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MOLECULAR SYSTEMATICS OF GONIODIDAE (INSECTA: PHTHIRAPTERA)

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ABSTRACT: The higher level phylogenetic relationships within the avian feather lice (Insecta: Phthiraptera: Ischnocera) are extremely problematic. Here we investigate the relationships of 1 family (Goniodidae), sometimes recognized as distinct within Ischnocera, using parsimony and likelihood analyses of nuclear and mitochondrial DNA sequences. These data support monophyly for a restricted definition of traditional Goniodidae, but recognition of this family would result in paraphyly of the large heterogeneous family Philopteridae. We show that the New World *Chelopistes* is not related to other members of Goniodidae, despite similarities in morphology, but rather is the sister taxon to *Oxylipeurus*. Within Goniodidae, genera are divided into those occurring on Galliformes (the *Goniodes* complex) and those occurring on Columbiformes (the *Coloceras* complex). Within the well-sampled *Coloceras* complex, or Physconelloidinae, several groups are identified. However, traditionally recognized genera such as *Coloceras* and *Physconelloides* appear to be paraphyletic. Whereas the phylogeny of Goniodidae reflects some aspects of host relationships, biogeography also influences coevolutionary history.

Parasitic lice (Insecta: Phthiraptera) are widely used as a model system for studies of host–parasite coevolutionary history (Hafner and Nadler, 1988, 1990; Barker, 1991; Hafner et al., 1994; Page et al., 1998; Johnson and Clayton, 2001). Although these and other studies have produced phylogenetic trees for 1 or more genera of lice, the higher level relationships among most major groups of lice remain largely uncertain. Identifying monophyletic groups for cophylogenetic study relies on robust phylogenetic information concerning genera of lice within families or subfamilies.

While classification of the order Phthiraptera into 4 suborders has been relatively stable over the past 50 yr, classification of families and genera within the suborder Ischnocera, containing about 60% of all described louse species, is troublesome. Most workers recognize 2 (Ward, 1957; R. Price, pers. comm.), 3 (Hopkins and Clay, 1952), or 4 (Smith, 2000) families of Ischnocera, while others recognize as many as 21 families (Eichler, 1963). Particularly problematic are relationships among genera of the avian Ischnocera, most of which are classified in Philopteridae. Some workers also recognize Heptapsogasteridae (e.g., Hopkins and Clay, 1952), for a group of distinctive genera of tinamou lice, and Goniodidae (e.g., Smith, 2000), for an apparently closely related group of louse genera occurring on Galliformes (chickens, quail, pheasants, partridges, etc.) and Columbiformes (pigeons and doves). Based on 138 morphological characters, Smith (2001) provided evidence for the monophyly of Goniodidae, as well as evidence for the close relationship between this family and Heptapsogasteridae. Earlier work by Smith (2000) used 62 morphological characters to produce a phylogenetic tree for the genera within each of these 2 families. Thus, morphological data provide some support for recognition of Heptapsogasteridae and Goniodidae. In contrast, Cruickshank et al. (2001) indicated polyphyly of both of these families based on sequences of a portion of the nuclear elongation factor 1-α (EF1α) gene. In both the morphological (Smith, 2001) and molecular (Cruickshank et al., 2001) studies, recognition of Goniodidae and Heptapsogasteridae creates paraphyly of Philopteridae. However, Philopteridae has long been problematic, and it seems desirable to eventually partition this very diverse group into multiple, monophyletic families.

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The goal of the present study is to investigate further the phylogenetic relationships of Goniodidae, as defined by Smith (2000), using both nuclear and mitochondrial DNA sequence data. Some authors include *Austrogoniodes* and/or *Osculotes* within Goniodidae (reviewed by Smith, 2000), and so we have included these genera to test these hypotheses. In addition to these 2 problematic genera, Clay (1976) considered *Chelopistes* and *Labicotes* (parasites of New World Galliformes) to be more closely related to *Oxylipeurus*, another galliform louse genus. Unlike *Chelopistes* (Fig. 1b), *Oxylipeurus* (Fig. 1c) has a long and slender body form occupying the wing niche (Clay, 1949) of its host. Based on her impressions, Clay (1976) suggested that *Chelopistes* and *Labicotes* should not be allied with the Goniodidae, despite the overall morphological similarity of these 2 genera to that group. In addition to testing for the monophyly of Goniodidae, we attempt to identify major groups within Goniodidae (sensu Smith, 2000). Clay (1976) loosely organized species occurring on Galliformes into the *Goniodes* complex and those occurring on Columbiformes into the *Coloceras* complex. Eichler (1963), following previous work by Kéler (1939) and Eichler (1941), proposed a more formal classification, recognizing 7 subfamilies, with galliform and columbiform lice somewhat interspersed between these subfamilies. We make a preliminary evaluation of this classification by including representatives from 5 of the 7 subfamilies in our study (Table I).

A final goal of our study is to test the monophyly of several goniodid genera, which was not done by Smith (2000) because he included only a single representative species of each genus. Within Goniodidae, generic level taxonomy has been quite unstable. Hopkins and Clay (1952) recognized 11 genera that could be classified within Goniodidae (although they did not recognize Goniodidae as a family). In contrast to the conservative approach adopted by Hopkins and Clay (1952), Eichler (1963) recognized 30 genera that he placed within Goniodidae, and this number has been expanded by subsequent workers following Eichler's lead (reviewed in Smith, 2000).

