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PHYLOGENETIC RELATIONSHIPS OF THE GENERA OF THE PRONOCEPHALIDAE LOOSS, 1902 (DIGENEA: PARAMPHISTOMIFORMES)

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ABSTRACT: Quantitative phylogenetic analysis of 20 nominal genera of the Pronocephalidae based on 47 morphological transformation series produced 6 equally parsimonious trees, each with a consistency index of 77.8%. All trees agree that *Adenogaster* is the sister group to the rest of the pronocephalids, and a new subfamily is proposed for it. The Pronocephalinae comprises *Pronocephalus*, *Ruicephalus*, *Neopronocephalus*, *Macravestibulum*, *Choanophorus*, *Cetiosaccus*, and *Metacetalabulum*. The Charaxicephalinae comprises *Charaxicephalus*, *Desmogonius*, *Diaschistorchis*, *Pleurogonius*, *Iguanacola*, *Renigonius*, *Parapleurogonius*, *Himasomum*, *Pyelosomum*, *Cricocephalus*, *Barisomum*, and *Pseudobarisomum*. An amended diagnosis for *Himasomum* is presented. The trees differ only in the placements of *Pleurogonius*, *Renigonius* + *Parapleurogonius*, *Iguanacola*, and *Himasomum* relative to each other. *Parapronocephalum* and *Notocotyloides* are members of the clade containing the Notocotyloidea. The phylogenetic tree supports interpretations of 3–4 transitions from marine to freshwater turtles, 3 host switches from marine turtles to the Galapagos marine iguana and 3 from marine turtles to the French angelfish, and widespread host switching among marine chelonians. No switches to non-chelonian hosts coincide with transitions from marine to freshwater.

Marine turtles host a diverse array of helminth parasites, dominated by members of the digenean family Pronocephalidae Looss, 1902. Looss (1901) established the family for monostomous digeneans with cephalic collars inhabiting marine turtles, particularly *Chelonia mydas* (L.). Species allocated to this family include parasites of marine fish, iguanas, birds, and freshwater turtles. Classification of the Pronocephalidae has been unstable. Price (1931) recognized 3 subfamilies: Opisthoporinae Price, 1931 for *Opisthoporus aspidonectes* (MacCallum, 1917) Fukui, 1931 (= *Teloporia aspidonectes*); Charaxicephalinae Price, 1931, including *Charaxicephalus* Looss, 1901 and *Desmogonius* Stephens, 1911; and Pronocephalinae Looss, 1902 including *Pronocephalus* Looss, 1899, *Cricocephalus* Looss, 1899, *Pyelosomum* Looss, 1899, *Adenogaster* Looss, 1901, *Glyphicephalus* Looss, 1901, *Pleurogonius* Looss, 1901, *Epibathra* Looss, 1902, *Barisomum* Linton, 1910 (including *Himasomum* Linton, 1931), *Diaschistorchis* Johnston, 1913 (which Price synonymized with *Wilderia* Pratt, 1914 and *Synechorchis* Barker, 1922), and *Astrorchis* Poche, 1926.

Mehra (1932) added the Hippocrepiinae Mehra, 1932 for *Hippocrepi* Travasos, 1922 and the Neopronocephalinae Mehra, 1932 for *Neopronocephalus* Mehra, 1932, placed *Macravestibulum* Mackin, 1930 in the Pronocephalinae, and transferred *Diaschistorchis* to the Charaxicephalinae. Ruiz (1946) synonymized the Notocotyloidea and Pronocephalidae, recognizing 7 subfamilies: Notocotylinae Lühe, 1909; Nudacotylinae Barker, 1916; Opisthotrematinae Poche, 1926; Pronocephalinae, including *Pronocephalus*, *Cricocephalus*, *Glyphicephalus*, *Epibathra*, *Pyelosomum*, *Adenogaster*, *Teloporia* Fukui, 1933, *Iguanacola* Gilbert, 1938, and *Renigonius* Mehra, 1939; Charaxicephalinae, including *Charaxicephalus*, *Desmogonius*, and *Diaschistorchis*; Neopronocephalinae, including *Neopronocephalus*; and Choanophorinae Caballero, 1942, including *Choanophorus* Caballero, 1942, *Macravestibulum*, *Metacetalabulum* Teixeira de Freitas and Lent, 1938, and *Cetiosaccus* Gilbert, 1938.

