used to clarify the phylogenetic relationships among species within the genus *Myrsidea*. Therefore, the morphological similarity of genital sclerites among *Myrsidea* parasitizing closely related hosts (Clay, 1970; Price & Dalgleish, 2007) suggest that

there is phylogenetic signal in host association. As a result, many authors have adopted the practice of conducting taxonomic revisions of *Myrsidea* from specific focal host families (e.g., Dalgleish & Price, 2003b; Kolencik et al., 2018; Kounek, Sychra, Čapek, Lipková, & Literák, 2011; Kounek, Sychra, Čapek, & Literák, 2011; Sychra et al., 2006). This taxonomic approach of focusing on single host families assumes that species of *Myrsidea* are always specific to particular host taxonomic groups (e.g., host families). Therefore, if major clades of *Myrsidea* are not confined to closely related host groups, then smaller taxonomic revisions based on host families could lead to the same louse species being described multiple times, artificially increasing the number of species in this genus. One major question that remains unanswered is whether, in a broad phylogenetic context, *Myrsidea* clades are restricted to avian host families.

Here we use a phylogeny of *Myrsidea* to determine the level of specificity of *Myrsidea* clades to major host groups. We examine this specificity at two scales, first at the avian family level and second at the level of host-species. Although 95% of *Myrsidea* species parasitize passerine birds (order Passeriformes; Price et al., 2003), there are representatives parasitizing a few non-passerine families, including barbets (order Piciformes, families Lybiidae and Capitonidae; Bueter et al., 2009; Soto-Patiño et al., 2018), woodpeckers (order Piciformes, family Picidae; Ilieva, 2009), toucans (order Piciformes, family Ramphastidae; Hellenthal et al., 2005; Price et al., 2004) and hummingbirds (order Apodiformes, family Trochilidae; Dalgleish & Price, 2003a). Although there are many taxonomic studies of *Myrsidea* from passerines (e.g., Dalgleish & Price, 2003b; Dalgleish & Price, 2005; Kolencik et al., 2016; Kolencik et al., 2017; Kolencik et al., 2018; Kounek, Sychra, Čapek, Lipková, & Literák, 2011; Kounek, Sychra, Čapek, & Literák, 2011; Kounek et al., 2013; Price et al., 2005; Price & Dalgleish, 2006; Sychra et al., 2006; Sychra et al., 2007; Sychra et al., 2009; Valim & Weckstein, 2013), only a few authors have focused their study on those from non-passerine birds (Carriker, 1949; Carriker & Diaz-Ungria, 1961; Hellenthal et al., 2005; Price et al., 2004). How these non-passerine avian host orders and families acquired their *Myrsidea* chewing lice remains a key unanswered question. Presumably, this has happened through the process of host-switching. Such host-switching events between unrelated hosts may have been possible at a location where birds with similar behaviour and ecology coexist (Sychra et al., 2014; Weckstein, 2004). Another unanswered question about the host distribution of *Myrsidea* is how many times *Myrsidea* may have host-switched from passerine to non-passerine groups. These questions can be answered with a phylogeny of *Myrsidea* that includes samples collected from both passerines and non-passerines.

Lastly, the taxonomy of *Myrsidea* from toucans has been based on morphological descriptions that compare the differences between toucan *Myrsidea* species and other *Myrsidea*. Carriker (1949) erected a new morphologically diagnosable genus from toucans named *Ramphasticola* Carriker. However, the status of this genus, and whether it is synonymous with *Myrsidea*, has been controversial (Hellenthal et al., 2005; Hopkins & Clay, 1952). A phylogeny including broad taxonomic sampling of *Myrsidea* and *Ramphasticola* will also shed light on the generic limits in this complex.

To address these questions, we sequenced portions of both nuclear and mitochondrial protein coding genes to reconstruct the phylogeny for the genus *Myrsidea*, including species sometimes placed in the genus *Ramphasticola*. We examined patterns in both host and geographic distribution of these parasites over this tree and discuss the implication of our results for the taxonomy of this genus.

MATERIALS AND METHODS

Taxon sampling, DNA extraction and slide mounting

Parasites were collected from avian hosts using the postmortem ethyl acetate fumigation and ruffling methods (Clayton et al., 1992; Clayton & Drown, 2001) and stored in 95% ethanol at −80°C. In most cases, host specimens were prepared as vouchers and deposited into one of several museum bird collections (Table S1). In some cases, the hosts were banded and released.

We extracted DNA from individual louse specimens using the Qiagen DNeasy micro-kit (Valencia, CA, U.S.A.), following the manufacturer's protocols either as described by Johnson et al. (2001) or Valim and Weckstein (2011). The only difference between these two modified protocols is that Johnson et al. (2001) separated the head and the body of each specimen and the Valim and Weckstein (2011) protocol involved making a small cut halfway across the meeting point of the head and thorax of the specimen. After DNA extraction from individual louse specimens, the exoskeletons were retained and mounted on microscope slides using the standard Canada Balsam method described by Palma (1978). Slide mounted specimens from this study were deposited in the insect collections at Field Museum of Natural History, Chicago, IL (FMNH) and Illinois Natural History Survey (INHS), Champaign-Urbana, IL (Table S1).

The final data set analysed for this study included 144 *Myrsidea* and 8 *Ramphasticola* specimens from 100 avian host taxa from 23 families and 59 genera within the orders Passeriformes and Piciformes, collected in 15 countries. We used *Dennyus* sp. for an outgroup taxon to root the phylogenies because based on previous studies it is considered the closest relative to the *Myrsidea* complex (Cruickshank et al., 2001; Marshall, 2003; Table S1).

Molecular methods

Three loci were amplified using the polymerase chain reaction (PCR), including a 379 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene (COI, Hafner et al., 1994), a separate 670 bp fragment of the COI gene (COI-L; Folmer et al., 1994) and a 347 bp fragment of the elongation factor 1-alpha gene (EF1α; Danforth & Ji, 1998), for a total of 1396 bp of DNA sequence data. All primers and their sequences are listed in Table S2.