To evaluate the phylogenetic relationships of goniodid taxa, we sequenced representatives of many of the genera for portions of the nuclear EF1α gene and the mitochondrial cytochrome oxidase I (COI) gene. Because of the limited availability of fresh material for sequencing, we primarily focus on the Goniodidae occurring on pigeons and doves (the *Coloceras* complex [Clay, 1976]) but also include a diversity of taxa oc-

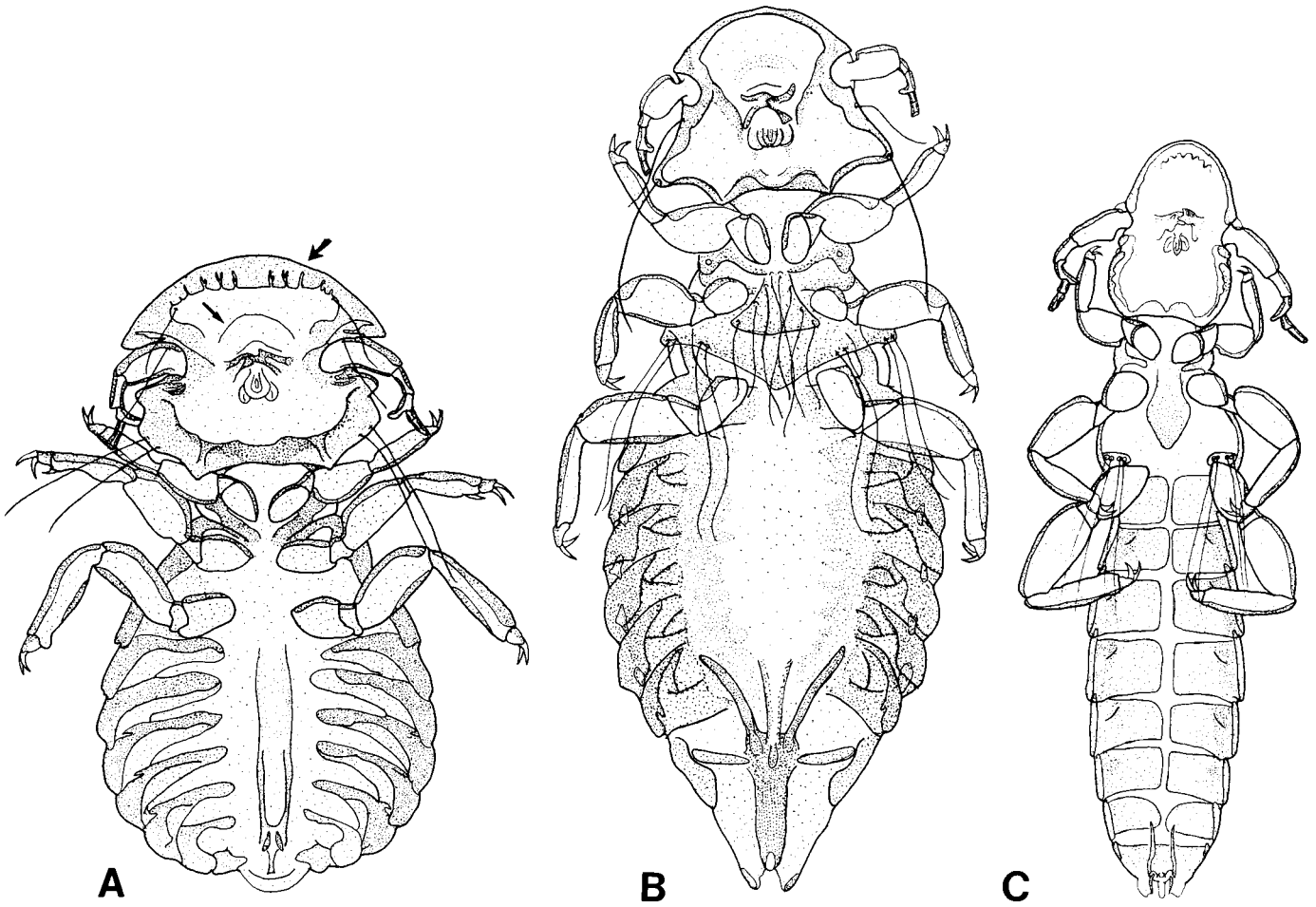

FIGURE 1. Males of (**A**) *Goniodes dissimilis* (body niche): thickened arrow, marginal carina; thin arrow, ventral carina forming a semicircular band around the oral cavity; (**B**) *Chelopistes lervicola* (body niche), (**C**) *Oxylipeurus ithaginis* (wing niche). Scale bar = 1 mm. Drawings are composite including both dorsal and ventral aspects.

curring on Galliformes. We use the generic level classification scheme of Hopkins and Clay (1952) for convenience; however, we comment on implications of our phylogenetic results for generic level taxonomy. We compare the resulting phylogeny to the morphological studies of Smith (2000, 2001) and to various classification schemes.

MATERIALS AND METHODS

Sampling and specimen preparation

We collected lice mainly using the ethyl acetate fumigation method described by Clayton (1990). We sampled a diversity of species of Goniodidae from Columbiformes and Galliformes as well as outgroups (Table I). Lice were stored either in 95% ethanol in a −20 C freezer or dry in a −70 C freezer. DNA was extracted from individual lice by carefully removing the head from the body of the louse and placing both parts in digestion buffer from a Qiagen tissue extraction kit. Digestion proceeded for 56 hr at 55 C. After digestion, the head and the body of the louse were removed from the buffer and mounted together in balsam on a microslide as a voucher and for species identification. Voucher slides were deposited in the Price Institute of Phthirapteran Research, University of Utah, Salt Lake City. We completed the DNA extraction procedure using manufacturer's protocols (Qiagen).