Yamaguti (1958) recognized the superfamily Notocotyloidea Kossack, 1911 comprising the Notocotyloidea, Nudacotyloidea, Opisthotrematidae, and Pronocephalidae. Within the Pronocephalidae, he recognized 10 subfamilies: Choanoporinae for *Choanophorus*, Metacetalabulinae Yamaguti, 1958 for *Metacetalabulum*, Cetiosaccinae Yamaguti, 1958 for *Cetiosaccus*, Macravestibulinae Yamaguti, 1958 for *Macravestibulum*, Teloporiinae Yamaguti, 1958 for *Teloporia*, Diaschistorchinae Yamaguti, 1958 for *Diaschistorchis*, Charaxicephalinae for *Charaxicephalus*, Desmogoniinae Yamaguti, 1958 for *Desmogonius*, Neopronocephalinae for *Neopronocephalus*, and Pronocephalinae for *Pronocephalus*, *Cricocephalus*, *Pyelosomum*, *Glyphicephalus*, *Epibathra*, *Adenogaster*, *Barisomum*, *Iguanacola*, *Renigonius*, *Medioporus* Oguro, 1936, *Myosaccus* Gilbert, 1938, and *Ruicephalus* Skrjabin, 1955. Yamaguti (1971) added *Pseudobarisomum* Siddiqi and Cable, 1960 and *Neocricocephalus* Gupta, 1962 to the Pronocephalinae.

Since 1971, 6 genera, 5 of them monotypic, have been described: *Paradenogaster* Fischthal and Kuntz, 1975, *Rameshwarotrema* Rao, 1975, *Parapleurogonius* Sullivan, 1976, *Charaxicephaloides* Groschafft and Tenora, 1978, *Raogaster* Groschafft and Tenora, 1981, and *Cortinasoma* Oshmann and Zharikova, 1984. The family thus comprises approximately 80 nominal species allocated to approximately 32 genera (22 species have been placed in *Pleurogonius*) in 10 subfamilies.

We present herein the first phylogenetic systematic analysis of supraspecific taxa comprising the Pronocephalidae.

MATERIALS AND METHODS

Specimens examined

We examined available published accounts and the following specimens (accession numbers follow species names, number of specimens examined is in parentheses): CHIBUNAM (Colección Helminológica del Instituto de Biología de la Universidad Nacional Autónoma de México, México City, México): *Parapronocephalum symmetricum*: 224-18 (1); *Adenogaster serialis*: 217-12 (20), 233-9 (1), 212-1 (1), 225-13 (5); *Pleurogonius sindhi*: 213-5 (1); *Pleurogonius americanus*: 243-7 (2); *Pleurogonius grocotti*: 212-8 (1); *Pleurogonius lobatus*: 250-11 (5); 213-6 (7); *Pleurogonius linearis*: 32-6 (1); *Pyelosomum cochlear*: 233-10 (6), 212-11 (1); *Pyelosomum posterorchi*: 213-4 (1); *Pyelosomum renicapite* (= *Astrorchis* r.): 250-12 (8); *Cricocephalus albus*: 212-13 (7), 234-16 (1); *Barisomum erubescens*: 234-15 (4); *Pronocephalus trigonocephalus*: 213-3 (1); *Choanophorus rovirosai*: 19-9 (3), 217-17 (7). UNSMHWML

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(University of Nebraska State Museum, Division of Parasitology, Harold W. Manter Laboratory, Lincoln, Nebraska, U.S.A.): *Pleurogonius malaclemys*: 23753 (7); *Pleurogonius trigonocephalus*: 1701 (5); *Glyphicephalus candidulus* (= *Pleurogonius candidulus* and *Barisomum candidulus*): 157 (10), 158 (7), 123913 (5), 123914 (2), 123915 (2), 123916 (2), 124287 (1); *Renigonius cuorensis*: 20867 (1); *Parapleurogonius breviaecum*: 20865 (1); *Pyelosomum renicapite* (= *Astrorchis r.*): 1700 (1); *Pyelosomum amblyrhynchi* (= *Myosaccus a.*): 1702 (2); *Cricocephalus albus*: 1711 (3); *Charaxicephalus robustus*: 1707 (1); *Desmogonius desmogonius*: 874 (15); *Diaschistorchis multitesticularis*: 20864 (1); *Neopronocephalus orientalis*: 20866 (3); *Macravestibulum obtusicaudatum*: 1706 (1), 22471 (3). USNMHC (United States National Museum Helminthological Collection, Beltsville, Maryland, U.S.A.): *Pleurogonius puertoricensis*: 73319 (2); *Pleurogonius laterouterus*: 73317 (5); *Pleurogonius longuisculus*: 9658 (3); *P. linearis*: 9653 (3), 73399 (9); *P. malaclemys*: 39052 (1); *P. trigonocephalus*: 9660 (3), 73340 (1); *Parapleurogonius breviaecum*: 74052 (7); *Iguanacola navicularis*: 43401 (4); *P. cochlear*: 9665 (1); *P. posterorchis*: 73331 (2); *Pyelosomum longicaecum*: 8910 (1); *P. renicapite* (= *Astrorchis r.*): 74860 (1); *Pseudobarisomum holacanthi*: 39310 (1); *Barisomum pomacanthi* (= *Pleurogonius p.*): 8087 (1), 8088 (3), 8089 (1), 8090 (1), 8091 (1); *C. robustus*: 9619 (2); *Cetiosaccus galapagensis*: 9215 (1); *Metacetabulum invaginatum*: 73333 (4); *Teloporia aspidonectes*: 61205 (1); *Paradenogaster selfi*: 73009, 73010, 73011 (10).