Each PCR tube was filled with 24 μl of a PCR master mix and 1 μl of template louse DNA, totalling 25 μl. PCR master mix in each tube included 2.5 μl PCR buffer (provided with the polymerase), 0.2 μl of

Platinum Taq DNA Polymerase (Invitrogen, Life Technologies, Carlsbad, California), 1 μl of 50 mM $MgCl_2$ (provided with polymerase), 1 μl of each primer after dilution to 10 μM, 1 μl of 100 μM DNTPs and 17.3 μl of sterile dH_2O. Negative controls were also included in each set of reactions. The PCR amplification protocol conditions are found in Table S3.

The success of amplification was verified by electrophoresing a subsample of each PCR product on a 1% agarose gel stained with ethidium bromide and visualized under UV light. Amplification products were purified by cutting a single band of product out of a low-melt agarose gel and digesting it with GELase (Epicentre Technologies, Madison, WI, U.S.A.) following the recommended manufacturer protocol. PCR products were then cycle-sequenced using the BigDye 3.1 Terminator kit (BigDye, Applied Biosystems, Foster City, CA, U.S.A.) and the same primers used during amplification. Sequencing reaction products were cleaned with an ethanol-EDTA precipitation and resuspended in Hi-Di formamide before being run on an ABI 3730 automated capillary DNA sequencer.

Phylogenetic reconstruction

Clean consensus sequences (GENEIOUS PRIME® 2020.0.4; https://www.geneious.com) from each gene fragment were aligned in SEAVIEW v4.7 (Gouy et al., 2010) using CLUSTAL OMEGA (Sievers et al., 2011) and aligned by eye. PARTITIONFINDER v2.1.1 (Lanfear et al., 2016) was used to determine the optimal partitioning scheme and the best-fit model of molecular evolution for each partition. Data partitions were defined according to the loci and their codon positions. We estimated a Maximum Likelihood (ML) phylogeny using RAXML (v8.2.12) and applied the GTR + G model of molecular evolution for each partition and assessed nodal support using 1000 bootstrap replicates (Felsenstein, 1985). We ran a Bayesian Inference analysis (BA) in MRBAYES (Ronquist & Huelsenbeck, 2003) with seven partitions and three models (Table S4). All model parameters except topology and branch lengths were set as unlinked between partitions and were estimated from the data, as in Johnson et al. (2011). We ran two parallel runs for 50 million generations with four Markov chains (Huelsenbeck & Bollback, 2001) and sampled the Markov chains every 1000 generations for a total of 50,000 parameter point estimates. All sampled parameter point estimates were examined in TRACER v 1.7.1 (Rambaut et al., 2018) to determine whether the chains had reached stationarity and 25% of these parameter point estimates were removed as burning. Finally, a 50% majority rule consensus tree with posterior probabilities was generated. The best ML tree and 100 randomly chosen posterior distributions of trees from the BA analysis were used in the following analyses to account for tree uncertainty.

Operational taxonomic units and test of phylogenetic signal

Operational taxonomic units (OTUs) were determined using two methods as in Bush et al. (2016). We combined the two different fragments of COI assuming they evolve at approximately the same rate. First, we used MOTHUR (Schloss et al., 2009), which applies a cut-off value and pairwise distances to determine OTU clusters. Here we used a 12% cutoff value estimated from interspecific variation in COI for *Myrsidea* as reported by Kolencik et al. (2017) and confirmed these results by examining the tree for closely related groups under this cut-off. For phylogenetic-based estimation, we used the Bayesian implementation of the general mixed yule-coalescent model (BGMYC) on 100 random trees from the Bayesian analysis, after removing the outgroup. We used a custom perl script (Bush et al., 2016) to calculate the mean value for the conspecificity probability threshold (p.div) from the results of the BGMYC analysis (Reid & Carstens, 2012) to calculate the number of OTUs within the dataset (Table S1).

To examine the phylogenetic signal (Maddison & Slatkin, 1991) with respect to the host family, the OTUs were associated with their host families (Clements et al., 2018), with only a single host family association character scored for each OTU. We tested for host family phylogenetic signal on the *Myrsidea* phylogeny using a Maddison and Slatkin (1991) test and the Bayesian consensus tree pruned to a single taxon per OTU for each of the clusters identified by both MOTHUR and BGMYC (Figure S2). We ran the Maddison and Slatkin (1991) test using a custom R script (Bush et al., 2016), with 1000 randomizations in both runs.

RESULTS

Of the 153 louse DNA samples tested, we successfully amplified and sequenced the short 379 bp fragment of COI from all 153 samples, the 670 bp fragment of COI (which we refer to as COI-L) from 127 samples, and the EF-1α gene from 140 samples. Both ML and BA analyses of these concatenated DNA sequences resulted in similar phylogenetic trees (Figures 1 and S1). For each of the trees, higher support was found for the more terminal nodes and lower support was found among the earlier branching nodes at the base of the tree, which is similar to phylogenetic studies of other louse genera based on this subset of loci (e.g., Bush et al., 2016). However, these phylogenetic reconstructions still yielded many clades with high support. The major differences between the ML and BA trees generally involved weakly supported basal rearrangements, whereas the major groupings of *Myrsidea* among hosts remained stable.