Sequencing

Using polymerase chain reaction (PCR), we amplified a portion of the nuclear EF1α and mitochondrial COI genes. For EF1α we used the primer combination EF1–For3 and EF1–Cho10 (Danforth and Ji, 1998), and for COI we used L6625 and H7005 (Hafner et al., 1994). We used reaction conditions as described by Johnson and Clayton (2000). Sequencing reactions included the PCR primers and were performed as described by Johnson and Clayton (2000). Complementary chromatograms were resolved using Sequencher 3.0 (GeneCodes), and we aligned sequences across species using this program. This produced 348 bp of sequence for EF1α and 383 bp of sequence for COI (GenBank accession numbers AF348643–AF348668, AF348836–AF348877).

Phylogenetic analysis

To thoroughly test the monophyly of Goniodidae, we selected a number of genera within Ischnocera to serve as a composite outgroup. We based this outgroup choice on phylogenetic analyses of major lineages of Ischnocera from morphological (Smith, 2001) and molecular (Cruickshank et al., 2001) data. We rooted all trees on a representative of Trichodectidae (*Geomydoecus craigi*), a family of mammalian Ischnocera believed to be the sister taxon to avian Ischnocera (Smith, 2001). All analyses were performed using PAUP* (Swofford, 2000).

To determine if the EF1α and COI sequences are consistent with a single underlying phylogeny, we conducted a partition homogeneity test (Farris et al., 1994, 1995; Swofford, 2000). Because this test indicated no significant conflict between genes over the phylogeny (see Results), we conducted the remainder of the analyses by combining these 2 gene regions.

We first used unordered parsimony with 100 random addition replicates to search for the most parsimonious trees from the combined data

TABLE I. Samples sequenced.

Species	Host	Locality	Subfamily (Eichler, 1963)
Core Goniodidae (Smith, 2000)			
Coloceras complex, lice from Columbiformes			
Campanulotes compar (a)	*Columba livia*	Utah	Physconelloidinae
C. compar (b)	*C. livia*	Utah	Physconelloidinae
Coloceras hilli	*Streptopelia decaocto*	Netherlands	Physconelloidinae
Coloceras laticlypeatus	*Turtur brehmeri*	Ghana	Physconelloidinae
Coloceras doryanus	*Macropygia tenuirostris*	Philippines	Physconelloidinae
Coloceras sp.	*Macropygia ruficeps*	Borneo	Physconelloidinae
Physconelloides spenceri 1	*Columba speciosa*	Mexico	Physconelloidinae
P. spenceri 2	*Columba fasciata*	Peru	Physconelloidinae
Physconelloides anolaimae 1	*Columba subvinacea*	Guyana	Physconelloidinae
P. anolaimae 2	*Columba plumbea*	Guyana	Physconelloidinae
Coloceras n. sp. (a)	*Streptopelia capicola*	South Africa	Physconelloidinae
Coloceras n. sp. (b)	*Streptopelia senegalensis*	South Africa	Physconelloidinae
Coloceras indicum	*Chalcophaps indica*	Philippines	Physconelloidinae
Coloceras clypeatum	*Phapitreron amethystina*	Philippines	Physconelloidinae
Coloceras savoi (a)	*Columba guinea*	South Africa	Physconelloidinae
C. savoi (b)	*C. guinea*	South Africa	Physconelloidinae
Coloceras n. sp.	*Phapitreron leucotis*	Philippines	Physconelloidinae
Auricotes rotundus	*Ptilinopus occipitalis*	Philippines	Physconelloidinae
Physconelloides ceratoceps 1 (a)	*Leptotila jamaicensis*	Mexico	Physconelloidinae
P. ceratoceps 1 (b)	*L. jamaicensis*	Mexico	Physconelloidinae
Physconelloides cubanus	*Geotrygon montana*	Mexico	Physconelloidinae
P. ceratoceps 2	*Leptotila megalura*	Bolivia	Physconelloidinae
P. ceratoceps 3	*Leptotila plumbeiceps*	Mexico	Physconelloidinae
P. ceratoceps 4	*Leptotila verreauxi*	Mexico	Physconelloidinae
Physconelloides eurysema 1	*Columbina passerina*	Mexico	Physconelloidinae
P. eurysema 2	*Columbina inca*	Mexico	Physconelloidinae
Physconelloides robbinsi	*Metriopelia ceciliae*	Bolivia	Physconelloidinae
Physconelloides n. sp.	*Uropelia campestris*	Bolivia	Physconelloidinae
Physconelloides galapagensis	*Zenaida galapagoensis*	Galapagos	Physconelloidinae
Physconelloides zenaidurae	*Zenaida macroura*	Texas	Physconelloidinae
Physconelloides wisemani	*Zenaida asiatica*	Arizona	Physconelloidinae
P. eurysema 3 (a)	*C. passerina*	Mexico	Physconelloidinae
P. eurysema 3 (b)	*Claravis pretiosa*	Mexico	Physconelloidinae
Goniodes complex, lice from Galliformes			
Goniodes isogenos	*Francolinus africanus*	South Africa	Goniodinae
Goniocotes sp.	*F. africanus*	South Africa	Goniocotinae
Goniodes sp.	*Callipepla californica*	Utah	Goniodinae
Passonomedea sp.	*Odontophorus gujanensis*	Brazil	Goniodinae
Chelopistes oculari	*Penelope purpurascens*	Mexico	Chelopistinae
Chelopistes sp.	*O. gujanensis*	Brazil	Chelopistinae
Chelopistes texanus	*Ortalis vetula*	Mexico	Chelopistinae
Taxa of uncertain status (Smith, 2000)			
Osculotes curta	*Opistocomus hoazin*	Brazil	Opisthocomiellinae
Austrogoniodes watersoni	*Eudyptula minor*	New Zealand	—
Heptapsogasteridae (Hopkins and Clay, 1952)			
Heptapsogaster minuta	*Nothura maculosa*	?	
Megapeostus asymmetricus	*Crypturellus cinnamomeus*	Mexico	
Pectenosoma verrucosa	*C. cinnamomeus*	Mexico	
Strongylocotes fimbriatus	*Crypturellus undulata*	Brazil	
Other outgroups			
Quadraceps punctatus	*Larus cirrocephalus*	South Africa	
Saemundssonia lari	*L. cirrocephalus*	South Africa	
Strigiphilus crucigerus	*Otus guatamalae*	Mexico	
Rallicola fuliginosa	*Dendrocincla anabatina*	Mexico	
Pseudolipeurus similis	*C. cinnamomeus*	Mexico	
Brueelia marginella	*Momotus momota*	Mexico	
Paragoniocotes sp.	*Aratinga astec*	Mexico	
Nyctibicola longirostris	*Nyctibius jamaicensis*	Mexico	
Oxylipeurus chiniri	*Ortalis vetula*	Mexico	
Oxylipeurus sp.	*O. gujanensis*	Brazil	