Taxa recognized

We used 20 of the nominal genera as terminal taxa. Ruiz (1946) considered *Epibathra*, *Myosaccus*, and *Astrorchis* as synonyms of *Pyelosomum*; we concur, and a species-level analysis of the genus (Pérez Ponce de León and Brooks, 1995) corroborates this decision. We found no consistent basis on which to separate *Pleurogonius*, *Glyphicephalus solidus* Looss, 1901 (the type species of *Glyphicephalus*), and *Medioporus*, so we have grouped them together as *Pleurogonius*. By contrast, *Himasomum* Linton, 1910, established for *Himasomum candidulum* Linton, 1910, was considered a synonym of *Barisomum* by Price (1931) and Yamaguti (1958, 1971), of *Pleurogonius* by Ruiz (1946) and Manter (1947) and of *Glyphicephalus* by Siddiqi and Cable (1960). In our study, *H. candidulum* and *Glyphicephalus lobatus* Looss, 1901 form a group distinct from *Pleurogonius* (including *G. solidus*) and *Barisomum*, so we consider *Himasomum* Linton, 1910 valid (see amended diagnosis below).

We did not use *Teloporia* Fukui, 1933, *Neocricicephalus* Gupta, 1962, *Paradenogaster* Fischthal and Kuntz, 1975, *Rameshwarotrema* Rao, 1975, *Charaxicephaloides* Groschafft and Tenora, 1978, *Raogaster* Groschafft and Tenora, 1981, or *Cortinasoma* Oshmarin and Zharikova, 1984 as separate taxa. *Teloporia* was proposed by Fukui (1933) for specimens described by MacCallum (1921) as *Paramphistomum aspidonectes* and transferred to *Opisthoporus* by Fukui (1929). Our examination of specimens deposited in the United States National Helminthological Collection convinced us that *Teloporia* lacks the cephalic collar characteristic of pronocephalids and that the arrangement of gonads and the structure of the terminal genitalia preclude its inclusion in Pronocephalidae. *Neocricicephalus*, established for *Neocricicephalus vitallani* from *C. mydas* from an unnamed locality in the Caribbean Sea (Gupta, 1962), purportedly possesses a muscular pharynx and lacks an esophagus. Because no other monostomes have a pharynx or lack an esophagus, and because many pronocephalids have a muscular esophagus or esophageal bulb, we are uncertain about the description of this material. Gupta did not compare the new species with the features that are diagnostic for *Cricocephalus*, and we are unable to discern any substantive differences between the 2 taxa. Finally, there is no indication in the publication that any type specimens were designated or deposited in any museum collection. *Rameshwarotrema* was proposed for 2 species described from 27 specimens collected in *C. mydas* from India (Rao, 1975). There is no evidence that type material was deposited for these species, and the original descriptions provide insufficient information to distinguish the proposed species or genus from a number of previously described taxa. Illustrations accompanying the original descriptions suggest specimens that have been excessively flattened, perhaps accounting for the "extracecal uterine loops" suggested to be diagnostic for the genus, but lacking in all other pronocephalids. *Charaxicephaloides polyorchis* in *C. mydas* from Cuba differs from