The analysis of 152 ingroup taxa indicated that there are between 83 (based on 12% delimitation cutoff in MOTHUR) and 98 (based on 0.57 conspecificity probability threshold in BGMYC) OTUs (Table S1). Both species delimitation analyses correspond well with species identified based on morphology. The densest taxon sampling in our dataset was *Myrsidea* from toucans (68/152 samples; 44.7%). There were either 11 (MOTHUR) or 22 (BGMYC) OTUs among these samples. This broad taxonomic, host and geographic sampling from toucans is important for assessing the status of the toucan-specific genus *Ramphasticola*, which has been considered synonymous with *Myrsidea* (Hopkins & Clay, 1952; Price et al., 2003). The results of our analysis show that there are two clades of toucan-associated lice that are

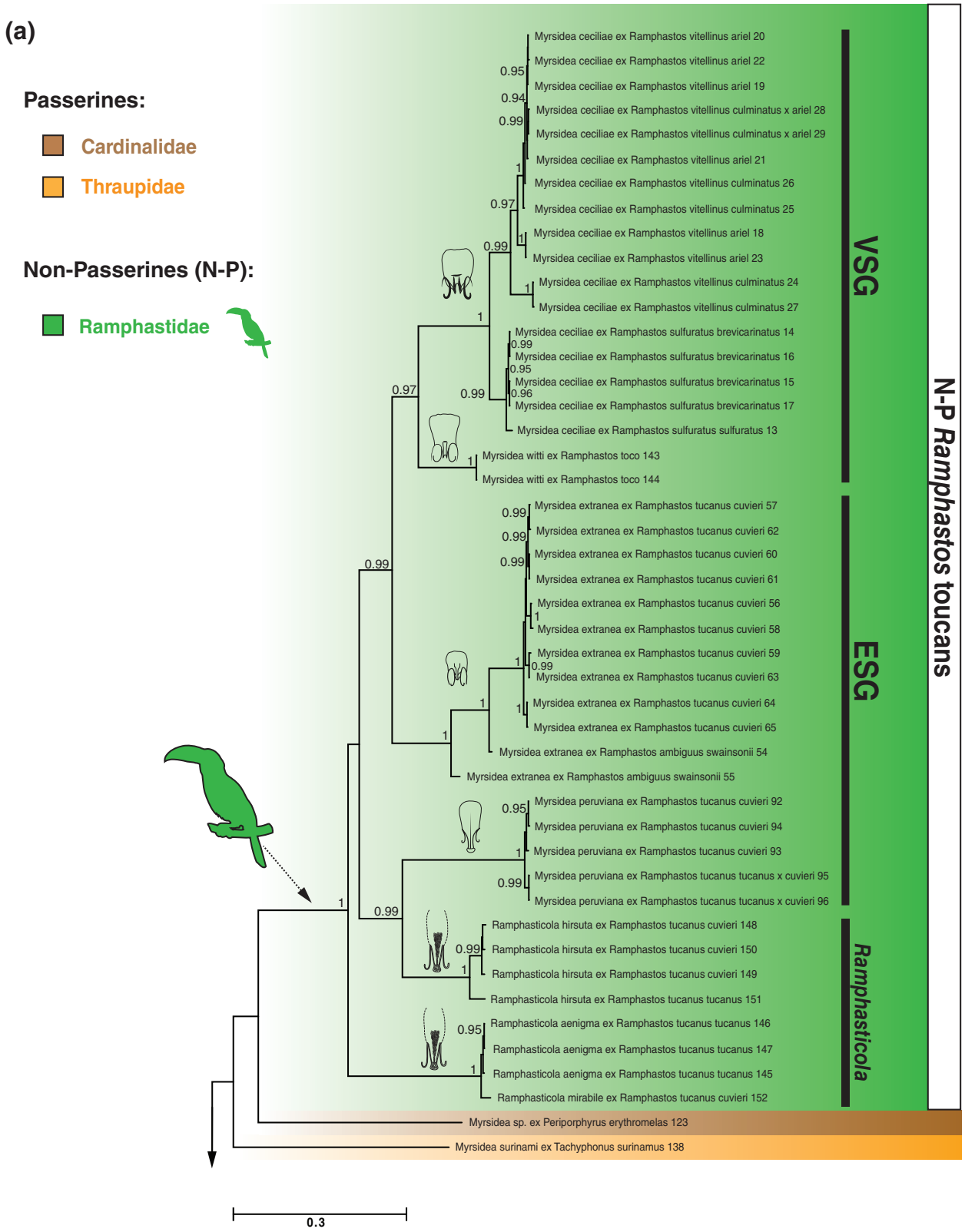

FIGURE 1 Bayesian phylogenetic tree of *Myrsidea* specimens based on mitochondrial cytochrome oxidase I and nuclear EF-1α sequences. Tree is rooted with the outgroup from the genus *Dennyus*. Nodes with posterior probability values above 90% are labelled with these values. The tree is coloured according to host families. Morphological groups as defined by Price et al. (2004) are also labelled on the tree, including the "*victrix* species group" (VSG: *M. victrix*, *M. ceciliae* and *M. witti*) the "*extranea* species group" (ESP: *M. extranea* and *M. peruviana*) and the "*abbreviata* species group" (ASG: *M. abbreviata*, *M. dorotheae*, *M. aleixoi* and *M. lanei*). Illustrations of male genital sclerites for toucan louse species are redrawn from Price et al. (2004) and Hellenthal et al. (2005) and are placed next to the nodes of the louse clades with these morphological features