TABLE I. Continued.

Species	Host	Locality	Subfamily (Eichler, 1963)
Austrophilopterus subsimilis	*Ramphastos sulfuratus*	Mexico	
Cuclotogaster hopkinsi	*Francolinus africanus*	South Africa	
Colinicola docophoroides	*Callipepla californica*	Utah	
Columbicola columbae	*C. livia*	Utah	
Columbicola gracilicapitis	*L. jamaicensis*	Mexico	
Geomydoecus craigi	*Thomomys talpoides*	Utah	

set. The support for this topology was evaluated by conducting 1,000 heuristic bootstrap replicates (Felsenstein, 1985).

We used 1 of the most parsimonious trees to evaluate what maximum likelihood model could not be rejected in favor of a more complex model using likelihood ratio tests according to the framework of Huelsenbeck and Crandall (1997). Using these tests, we found that a model incorporating 6 substitution categories (general time reversible), unequal base frequencies, and rate heterogeneity according to a gamma distribution (8 rate categories) was appropriate. We used the estimated parameters from this model to search for a maximum likelihood tree, using neighbor joining to obtain starting trees and NNI branch swapping. This search produced a new tree over which the parameters of the likelihood model were re-estimated. These new parameter estimates were then used in new tree searches and this procedure was repeated until the tree topology did not change between one iteration and the next.

RESULTS

We obtained sequences of multiple individuals of a few louse species from the same species of host and found them to be identical or nearly so (<1% sequence divergence). In several cases where the same species of louse was sequenced from multiple host species, we found large sequence divergences in the COI gene (between 8.8 and 17.2%), but these divergences were generally not evident in EF1α (0.0–1.3%). In a more comprehensive analysis of genetic variation in COI within *Physconelloides ceratoceps* and *Physconelloides eurysema* (Johnson et al., unpubl. data), these divergent haplotypes generally clustered by host. Very little divergence in COI was observed within each haplotype cluster (<1%). These divergent haplotypes may well represent cryptic species, but more work is needed at the morphological level to verify this. In the trees that follow, we indicate divergent haplotypes within a species of louse by numbers, and we indicate multiple individuals within a haplotype cluster using letters (see also Table I). When sequences are available for both genes for multiple individuals of the same haplotype cluster, the phylogenies presented here include multiple individuals (indicated by letters). However, in cases where variation in COI sequences is minor, single individuals will generally be good representatives of the species or haplotype cluster.

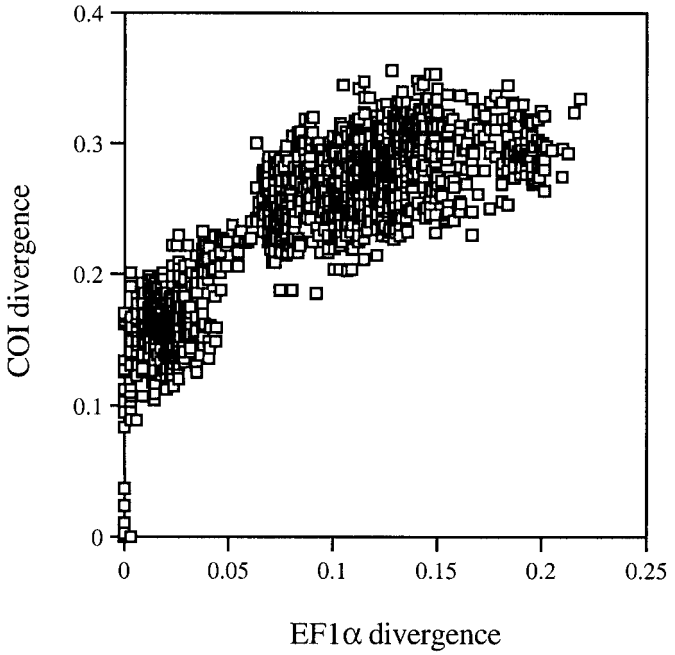

FIGURE 2. Plot of pairwise sequence divergences for COI against those for EF1α.