Charaxicephalus by having testes arranged in irregular double rows and not separated by uterine loops (Groschafft and Tenora, 1978), but we have been unable to obtain specimens. Based on the characters we have used, *Charaxicephaloides* would be the sister species of *Charaxicephalus*, and because it is monotypic, treating both taxa as a single genus should not affect the phylogenetic analysis. Groschafft and Tenora (1981) proposed *Raogaster* for the species described by Rao (1975) as *Adenogaster indica* in *C. mydas* from India, based on a single specimen that we have been unable to locate. We can discern no significant differences between the specimen upon which *A. indica* was based and *Adenogaster serialis*, a cosmopolitan species inhabiting *C. mydas* throughout the world. *Paradenogaster* was proposed for *Paradenogaster selfi* in the freshwater turtles *Ocadia sinensis* and *Geoclemys reevesi* from Taiwan (Fischthal and Kuntz, 1975). *Cortinasoma* was proposed for *Cortinasoma ocadiae*, in *Ocadia quadriocellata* from Vietnam (Oshmarin and Zharikova, 1984). Both species resemble *Adenogaster* by having ventral glands but are distinctive in lacking cecal diverticula (the plesiomorphic condition for character 5) and in having 2 polar filaments on each egg (the plesiomorphic condition for character 10). *Paradenogaster selfi* is further described as having a unipartite cirrus sac with an internal seminal vesicle. The holotype (USNM Helm. Coll. no. 73009) and paratypes (USNM Helm. Coll. no. 73010, 73011) of *P. selfi* exhibit cirrus sacs containing a vesiculate pars prostatica, elongate ductus Caballeroi (see character 47, below), and cirrus, similar to *Adenogaster*. *Cortinasoma ocadiae* is described as having very small rather than relatively large vitelline follicles (an autapomorphic condition); otherwise, it does not differ markedly from *Paradenogaster*. We have been unable to obtain specimens of *C. ocadiae*, so we are uncertain about the status of the ductus Caballeroi in that species. Based on the characters presented in the descriptions and those that we could confirm by examining specimens, we consider *Cortinasoma* a junior synonym of *Paradenogaster*. Furthermore, the 2 characters distinguishing *Paradenogaster*, with 2 species, from *Adenogaster*, which is monotypic, are plesiomorphies; there is therefore no basis for recognizing more than a single genus for the 3 species. We thus consider *Paradenogaster* a synonym of *Adenogaster*.

Analyses performed

Phylogenetic analyses were performed using phylogenetic systematics (Hennig, 1966; Wiley, 1981; Brooks and McLennan, 1991; Wiley et al., 1991). Results were confirmed using the PAUP (phylogenetic analysis using parsimony) computer program version 3.1.1 (Swofford, 1993), run on a Macintosh IIX computer. The following options were examined: Characters: All unordered, or multistate characters unordered; outgroups: Deuterobarididae only, Deuterobarididae + Notocotylidae, Deuterobarididae + Notocotylidae + *Parapronocephalum*; optimization: Acctran, Deltran; tree-building algorithm: heuristic search/branch swapping, branch and bound.

Character argumentation

Phylogenetic systematic analyses (Brooks et al., 1985, 1989; Brooks and McLennan, 1993a) have placed Notocotylidae and Pronocephalidae as sister groups at the terminal portion of the Paramphistomiformes. Their closest relatives, based on absence of a ventral sucker and the possession of ventral body glands, appear to be the Deuterobaridinae in the paraphyletic Microscaphidiidae.

Two monotypic genera, *Parapronocephalum* Belopol'skaia, 1952 and *Notocotylodes* Dollfus, 1966, have been problematic. Yamaguti (1958) listed *Parapronocephalum* as a member of the Notocotylidae (Yamaguti, 1958: 791) and of the Pronocephalidae (Yamaguti, 1958: 976). Later, Yamaguti (1971) placed *Parapronocephalum* in the Pronocephalidae and *Notocotylodes* in the Notocotylidae. Both species possess collars surrounding the oral sucker and inhabit shorebirds. All analyses we performed, including those in which we placed *Parapronocephalum* as a member of the ingroup and asked PAUP to try to force the ingroup to be monophyletic, placed *Parapronocephalum* and *Notocotylodes* as sister groups forming a clade that is the sister group of the Notocotylidae. Therefore, the notocotylids and *Parapronocephalum* plus *Notocotylodes* served as the primary outgroups and the deuterobaridines as a paraphyletic secondary outgroup. Unless stated otherwise, all outgroups agreed on the plesiomorphic condition. We identified the following characters

1) Distribution of vitelline follicles. The plesiomorphic condition is vitelline follicles extending along the lateral (extracecal) margins of the body from the level of the anterior margin of the testes to near the level of the cirrus sac (0). In *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus*, the vitellaria are restricted to near the testicular area (1).

2) Location of testes in body. Notocotyliids, *Notocotyloides* and *Parapronocephalum*, like most species of pronocephalids, have testes located very close to the posterior end of the body (0). *Charaxicephalus*, *Desmogonius*, and *Diaschistorchis* have testes fragmented into relatively large follicles distributed roughly linearly from near the posterior end of the body to as far anteriorly as midbody (1). *Pronocephalus*, *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* have testes located in the posterior 1/3 of the body, but some distance from the posterior end (2).

3) Location of testes with respect to the ceca. Notocotylids and *Notocotyloides*, like most species of pronocephalids, have extracecal testes (0). *Parapronocephalus*, *Pronocephalus*, *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* have intercecal testes (1); this trait is characteristic of many microscaphidiids and paramphistomids, the second-level outgroups, but is coded as apomorphic for the pronocephalids using the alternating outgroup rule (Wiley et al., 1991).