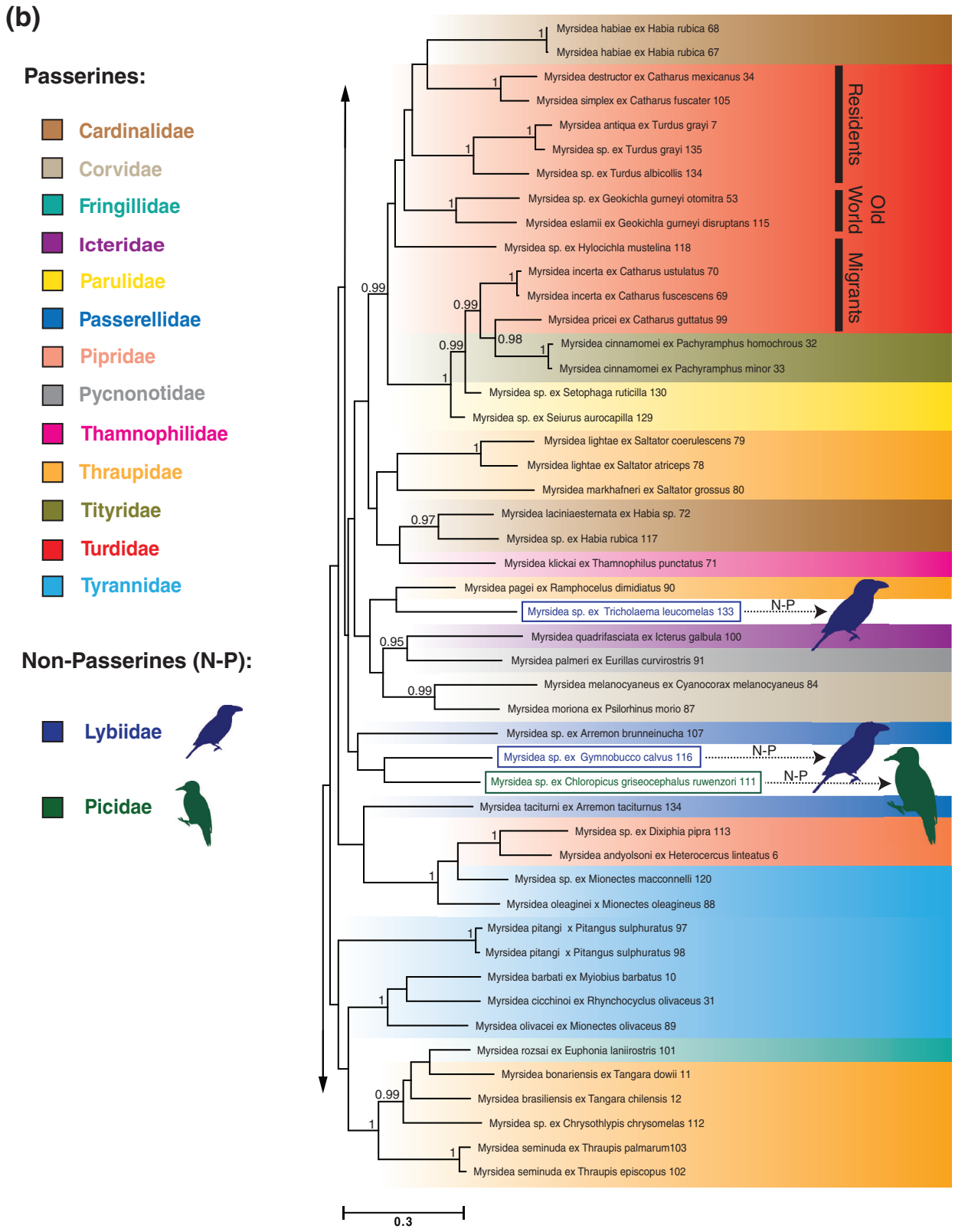

FIGURE 1 (Continued)

likely not sister groups, and both are nested within *Myrsidea* found on passeriform birds (Figure 1). One clade of louse species parasitizing *Ramphastos* Linnaeus toucans includes lice that are currently placed in *Ramphasticola*; a second clade includes *Myrsidea* hosted by *Pteroglossus* araçaris. In general, the OTUs identified by Mothur-matched species limits based on the toucan louse species