Sequences of COI showed much greater divergence than sequences for the EF1α gene. Based on plots of pairwise sequence divergence in the COI gene against those for EF1α (Fig. 2), we estimated the relative rate of substitution between the 2 genes to be approximately 10:1. Given these large rate differences, and the probable differences in level of homoplasy that result, methods that take into account these rate differences (such as weighted parsimony or maximum likelihood) should generally provide a better estimate of the tree.

The partition homogeneity test (Farris et al., 1994, 1995; Swofford, 2000) indicated that the 2 genes did not support significantly different trees ($P = 0.50$). Thus, we chose to combine the 2 gene regions into 1 data set in the analyses that follow. Unordered parsimony analysis of the combined gene regions resulted in 47 trees. The strict consensus of these trees (Fig. 3) still showed considerable resolution. In these trees, Goniodidae as recognized by Eichler (1963) is polyphyletic. More specifically, *Chelopistes* is sister to *Oxylipeurus,* and together these 2 occupy a relatively basal position within avian Ischnocera. In addition, *Austrogoniodes* and *Osculotes* appear not to be closely related to other goniodid taxa. Finally, the placement of the goniodid genus *Passonomedea* is uncertain, and it does not group strongly with other goniodids. However, monophyly of the remainder of Goniodidae is supported. This group contains *Goniodes, Goniocotes,* and all the genera from Columbiformes (*Physconelloides, Coloceras, Auricotes,* and *Campanulotes*).

Although Goniodidae as recognized by Eichler (1963) appears to be polyphyletic, the *Coloceras* complex (Clay, 1976) is monophyletic. Within this complex, several recognized groups are evident. For example, within *Physconelloides,* several species groups recognized by Price et al. (1999) are monophyletic (see also Fig. 4), including the *spenceri, ceratoceps,* and *galapagensis* species groups. Neither *Physconelloides* nor

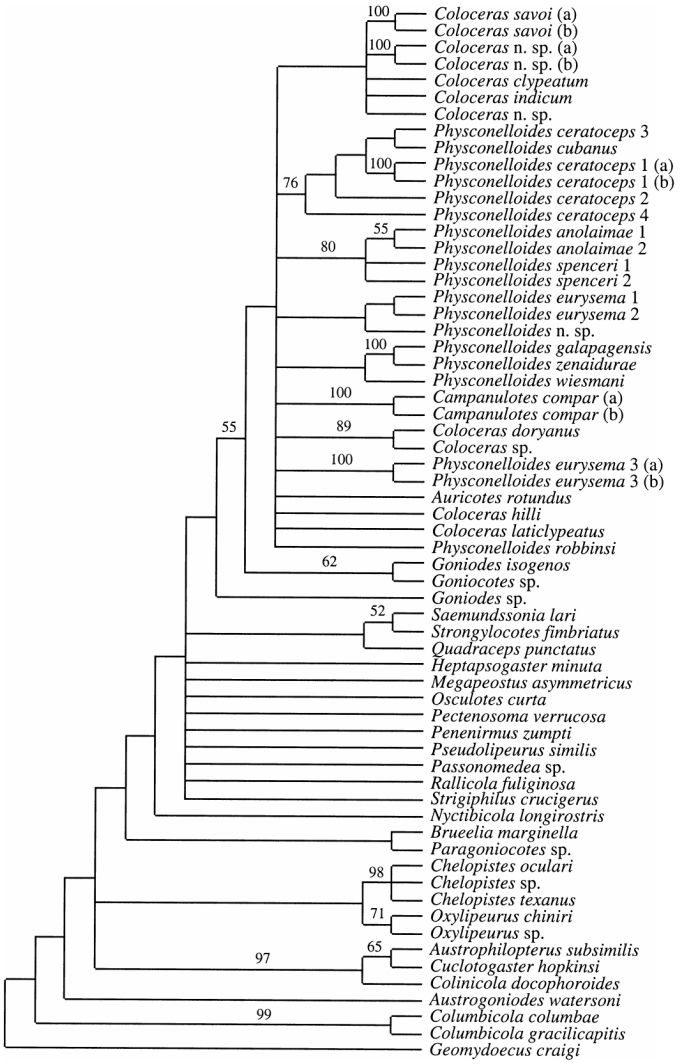

FIGURE 3. Strict consensus of 47 most parsimonious trees resulting from searches on the combined gene data set using 100 heuristic random addition replicates (length = 3,361, RC = 0.087). Multiple individuals from the same haplotype cluster (<1.0% COI sequence divergence) are indicated with small letters. Individuals representing highly divergent haplotypes (>8% COI sequence divergence) are indicated by numbers. Numbers above nodes indicate support from 1,000 bootstrap replicates. Unlabeled nodes received <50% bootstrap support.