4) Location of Mehlis' gland with respect to ovary. Deuterobarids and most pronoccephalids exhibit postovarian Mehlis' glands (0). Notocotyliids and *Pronoccephalum* exhibit preovarian Mehlis' glands (1), an apparent synapomorphy for those 2 groups. *Desmogonius*, *Adenogaster*, and *Cricoccephalus* are characterized by Mehlis' glands located laterally to the ovary (2). Two species of *Pyelosomum* also exhibit lateral Mehlis' glands, but this is a derived condition within the genus (Pérez Ponce de León and Brooks, 1995).

5) Cecal diverticula. We have observed 7 different cecal morphologies (Fig. 1). The plesiomorphic condition is that in which the ceca are smooth-walled throughout their entire length (0); this condition occurs in members of *Parapleurogonius*, *Ruicephalus*, *Cetiosaccus*, *Metacetabulum*, and *Neopronocephalus*, and in some species of *Pleurogonius* and *Adenogaster*. Derived conditions include: ceca with irregular diverticula in anterior portions, found in *Parapronocephalum*, *Iguanacola*, some members of *Pleurogonius*, 1 species of *Renigonus*, *Macravestibulum*, and *Choanophorus* (1); ceca with irregular diverticula throughout their length, found in *Pronocephalus*, *Diaschistorchis*, the second species of *Renigonus*, *Pyelosomum*, and *Pseudobarisomum* (2); ceca with regular diverticula spaced equally on the medial and lateral surfaces of the ceca, found in *Charaxicephalus* (3); ceca with regular diverticula found only on medial surface of ceca, found in *Adenogaster* (4); ceca with regular diverticula spaced alternately on the medial and lateral surfaces of the ceca, found in *Desmogonius* and *Barisomum* (5); and ceca with regular diverticula found only on the lateral surface of the ceca, found in *Cricocephalus* (6). This character was not used in constructing the phylogenetic tree, but we include it for future reference.

6) Posterior body projections. The plesiomorphic condition is the absence of posterior body projections in adults, exhibited by *Adenogaster*, *Pronocephalus*, *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* (0). All other pronocephalids exhibit posterior body projections (1).

7) Cephalic collar. The presence of a cephalic collar has been used to distinguish the Pronocephalidae from the Notocotyloidae and Microsaphidiidae. The absence of cephalic collars is clearly plesiomorphic (0) and the presence of such collars is apomorphic (1). We have concluded that *Parapronocephalum* and *Notocotyloides*, as well as all pronocephalids except for *Pseudobarisomum* possess cephalic collars. All phylogenetic analyses we performed placed *Parapronocephalum* and *Notocotyloides* as members of the Notocotyloidae clade, and not as members of the Pronocephalidae. Therefore, the presence of the cephalic collar may be plesiomorphic for the Notocotyloidae + Pronocephalidae clade, with independent losses in the Notocotyloidae (minus *Parapronocephalum* and *Notocotyloides*) and in *Pseudobarisomum*, or the cephalic collars of *Parapronocephalum* and *Notocotyloides* may not be homologous with those of the pronocephalids (in either case, the condition found in *Pseudobarisomum* is best explained as a secondary loss).

Modifications of the cephalic collar, such as whether or not it is

DT	000
NO	0011001201000000000011000001000001000000000000000
PA	000111120100000000000000001000001000000000000000
NT	001111020100000000000000001000001000000000000000
AD	001240111000001001000000000100000100000000000200
PL	0010011110110000011000000001000001000000000000000
HI	0010111111110000110001000010000010000000000000000
IG	0010111111110011111000100001000001000000000000000
RE	0010111111111000001110001001000001000000000000000
PP	0010011111111000011012000001000001000000000000000
PY	001021111011110001000011001100000100000000011000
CR	001261111111100012000010110100000100000000001100
PS	001021011111101002000010001100000100000000001100
BA	011051111111101002000010001100000100000000001100
CH	0110311112110000010000000000110001000000000000000
DE	0112511112111000010000000000101101000000000000000
DI	0210211112111000010000000000101011000000000000000
PR	0200201111100000010000000001000000000000000010000
RU	120000111110000001000000000100000111101001110011
CE	120000111110000001000000000100000211010000010010
ME	120000111010000001000000000100000211010000010011
NE	120000101010000001000000000100010111001001110010
MA	1200101111110000001000000000100000111001100010011
CP	120010111110000001000000000100000111001110010011

* DT = Deuterobaridinae; NO = Notocotylidae; PA = *Parapronocephalum*; NT = *Notocotylidae*; AD = *Adenogaster*; PL = *Pleurogonius*; HI = *Himasomum*; IG = *Iguanacola*; RE = *Renigonus*; PP = *Parapleurogonius*; PY = *Pyelosomum*; CR = *Cricocephalus*; PS = *Pseudobarisomum*; BA = *Barisomum*; CH = *Charaxicephalus*; DE = *Desmogonius*; DI = *Diaschistorchis*; PR = *Pronocephalus*; RU = *Ruicephalus*; CE = *Cetiosaccus*; ME = *Metacetabulum*; NE = *Neopronocephalus*; MA = *Macravestibulum*; CP = *Choanophorus*. 0 = plesiomorphic state; 1–6 = derived states.