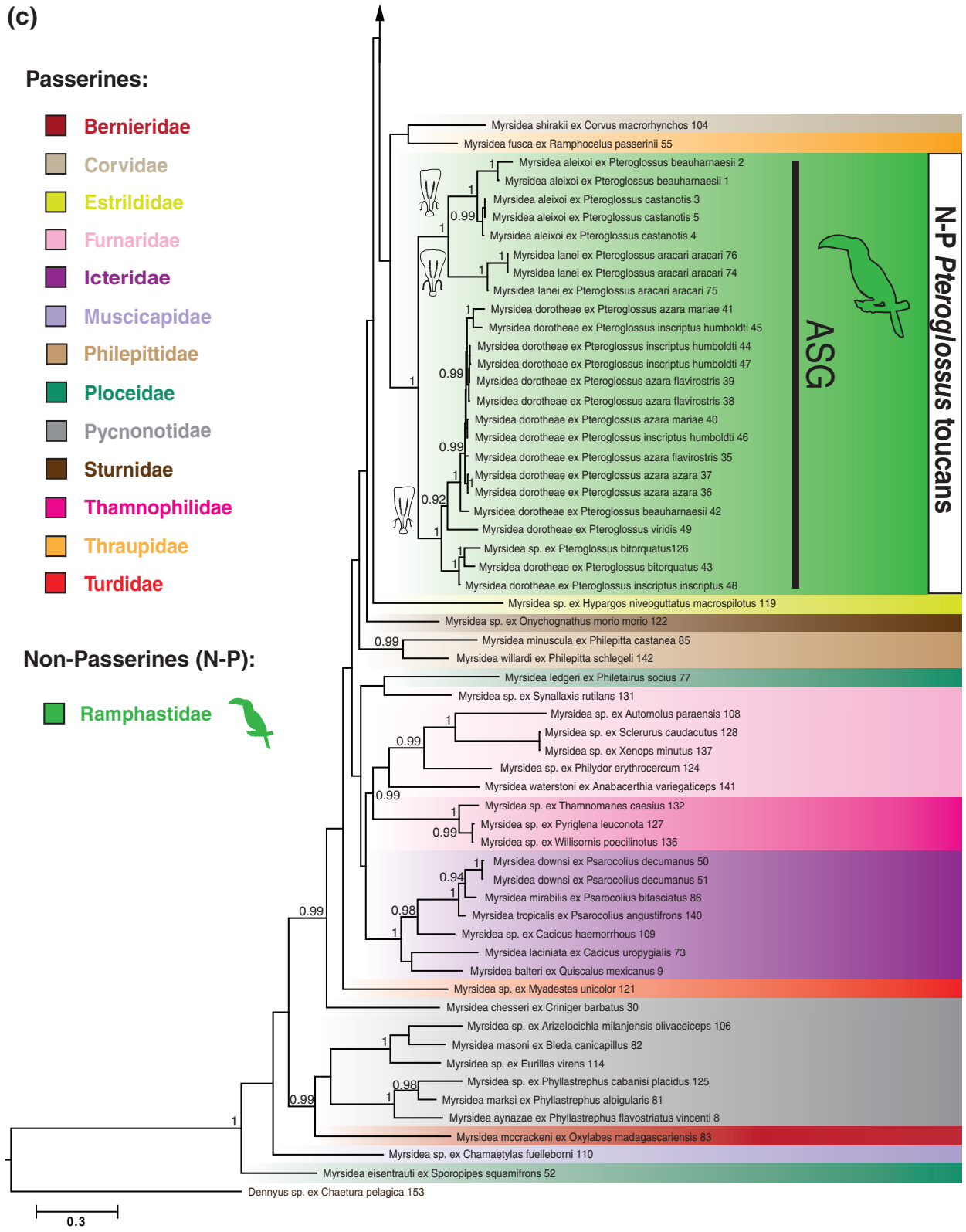

FIGURE 1 (Continued)

morphological descriptions, with two exceptions. *Myrsidea dorotheae* Eichler *was separated into three different OTUs; and a single Ramphasticola mirabile* (Carriker) specimen was placed in the same OTU with three *Ramphasticola aenigma* (Carriker) specimens. OTUs from the BGMYC analysis split most terminal clades and subclades from novel hosts into terminal OTUs. Thus, based on the BGMYC results, we find multiple cases where a single louse morphospecies species harbours multiple OTUs and each OTU is usually associated with a

different host species and/or localities. For example, in the BGMYC analysis, *M. lanei* Price, Hellenthal & Weckstein has two OTUs, *M. ceciliae* Carriker & Diaz-Ungria and *M. extranea* (Carriker) each have three OTUs and *M. dorotheae* has six OTUs. Furthermore, all four *R. hirsuta* (Carriker) specimens belong to different OTUs. However, *R. aenigma* and *R. mirabile* are lumped into the same OTU and parasitize the same host species, *Ramphastos tucanus* Linnaeus (Table S1).

The result of the Maddison and Slatkin (1991) test to assess phylogenetic signal of host family associations was significant ($p < 0.001$), supporting that most clades of *Myrsidea* have a strong level of specificity to host family (Figure S2). There are several well-supported *Myrsidea* clades or sister pairs that are specific to particular avian families (Figure 1). For example, there are two clades parasitizing the members of Ramphastidae (both with a posterior probability value of 1.00), a clade including most of the species from Icteridae (1.00), a small clade of *Myrsidea* parasitizing the members of Philepittidae (0.99) and a sister clade of *Myrsidea* consisting mostly of species parasitizing the members of Pycnonotidae, with one species hosted by the Malagasy host family Bernieridae (0.99).

Host switching events are supported by several closely related clades of *Myrsidea* that are hosted by multiple avian families, suggesting switching between those families. For example, a clade of lice (0.99) whose hosts include migrant New World thrushes (Turdidae) also includes Neotropical resident thrushes, as well as resident hosts from several other families (Cardinalidae, Parulidae and Tityridae). Furthermore, species of *Myrsidea* from the host family Thraupidae are found in five different clades across the tree, suggesting widespread host-switching between members of Thraupidae and other host families.

Non-passerine *Myrsidea* form four distinct clades in the tree (Figure 1), including two well supported monophyletic clades of toucan lice (Ramphastidae), one for *Myrsidea* (and *Ramphasticola*) hosted by *Ramphastos* toucans and one clade for *Myrsidea* hosted by *Pteroglossus* araçaris, and a weakly supported clade including one African barbet louse (ex *Gymnobucco calvus* Lafresnaye; Lybiidae) and an African woodpecker louse (ex *Chloropicus griseocephalus rewenzori* Sharpe, 1902; Picidae), and a separate African barbet louse (ex *Tricholaema leucomelus* Boddaert; Lybiidae). These findings suggest that there have been multiple interordinal host-switching events. However, none of the basal relationships among these toucan, barbet and woodpecker lice in relation to passerine lice are well supported. Thus, although the monophyly of the two toucan *Myrsidea* clades is well established, the relationship among these clades and non-passerine hosted *Myrsidea* is unclear in this phylogenetic reconstruction due to relatively low support (posterior probabilities <0.90).

Lastly, our results have implications for generic level taxonomy of *Myrsidea*. Within the well-supported monophyletic clade of *Myrsidea* and *Ramphasticola* parasitizing *Ramphastos* toucans, there are two clades of *Myrsidea* species, which are typical in that they have the sternal aster of setae and match the morphologically defined species-groups identified by Price et al. (2004) (Figure 1). For example, the *victrix* species group, which parasitizes channel-keel-billed croaking *Ramphastos* toucans (Weckstein, 2004), is recovered as monophyletic (VSG; Figure 1a). The *extranea* species group, which parasitizes smooth billed/yelping *Ramphastos* toucans (Weckstein, 2004) is a bit more complicated and forms two separate clades in the tree, the *extranea* clade and the *peruviana* clade, which are apparently not sisters (ESG; Figure 1a). However, *M. peruviana* Eichler is strongly supported (0.99; Figure 1) as sister to a morphologically distinct species that lacks well-developed typical aster—*Ramphasticola hirsuta*. Moreover, comparisons of the morphology of male genital sclerites of *Myrsidea* (including *Ramphasticola*) from toucans indicate there are three different types, all corresponding to particular clades in the tree (Figure 1). Interestingly, *M. peruviana* shares the same type of genital sclerite as *Ramphasticola* species. Furthermore, a second clade of *Ramphasticola* that includes *R. aenigma* and *R. mirabile* is sister to all other *Myrsidea* and *Ramphasticola* parasitizing *Ramphastos* toucans (Figure 1). Thus, *Ramphasticola* and *Myrsidea* are not reciprocally monophyletic. Additionally, the *abbreviata* species group (ASG), parasitizing *Pteroglossus* araçaris, is monophyletic in our tree (1; Figure 1c).