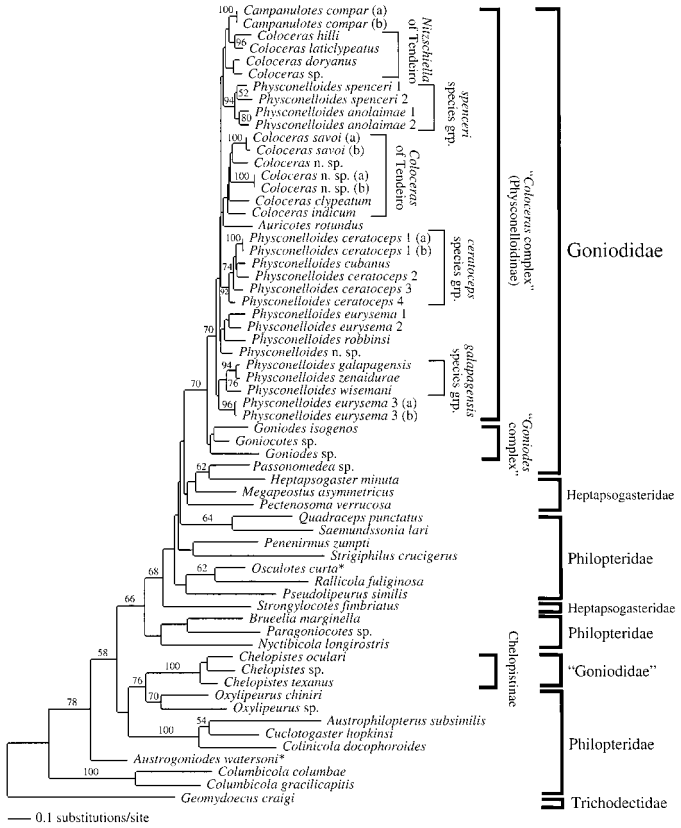

FIGURE 4. Tree resulting from iterative maximum likelihood search strategy (see Materials and Methods). Ln likelihood = −13,622.55. Model parameters: empirical base frequencies, general time reversible (A–C = 0.325, A–G = 10.286, A–T = 2.786, C–G = 1.613, C–T = 6.334, G–T = 1.0), rate heterogeneity according to a gamma distribution (shape parameter = 0.177, with 8 rate categories). Branch lengths are proportional to the branch lengths estimated under the maximum likelihood model (scale indicated). Multiple individuals from the same haplotype cluster (<1.0% COI sequence divergence) are indicated with small letters. Individuals representing highly divergent haplotypes (>8% COI sequence divergence) are indicated by numbers. Numbers associated with nodes indicate bootstrap support from 100 bootstrap replicates. Unlabeled nodes received <50% bootstrap support. Major groupings discussed in the text are indicated with brackets. Two taxa indicated with an asterisk are placed within Goniodidae by some authors.

Coloceras is monophyletic in these trees. However, several authors partition *Coloceras* into *Nitzschiella* and *Coloceras* (e.g., Tendeiro 1969a, 1973), and our results support monophyly of this more restricted definition of *Coloceras.* Although representation of taxa within the *Goniodes* complex is not as complete in our study, *Goniodes* does not appear to be a monophyletic genus.

Parsimony analysis weighting the EF1α gene by 10:1 over COI produces a more resolved tree (not shown). The relationships among the goniodid taxa are similar to those in the unweighted parsimony analysis, with monophyly of Goniodidae, exclusive of *Chelopistes, Passonomedea, Austrogoniodes,* and *Osculotes,* supported.

The tree resulting from maximum likelihood searches (Fig. 4) was generally better resolved and better supported than the parsimony trees. Again, Goniodidae (sensu Eichler, 1963) is polyphyletic. The sister relationship between *Chelopistes* and *Oxylipeurus* received 75% bootstrap support. Similar to the parsimony analysis results, *Osculotes* and *Austrogoniodes* do not appear to be at all closely related to Goniodidae. *Passonomedea* appears in a group with several representatives of Heptapsogasteridae, and this group is sister to Goniodidae. Like the parsimony analysis, the monophyly of a group containing the remainder of Goniodidae is supported, as is the monophyly of the *Coloceras* complex. Within the *Coloceras* complex, *Physconelloides* and *Coloceras* do not seem to be monophyletic, although this does not have strong support. The maximum likelihood tree does contain a monophyletic group comprising *Goniodes* and *Goniocotes* (the *Goniodes* complex in the strictest definition), but the monophyly of *Goniodes* itself is again not supported.

Like the parsimony trees, the maximum likelihood tree con-

tains several groups within the *Coloceras* complex that correspond to morphologically recognized groupings. Three species groups within *Physconelloides*, as identified by Price et al. (1999), are monophyletic in this tree: *spenceri, ceratoceps,* and *galapagensis.* The fourth species group included in our study (*eurysema*) is nearly monophyletic, with a single haplotype of *P. eurysema* clustering with the *galapagensis* species group. *Coloceras* as defined by Hopkins and Clay (1952) is paraphyletic, but a more restricted definition of *Coloceras* (Tendeiro, 1973) results in monophyly for the genus. The species classified as *Nitzschiella* by Tendeiro (1969a) form a group together with the single representative of *Campanulotes.*