"continuous" or "interrupted" ventrally, or whether it is "strongly" or "weakly" developed, have been used to distinguish major groups within the family. We have been unable to characterize these modifications consistently due to variations caused by different modes of fixation and preparation and degree of development of individual worms. Future studies based on a large series of new specimens from many species, perhaps using scanning electron microscopy, might provide useful information.

8) Position of ovary with respect to testes. Deuterobarids and paramphistomids exhibit posttesticular ovaries (0). Notocotylids and *Parapronocephalus* exhibit intertesticular ovaries, a trait exhibited by *Pronocephalus* (2). All other pronocephalids except *Neopronocephalus* exhibit pretesticular ovaries (1), while *Neopronocephalus* exhibits posttesticular ovaries reminiscent of the condition found in deuterobarids (0).

9) Number of genital pores. The plesiomorphic condition is a single genital pore (0), whereas all pronocephalids possess 2 genital pores (1).

10) Number of polar filaments in eggs. Notocotyliids and *Parapronocephalum* exhibit 2 polar egg filaments (1 on each pole), a condition also exhibited by most pronocephalids (0). Polar filaments were not observed in *Notocotyloides*. *Adenogaster*, some species of *Pyelosomum*, some species of *Pleurogonius*, *Metacetabulum*, and *Neopronocephalus* lack egg filaments (1), while *Charaxicephalus*, *Desmogonius*, *Diaschistorchis*, and some species of *Pyelosomum* have multiple polar filaments (2). This character was not used in constructing the phylogenetic tree, but we include it for future reference.

11) Presence or absence of ventral glands. The plesiomorphic condition, exhibited by *Adenogaster*, most notocotylids, and deuteroberidines, is the possession of glands on the ventral body surface (0). All other pronoccephalids lack ventral glands, which we interpret as an apomorphic secondary loss (1). *Notocotyloides* also reportedly lacks ventral glands, presumably a convergent secondary loss in that group.

12) Shape of posterior end of body. The plesiomorphic condition,

exhibited by *Adenogaster*, *Pronocephalus*, *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus*, is rounded posterior ends (some have posterior invaginations of the body as well; see character 36) (0). All other pronocephalids have truncated posterior ends (1).

13) Position of genital pores with respect to ceca. The plesiomorphic condition, exhibited by most pronocephalids, is intercecal genital pores (0). *Himasomum*, *Renigonius*, *Parapleurogonius*, *Pyelosomum*, *Criccephalus*, *Barisomum*, and *Pseudobarisomum*, as well as *Desmogonius* and *Diaschistorchis*, exhibit extracecal genital pores (1).

14) Body shape. Notocotylids, *Parapronocephalum* and almost all pronocephalids exhibit slender, elongate bodies (0). *Pyelosomum* species have bodies that are relatively more plump and rounded (1).

15) Anterior arms of excretory vesicles. Notocotylids, *Notocotylodes* and *Parapronocephalum*, and most pronocephalids exhibit arms of the excretory vesicle that are united anteriorly (0). *Adenogaster*, *Iguanacola*, *Barisomum*, and *Pseudobarisomum* all exhibit separate anterior arms of the excretory system (1).

16) Location of genital pores. In the outgroups and most pronocephalids, the genital pore(s) lie(s) in the anterior 1/3 of the body (0). In *Iguanacola*, the genital pores are equatorial (1).

17) Cirrus sac shape constrictions. The plesiomorphic condition is a cirrus sac without constrictions (0). *Iguanacola* and *Criccephalus* have a cirrus sac with a constriction in the middle (1).

18) Cirrus sac shape. Notocotylids, *Notocotylodes* and *Parapronocephalum*, and *Renigonius* possess narrow, elongate cirrus sacs (0). Most other pronocephalids possess relatively short and spherical, but weakly muscled, cirrus sacs (1), except or *Criccephalus*, *Barisomum*, and *Pseudobarisomum*, which possess relatively short and spherical, but strongly muscled, cirrus sacs (2).

19) Relative length of esophagus. The plesiomorphic condition is relatively short esophagi (0). Members of *Pleurogonius*, *Iguanacola*, *Renigonius*, and *Parapleurogonius* possess relatively long esophagi (1).