DISCUSSION

The avian chewing louse genus *Myrsidea* is the most species-rich avian louse genus with likely many undiscovered species (Valim & Weckstein, 2013). This diversity is thought to be due in part to the high host-specificity of the genus *Myrsidea*. Here we conducted a large molecular phylogenetic reconstruction of 152 *Myrsidea* specimens collected from 23 host families and 59 host genera to examine *Myrsidea* host specificity, interordinal and interfamilial host switching, and generic limits with respect to the avian louse genus *Ramphasticola*. In general, our tree was well resolved and well supported at more terminal clades but lacked highly supported branches among the more ancestral nodes of the tree.

Patterns of host distribution

The phylogenetic tree indicates that most *Myrsidea* species from closely related host groups cluster together phylogenetically. For example, there are many well-supported (>0.99) clades of *Myrsidea* that cluster by host family. These include two monophyletic toucan louse clades (Ramphastidae), a New World blackbird (Icteridae) louse clade and small antbird (Thamnophilidae) and asity (Philepittidae) louse clades, among others (Figure 1). In many of the recently published *Myrsidea* taxonomic revisions, authors have used host families to circumscribe their taxonomic descriptions of *Myrsidea* (e.g., Dalgleish & Price, 2003b; Johnson & Price, 2006; Kolencik et al., 2018; Price et al., 2005; Sychra et al., 2006). In many cases in which lice are less host-specific, this strategy of delimiting taxonomic revisions based on host taxonomic groups could be risky and could result in the description of synonyms. However, our analysis of phylogenetic signal, using the Maddison and Slatkin (1991) test, indicates that host family has significant phylogenetic signal on the *Myrsidea* phylogeny (Figure S2), and therefore confirms that in general *Myrsidea* lineages are highly specific to clades (families) of hosts. Thus, our

results suggest that *Myrsidea* taxonomic revisions focused on lice from individual host families are a reasonable strategy for tackling the taxonomic impediment of this immensely diverse louse genus.

An examination of the *Myrsidea* phylogenetic tree suggests a history of host switching between migratory thrushes (Turdidae) and tropical resident birds (Figure 1b). In a well-supported clade (0.99) that consists of lice from multiple host families, including thrushes (Turdidae), cardinals (Cardinalidae), tityras (Tityridae) and wood warblers (Parulidae), the lice parasitizing *Catharus* Bonaparte thrushes are not each other's closest relatives. Specifically, one clade of lice parasitizing the Neotropical migrant *Catharus* thrushes (*C. ustulatus* Nuttall, *C. fuscescens* Stephens and *C. guttatus* Pallas) is closely related to *Myrsidea cinnamomei* Dalgleish & Price, which parasitizes a Neotropical resident species from the distantly related host family Tityridae (*Pachyramphus homochrous* Sclater and *P. minor* Lesson). This same clade is similarly closely related to *Myrsidea* sp. from two parulid warbler species (*Setophaga ruticilla* Linnaeus and *Seiurus aurocapilla* Linnaeus), which are also not closely related to *Catharus* thrushes. This clade of lice is sister to a second clade that is not well supported basally, but also includes *Myrsidea* that parasitize Neotropical resident (non-migratory) *Catharus* (*C. mexicanus*, *C. fuscater*) and *Turdus* Linnaeus thrushes (*Turdus grayi* Bonaparte, *T. albicollis* Vieillot), Old World Thrushes (*Geokichla gurneyi* Hartlaub) and one cardinalid louse (*Myrsidea habiae* Kolencik & Sychra ex. *Habia rubica* Vieillot). Thus, the polyphyly of lice from thrushes implies that at a macroevolutionary scale there were likely multiple host-switching events between migratory thrushes and tropical residents, and perhaps tropical resident thrushes and other tropical resident birds. The relationships within this clade need to be tested with additional molecular data to be certain of many of the basal relationships. Furthermore, additional specimens from a broader sampling of host species will help to test whether the few cases where *Myrsidea* lice appear to lack host family specificity are simply sampling artefacts.

Our phylogenetic analyses of the genus *Myrsidea* also implies major host-switching events between different orders of birds, for example, between Passeriformes and Piciformes. Here we found five lineages of *Myrsidea* from three non-passerine host families in the order Piciformes, including the toucans (Ramphastidae), African barbets (Lybiidae) and woodpeckers (Picidae). The species of *Myrsidea* found on toucans form two well supported (BI = 1 respectively) and reciprocally monophyletic clades, one hosted by *Ramphastos* toucans and the other hosted by *Pteroglossus* araçaris (Figure 1). In our phylogenetic reconstruction, these two *Myrsidea* clades hosted by toucans are not sister groups. Therefore, taken at face value, this implies that toucans acquired their *Myrsidea* chewing lice independently from host-switching events from perching birds. Although two *Myrsidea* from African barbets are sister taxa, the *Myrsidea* from another African barbet host, *Tricholaema leucomelas* (Boddaert), is sister to a louse from a tanager. Support for nodes basal to these toucan louse and African barbet louse clades is weak and thus these relationships need to be tested with genomic scale data to obtain better phylogenetic support to assess how many times toucans acquired their *Myrsidea*.