DISCUSSION

Phylogeny and implications for classification

Phylogenetic analyses of combined mitochondrial COI and nuclear EF1α gene sequences produce considerable resolution for relationships among Goniodidae and outgroup taxa. These trees support monophyly for a restricted Goniodidae. This group contains *Goniodes, Goniocotes, Physconelloides, Auricotes, Coloceras, Campanulotes,* and by association *Pachyskelotes* and *Kodocephalon. Pachyskelotes,* and *Kodocephalon* were not examined in our study, but based on strong morphological similarity to other goniodids, we feel they will fall in this group. *Osculotes* and *Austrogoniodes* do not appear to have any relationship to this group, other than limited morphological resemblance. In addition, *Chelopistes* that occurs on New World Galliformes appears to be related closely to *Oxylipeurus* rather than to Goniodidae. The close relationship of *Chelopistes* and *Oxylipeurus* is also evident in pairwise divergences for the EF1α gene. For these sequences, *Chelopistes* and other goniodid taxa are 12–13.5% divergent, while *Chelopistes* is only 7.5–9% divergent from *Oxylipeurus.* This relationship was suggested by Clay (1976) and is further born out by identical chromosome numbers in these 2 genera (Kettle, 1977). Thus, *Chelopistes* (and by association *Labicotes*) should be removed from conceptions of Goniodidae.

The relationships of *Passonomedea* are more unclear in analyses of our DNA sequence data set. *Passonomedea,* an exclusively New World genus, appears to have some relation to tinamou lice (Heptapsogasteridae) that in turn seem to be related closely to Goniodidae (Smith, 2001; Fig. 4). However, Smith (2000) found support for exclusion of *Passonomedea* from within Heptapsogasteridae on the basis of morphological characters. Indeed, the inclusion of *Passonomedea* within the Goniodidae on the basis of molecular data cannot be ruled out at this time, but this genus appears to be quite divergent, at least from other goniodids.

Within our more restricted circumscription of Goniodidae, there appear to be 2 major groups (Fig. 4). One of these is the *Coloceras* complex (sensu Clay, 1976), consisting exclusively of taxa parasitizing pigeons and doves (Columbiformes). The subfamily Physconelloidinae of Eichler (1941) could also be redefined to include *Auricotes* and would more formally identify this group. Monophyly of such a Physconelloidinae is supported in both parsimony and maximum likelihood analyses and receives bootstrap support in the likelihood analysis. However, monophyly of columbiform goniodids was not obtained by Smith (2000) who placed *Goniocotes* within the *Coloceras* complex, based on cladistic analysis of morphological characters.

Monophyly of the *Goniodes* complex (sensu Clay, 1976) restricted to include only *Goniodes, Goniocotes,* and by association *Pachyskelotes* is less certain. This group is monophyletic in the likelihood tree (Fig. 3) and appears as a grade in the parsimony analyses (Fig. 2). Considering 5 of the 7 subfamilies of Goniodidae (Eichler, 1963) from which we have samples, only Chelopistinae receives support, but not as a member of Goniodidae. Goniodinae and Goniocotinae do not appear to be supported, and Physconelloidinae would only be monophyletic upon inclusion of *Auricotes.* We cannot comment on the monophyly of Opisthocomiellinae because we included only a single representative species, but this subfamily should be removed from Goniodidae.

Given the polyphyly of Goniodidae, is there any reason to recognize this family within Ischnocera? While a more restricted definition of Goniodidae could identify a monophyletic clade, recognition of Goniodidae as a family would result in paraphyly of the traditionally recognized Philopteridae, because Goniodidae falls within Philopteridae. An alternative would be to partition Philopteridae into a number of smaller families, each of which was monophyletic. However, identification of these smaller groups has been an extremely difficult enterprise for louse taxonomists in the past (Clay, 1951; Ward, 1957; Ledger, 1980), and limited molecular data have, to date, provided only limited support for other major groupings within Ischnocera (Cruickshank et al., 2001). Thus, in comprehensive classifications of Ischnocera, for the time being it seems prudent not to assign family status to Goniodidae, although this name is likely to have validity as a more complete understanding of ischnoceran relationships is developed. Likewise Physconelloidinae (as redefined here) appears to have validity as a major group within Goniodidae; however, we feel that a comprehensive subfamilial classification of Goniodidae would be premature at this time because of a need for more sampling within the *Goniodes* complex.

Within the *Coloceras* complex (or Physconelloidinae) several relationships are apparent. The well sampled *Physconelloides* and *Coloceras* appear to be paraphyletic; however, major groupings within these genera correspond to traditionally recognized groups. *Coloceras* has been partitioned by Tendeiro (1969a, 1973) and others into *Coloceras* and *Nitzschiella.* However, Clay (*in* Ledger, 1980) argued that *Coloceras* and *Campanulotes* probably grade into each other, and thus recognition of *Nitzschiella* was probably not warranted. Tendeiro (1969a) recognized *Nitzschiella* based on the proportions of the head and abdomen as well as other metric features. Indeed, he found 1 species that possessed ''the head of the *Campanulotes* type and the abdomen of the *Nitzschiella* type'' that caused him to recognize a new genus (*Nitzschielloides*) rather than synonymize *Nitzschiella* and *Campanulotes* (Tendeiro, 1969b). Relevant to these problems, we found 2 major groups of *Coloceras.* One of these groups corresponds to the more restricted definition of *Coloceras* of Tendeiro (1973), and this group was identified in both the parsimony and likelihood trees (Figs. 3, 4). In the likelihood analysis a group containing species described as *Nitzschiella* by Tendeiro (1969a) was identified (Fig. 4). However, this group also contained our single representative of *Campanulotes,* creating paraphyly for *Nitzschiella.* Thus, while

monophyly of *Coloceras* as defined by Tendeiro is supported, monophyly of *Nitzschiella* is not. One possible solution is to recognize a more restricted *Coloceras,* following Tendeiro (1973), but to merge *Nitzschiella,* and by association the problematic *Nitzschielloides,* into *Campanulotes.* However, more representatives of *Campanulotes* and *Nitzschiella* are needed before these major taxonomic changes can be proposed.