20) Development of eggs. The plesiomorphic condition is nonembryonated eggs (0). *Renigonius* has embryonated eggs (1).

21) Position of ceca with respect to testes. The plesiomorphic condition is ceca extending to near the posterior end of the body (0). *Renigonius* and *Parapleurogonius* possess ceca that terminate at the anterior margins of the testes (1).

22) Internal seminal vesicle. Deuterobaridines, *Notocotylodes* and *Parapronocephalum*, and almost all pronocephalids lack a true internal seminal vesicle (0). Notocotylids possess relatively large internal seminal vesicles (1). *Parapleurogonius* possesses a small internal seminal vesicle (2).

23) Extent of uterine loops with respect to ceca. The plesiomorphic condition is intercecal uterine loops (0). Cecal and extracecal uterine loops (1) occur in *Himasomum*, *Iguanacola*, *Pyelosomum*, *Criccephalus*, *Barisomum*, and *Pseudobarisomum*. cecal and extracecal uterine loops occur in some other species of *Pleurogonius*, but we code *Pleurogonius* as plesiomorphic for the trait pending a more detailed analysis of the genus.

24) Cecal configuration. The plesiomorphic condition is ceca that extend posteriorly in essentially a straight line (0). Four species of *Pyelosomum* exhibit sinuous ceca (1), and the other 2 species exhibit serpentine ceca (2). The transformation series for this character was determined as a result of a species-level analysis of *Pyelosomum* (Pérez Ponce de León and Brooks, 1995); in this study, *Pyelosomum* is coded as (1).

25) Position of genital pore with respect to cecal bifurcation. The plesiomorphic condition is genital pores positioned immediately posterior to the cecal bifurcation (0). *Renigonius* and *Criccephalus* exhibit prebifurcal genital pores (1).

26) Metraterm structure. The plesiomorphic condition is "simple," i.e., relatively short and straight, metraterms. *Criccephalus* exhibits a

very long metraterm with 2 prominent muscular and glandular dilations (1).

27) Orientation of cirrus sac. The plesiomorphic condition is cirrus sacs oriented longitudinally in the body (0). *Pyelosomum*, *Barisomum*, and *Pseudobarisomum* exhibit transverse cirrus sacs (1).

28) Testes condition. The plesiomorphic condition is paired entire testes (0). *Charaxicephalus*, *Desmogonius*, and *Diaschistorchis* have fragmented testes (1).

29) Esophageal bulb. Notocotylids, *Notocotylodes* and *Parapronocephalum*, and most pronocephalids lack any muscular swelling at the juncture of the cecal bifurcation, called an esophageal bulb (0). *Charaxicephalus*, *Desmogonius*, and *Diaschistorchis* exhibit esophageal bulbs (1), which would appear to be convergent with similar structures found in many paramphistomids and microscaphidiids.

30) External seminal vesicle structure. The plesiomorphic condition is external seminal vesicles with smooth walls (0). In *Charaxicephalus* the external seminal vesicle has constrictions (1).

31) External seminal vesicle shape. The plesiomorphic condition is relatively short and winding external seminal vesicles (0). *Desmogonius* and *Diaschistorchis* exhibit long and straight external seminal vesicles (1).

32) Position of vitellaria with respect to testes. The plesiomorphic condition is pretesticular vitellaria (0). In *Neopronocephalus* and *Desmogonius* the vitellaria are posttesticular (1).

33) Position of testes with respect to ceca. The plesiomorphic condition is testes lying dorsal to the plane of the cecal, whether they are extracecal or intercecal (0). In *Diaschistorchis*, the testes lie ventral to the ceca (1).

34) Arrangement of testes. Deuterobaridines, some microscaphidiids, and some paramphistomids exhibit obliquely arranged testes, which we code as plesiomorphic (0); *Pronocephalus* also exhibits this condition. Notocotylids, *Parapronocephalum*, and almost all pronocephalids have symmetrically arranged testes (1), while *Cetiosaccus* and *Metacetabulum* exhibit tandem testes (2).

35) Relative length of ceca. The plesiomorphic condition is relatively long ceca extending at least to within 1 testis diameter of the posterior end of the body (0). *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* have relatively short ceca that terminate more than 1 testis diameter from the posterior end (1).

36) Posterior vestibule. The plesiomorphic condition is posterior ends terminating simply with a terminal or dorso-terminal excretory pore (0). *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* possess prominent invaginations of the posterior end of the body, which has been called the "posterior vestibule" (1).

37) Position of vitelline follicles with respect to the ovary. The plesiomorphic condition is preovarian vitelline follicles (0). *Ruicephalus* exhibits postovarian vitelline follicles (1).