Taxonomic implications

The results of our phylogenetic analysis have implications for *Myrsidea* taxonomy, particularly regarding the *Myrsidea* and *Ramphasticola* found on toucans. Initially, Carriker (1949) described a morphologically distinct genus of Amblycera from the large-bodied *Ramphastos* toucans, which he named *Ramphasticola*. However, Hopkins and Clay (1952) did not recognize *Ramphasticola* as a genus distinct from *Myrsidea*, and thus, Price et al. (2003) also maintained it as a junior synonym of *Myrsidea*. The synonymy of these two genera is also supported by a comparison of the genital sac sclerite from what Price et al. (2004) referred to as "typical *Myrsidea*" from toucans (figure 14 in Price et al., 2004) with the genital sac sclerite found in *Ramphasticola* (figure 7 in Hellenthal et al., 2005). Yet, Hellenthal et al. (2005) suggested *Ramphasticola* should be treated as a unique genus and indicated four important features which separate them from all other *Myrsidea*. Among these features, perhaps the most important was the absence of the distinctive well-developed sternal aster (Figure 2c,d), which is a spine-like cluster of setae on both posterio-lateral margins of sternite II (Figure 2a,b), often considered as one of the defining morphological features of the genus *Myrsidea* (Zlotorzycka, 1964). Contrary to Zlotorzycka (1964) who described several genera based on differences in this character, Clay (1966) did not consider the aster to be a main generic character and did not include it in her key for the family Menoponidae (Clay, 1969). Our phylogenetic reconstruction confirms that this character is not important for defining the generic limits in this complex.

Our results indicate that *Ramphasticola* is phylogenetically nested within typical *Myrsidea* and therefore our results are consistent with the synonymy of these taxa proposed by Hopkins and Clay (1952) and Price et al. (2003). In this case, *Ramphasticola* are found in two well supported clades (Figure 1), one with *R. hirsuta* (BI – 1.00), which is sister to *M. peruviana* (BI = 0.99), and this clade is sister to all *Myrsidea* hosted by *Ramphastos* toucans (BI = 0.99). Furthermore, based on the results in this study we find that some species in the *Myrsidea* complex (e.g., those currently considered *Ramphasticola*) lack the sternal aster of spine-like setae on sternite II, confirming that it is not a synapomorphy for all lice found in the broader *Myrsidea* clade. Our phylogenetic results suggest a loss of this feature in some members of the broader *Myrsidea* clade. In general, focusing only on one or few characteristics can be problematic in defining a new genus. As suggested in Kolencik et al. (2017), morphological description of a new species in combination with molecular phylogenetic data can help determine the value of specific characters in defining monophyletic groups. This approach was adapted by Kolencik et al. (2021), who found that three species in the *Myrsidea* complex with the sternal asters were sufficiently unique in morphology and genetics to treat them as members of a new genus, *Apomyrsidea* Kolencik & Sychra & Allen.

To distinguish the two different morphotypes of "*Myrsidea*" occurring on toucans, we use the terms "typical" (with aster) and "atypical" (aster absent) *Myrsidea*. The term "typical *Myrsidea*" was previously mentioned by Price et al. (2004) and Hellenthal et al. (2005)

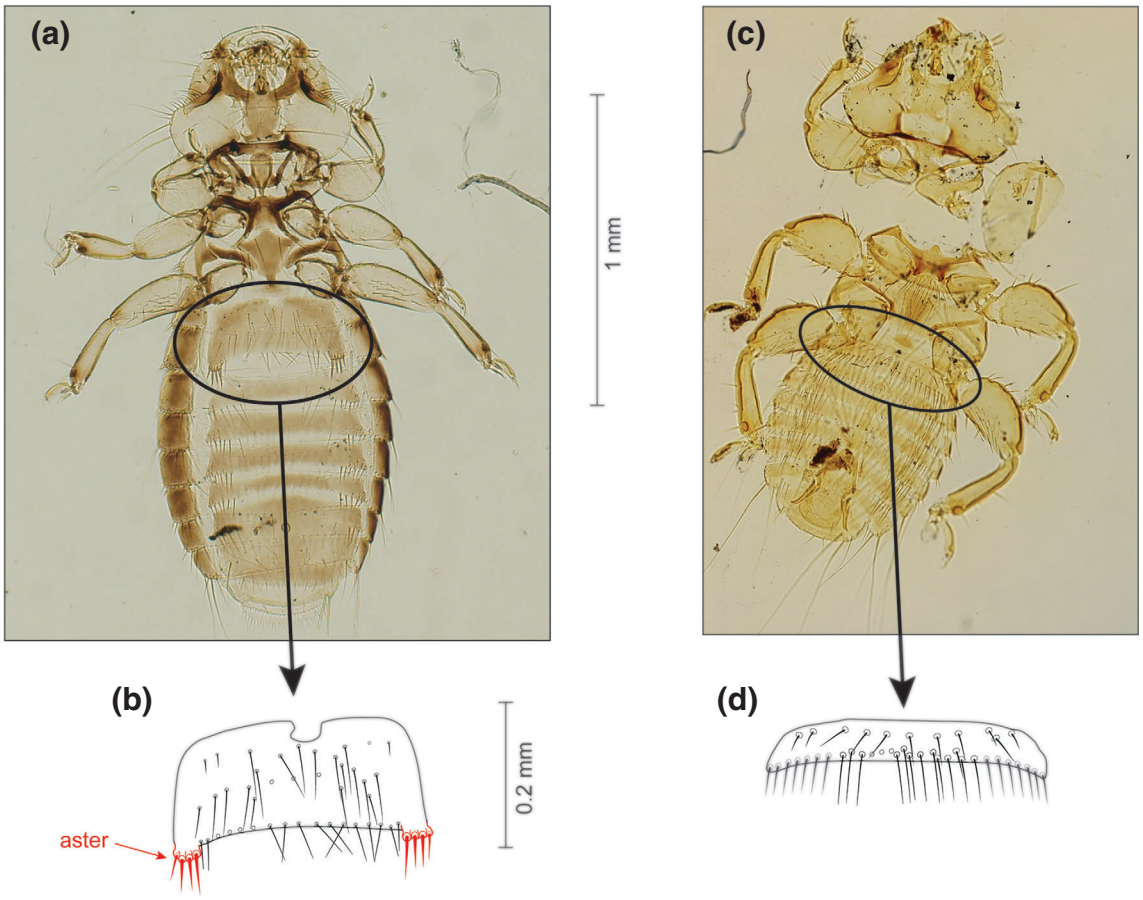

FIGURE 2 (a) Ventral view of female *Myrsidea seminuda* Eichler (as typical *Myrsidea*), with the ellipse marking sternite II and the distinctive sternal asters; (b) An illustration of sternite II with sternal asters highlighted in red; (c) Ventral view of male *Ramphasticola* (now as *Myrsidea*) *aenigma* (as atypical *Myrsidea*); (d) An illustration of sternite II without well-developed sternal asters of setae