An even more difficult problem is the relationship of *Physconelloides.* While the species groups recognized by Price et al. (1999) generally bear up under this study, these groups appear to form a grade at the base of Physconelloidinae. One possible solution is to recognize each species group as a separate genus. However, the support for the arrangement among the species groups is weak, owing to very short branches connecting these species groups (see Fig. 4). More data are needed before alterations of generic level classification can be proposed, because it is not yet certain that *Physconelloides* is paraphyletic.

We included a much more limited sample of the *Goniodes* complex. However, even in this limited sample, *Goniodes* appears to be paraphyletic, and this was evident in both parsimony and likelihood trees. *Goniodes isogenos* from a francolin (*Francolinus africanus*) is sister to a species of *Goniocotes* from the same host, while a species of *Goniodes* from New World quail (*Callipepla*) falls outside the francolin lice. Clay (1951) suggested that *Goniodes* and *Goniocotes* grade into one another when a large enough sample of both genera is examined, and this appears to be evident in the molecular data.

Morphological convergence

The polyphyly of the Goniodidae highlights the difficulties that morphological convergence creates for classification schemes based on morphology of ischnoceran lice. While carefully constructed morphological character matrices have the potential to identify such convergence by identifying homoplasious characters, the only study of this type within avian Ischnocera (Smith, 2001) placed *Chelopistes* with Goniodidae.

What is the source of this morphological convergence? Much of the morphological diversity within avian Ischnocera appears to correspond to specialized niches on the body of the host. For example, members of Goniodidae, and other lice of this form, possess a short rounded body shape and a head shape consisting of an uninterrupted marginal carina and a ventral carina forming a semicircular band around the oral cavity (Ledger, 1980; Fig. 1a). These lice generally occur on the body of the host exclusive of the head. In contrast, individuals of other genera of avian Ischnocera specialize by inserting themselves between the feather barbs of the wing feathers to escape preening defenses of the host (Clayton, 1991; Clayton et al., 1999). Individuals belonging to these wing-specialist genera have a long and slender body form as typified by *Columbicola* on Columbiformes and *Oxylipeurus* on Galliformes (Fig. 1c). Thus, niche specializations, if independently derived in various groups of avian Ischnocera could lead to convergence in overall body form, obscuring evolutionary relationships. This convergence is likely to be especially problematic when it occurs in different lineages of lice parasitizing the same host taxa. Convergence of ischnoceran head and body form has been recognized by a number of workers on the basis of morphology alone (Clay, 1949; Eichler, 1963), but the number of avian louse lineages that exhibit such convergence is still uncertain. As evident in our molecular study, the galliform body louse lineage including *Chelopistes* (Fig. 1b) has converged on the body form and head shape of other galliform body lice (*Goniodes* and *Goniocotes*), despite being closely related to the wing louse, *Oxylipeurus* (Fig. 1c). In contrast, the body lice of Columbiformes form a monophyletic group distantly related to the wing lice (*Columbicola*) on these same hosts.

Biogeography and host relationships

Several biogeographic patterns are evident in the phylogeny for these groups of lice. *Chelopistes* is essentially restricted to New World Galliformes, while *Goniodes* and *Goniocotes* are much more prevalent in the Old World. The historical isolation of South America, and the presumed absence of body lice on Galliformes there in the past (Clay, 1976) may have provided an opportunity for niche specialization of lineages within *Chelopistes* arising from an *Oxylipeurus*-like ancestor in the absence of any competition. Based on current understanding of galliform relationships (Kimball et al., 1999), it also appears that some lineages within *Chelopistes* may have switched onto some galliform hosts, such as turkeys (Melagridinae) who colonized the New World from the Old.

The close relationship between species Goniodidae on Galliformes and Columbiformes suggests that these lice may have switched from one of these host groups to the other, given that these host groups are very distantly related (Sibley and Ahlquist, 1990), and that the genetic divergences between the *Coloceras* and *Goniodes* complexes are relatively small. The direction of the hypothesized switch is currently unclear. Other groups of lice on Galliformes (*Chelopistes, Oxylipeurus, Cuclotogaster, Colinicola*) and Columbiformes (*Columbicola*) do not appear to be related closely to Goniodidae; these host orders thus appear to carry multiple lineages of Ischnoceran lice with independent evolutionary histories.

Within Physconelloidinae, relationships among louse species generally reflect relationships among host species (Johnson and Clayton, 2000, 2001). However, biogeography also appears to be important. At the generic level, *Physconelloides* is restricted to the New World and Australasia. While this genus may be paraphyletic, the occurrence of closely related groups within this genus on distantly related hosts occupying the same areas suggests that biogeographic opportunities for host switching may play an important role. Additionally, both groups within *Coloceras* are restricted to Africa and Eurasia, despite their presence on distantly related hosts. For example, louse species on birds in the largely sympatric host genera *Streptopelia* and *Turtur* appear closely related, in contrast to their hosts, which are not closely related (Johnson and Clayton, 2000). Both host phylogeny and biogeography appear to have an important influence on the patterns of speciation within Physconelloidinae.

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