and referred to *Myrsidea* from toucans which shared the typical morphological characteristics such as the sternal aster of spine-like setae on sternite II, which is lacking in *Ramphasticola*. This division is not related to the type species for these genera, which are *M. victrix* Waterston for *Myrsidea* – here represented by the close relative and erstwhile subspecies *M. ceciliae*, and *M. hirsuta* for *Ramphasticola*. The results of our phylogenetic study strongly support that rather than being a unique genus, *Ramphasticola* is a morphologically atypical *Myrsidea* (Figure 1). Although *Ramphastos* toucans can harbour both typical and atypical *Myrsidea*, there is no evidence of atypical *Myrsidea* parasitizing *Pteroglossus* araçaris or channel-keel-billed/croaking *Ramphastos* toucans. Atypical *Myrsidea* (i.e., *Ramphasticola*) are only known from smooth-billed/yelping *Ramphastos* toucans. Furthermore, there are two clades of *Ramphasticola* present in the tree, indicating that *R. hirsuta* phylogenetically falls within the *Myrsidea* "*extranea* species group" (in sensu Price et al., 2004). The non-monophyly of *Ramphasticola*, with respect to *Myrsidea*, suggests that there may be morphological plasticity within this group with respect to the development of sternal asters.

Furthermore, Price et al. (2004) distinguished three species groups (*victrix*, *extranea* and *abbreviata*) that were based mainly on modifications of the metanotum and/or tergites of females. However, they noted that this classification introduces a degree of heterogeneity, by including males with conspicuously different male genital sclerites. Clay (1966) suggested that females show important characteristics that can be used to distinguish species, but that these characteristics appear to be of little phylogenetic importance, whereas the genital sclerites of males could be used to clarify the phylogenetic relationships among species within the genus *Myrsidea*. Although our results do not completely follow species groups according to Price et al. (2004), they do support the conclusions of Clay (1966) and show that closely related *Myrsidea* share the same type of genital sclerites (Figure 1). While the genital sac sclerites of the *abbreviata* species group (ASG) appear to be similar to those in VSG, they differ in the structure of the lateral arms, which leading up along the postero-lateral margin are rounded and partially bifurcated, in comparison to broken at an acute angle in VSG (Figure 1a,c). Thus, these morphological characters are an important phylogenetic indicator of relationships within *Myrsidea*.

CONCLUSIONS

Our phylogenetic study yielded three main results. First, host family exhibited significant phylogenetic signal on the *Myrsidea* phylogeny,

which suggests that focusing taxonomic revisions on these lice from specific host families is a reasonable approach to tackling the taxonomic impediment of describing species in this hyper diverse genus. Even with molecular phylogenetic data, the importance of morphological revisions should not be underestimated. Herein, from 152 ingroup samples, 32 (21.1%) specimens could not be assigned to a species designation based on current taxonomic literature, mostly due to a lack of adequate material necessary for complete species level revision (including well-preserved specimens with both adult sexes). This study found 27 previously undescribed louse-host associations that may be new species. With many *Myrsidea* species still undiscovered (Valim & Weckstein, 2013), our knowledge about this highly diverse genus is limited. Second, well-supported clades of lice from different host orders (Passeriformes and Piciformes) are not reciprocally monophyletic, suggesting that they may have arisen due to intraordinal host switching events. However, the genetic data analysed here are mostly partial sequences from one or few genes, and do not provide strong support among more basal nodes in the phylogeny (e.g., Kolencik et al., 2017; Valim & Weckstein, 2013). Future work on this genus should improve both the genome level of sequence data combined with increased taxon sampling to provide more robust phylogenetic reconstructions at basal nodes. Lastly, our phylogenetic data indicate that *Ramphasticola* is nested within *Myrsidea* and that these genera as currently defined are not reciprocally monophyletic and therefore *Ramphasticola* is best treated as a junior synonym of *Myrsidea*. In addition to phylogenomic scale data and broader taxon sampling, detailed morphological descriptions are needed for many unnamed taxa in this relatively unknown hyper diverse group of lice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure S1. Maximum Likelihood phylogenetic tree, using the GTR + G model of molecular evolution and 1000 bootstrap replicates. Bootstrap values are next to the nodes (values below 50% are not shown). Green coloured are lice species from toucans (Ramphastidae), blue from barbets (Lybiidae), and purple from woodpecker (Picidae). R - previously described as *Ramphasticola*, now synonymized as *Myrsidea*.

Figure S2. The results of Maddison and Slatkin (1991) test of host family phylogenetic signal based on taxon sampling delimited by both OTU analyses. A-83 OTUs from 12% cutoff Mothur analysis; B-98 OTU from mean conspecificity probability threshold result of the bGMYC species delimitation analysis.

Table S1. List of louse specimens included in our phylogenetic analyses, with their voucher numbers, hosts, host families, OTU numbers, GenBank accession numbers, country of collection and host catalogue id/voucher number. NA = missing molecular data, † = new louse-host association, * = now synonymized as *Myrsidea*.

Table S2. Primer pairs used for DNA amplification and sequencing.

Table S3. PCR protocols.

Table S4. Partitions and their models used in Bayesian Analysis (BA).

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