38) Relative size of the excretory vesicle. The plesiomorphic condition is a relatively small excretory vesicle that is often difficult to see in preserved material (0). *Cetiosaccus* and *Metacetabulum*, by contrast, exhibit very large and prominent excretory vesicles (1).

39) Thickening of esophageal walls. The plesiomorphic condition is relatively thin-walled esophagi (0). *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* all possess thickened esophageal walls (1).

40) Posterior digitiform processes. The plesiomorphic condition is no digitiform processes associated with the posterior end of the body (0). *Macravestibulum* and *Choanophorus* exhibit such processes (1).

41) Copulatory pouch. The plesiomorphic condition is genital pore(s) opening directly at the surface of the worm (0). In *Choanophorus* the genital pores open into a copulatory pouch (1).

42) Extent of uterine loops with respect to ovary and testes. The

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FIGURE 1. Diagrammatic representation of the diversity of cecal morphologies for pronocephalid digeneans. 0, Smooth-walled throughout their entire length; 1, with irregular diverticula in anterior portion; 2, with irregular diverticula throughout their length; 3, with regular diverticula paired on medial and lateral surfaces of ceca; 4, with regular diverticula only on medial surface of ceca; 5, with regular diverticula alternating on medial and lateral surfaces of ceca; 6, with regular diverticula only on lateral surface of ceca. 0 is the plesiomorphic condition; all other numbers are arbitrary and do not represent a linear transformation series.

plesiomorphic condition is no uterine loops between the testes and ovary (0). *Pronocephalus* and *Ruicephalus* exhibit uterine loops between the testes and ovary (1).

43) Relative number of vitelline follicles. The plesiomorphic condition is numerous vitelline follicles in each vitellarium (0). In *Ruicephalus* and *Neopronocephalus* there are very few follicles in each vitellarium (1).

44) Relative size of cirrus sac. The plesiomorphic condition is relatively long cirrus sacs (0). *Pyelosomum*, *Pronocephalus*, *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, *Choanophorus* possess very short cirrus sacs (1).

45) Relative size of excretory pore. The plesiomorphic condition is very small excretory pores, which are sometimes difficult to find in preserved material (0). *Pyelosomum*, *Cricocephalus*, *Barisomum*, and *Pseudobarisomum* all possess prominent excretory pores (1).

46) Ductus Caballeri. The plesiomorphic condition is a vesiculate pars prostatica joined closely to the ejaculatory duct (0). In *Adenogaster*, *Cricocephalus*, *Barisomum*, and *Pseudobarisomum*, the pars prostatica is separated from, and connected to, the ejaculatory duct by a distinct winding tube, which we are calling the ductus Caballeri, in honor of the late Dr. Eduardo Caballero y Caballero (Fig. 2). The ductus Caballeri has 2 forms in the Pronocephalidae. In *Adenogaster*, it is long and relatively straight (1), whereas in *Cricocephalus*, *Barisomum*, and *Pseudobarisomum*, it is very short and coiled (2).

47) Position of excretory pore. The plesiomorphic condition is excretory pores that open dorsally near the posterior end of the body (0). In *Ruicephalus*, *Metacetabulum*, *Cetiosaccus*, *Neopronocephalus*, *Macravestibulum*, and *Choanophorus* the excretory pores open at the posterior end of the body (1).

RESULTS

All combinations of PAUP options listed above produced the same 6 equally parsimonious trees with consistency indices of 70.1% (77 changes for 53 possible apomorphies) for the Pronocephalidae and the Notocotyloidea + *Notocotyloides* and *Parapronocephalum*, and 77.8% for the Pronocephalidae alone (63 changes for 49 possible apomorphies). The trees differ only in the placement of *Pleurogonius*, *Renigonus* + *Parapleurogonius*, *Iguanacola*, and *Himasomum*. We have been unable to find a synapomorphy for *Pleurogonius*, suggesting that it is probably paraphyletic, and this may account for the ambiguity in the analysis. Adding characters 5 and 10 and treating all members of *Pleurogonius* as plesiomorphic for both characters lowers the consistency index to approximately 66% and produces 32 equally parsimonious trees, 6 of which are those found when the 2 characters are excluded. When character 5 is included and 10 excluded, the same 32 equally parsimonious trees result, with a consistency index of approximately 69%. Excluding character 5 and including character 10 produces 12 equally parsimonious trees, 6 of which are those found when the 2 characters are excluded, with a consistency index of approximately 68%.

Despite the ambiguity produced by *Pleurogonius*, our analysis supports a more informative and stable classification than previous treatments of the family. The relative relationships among *Adenogaster*, *Pronocephalus*, *Ruicephalus*, *Neopronocephalus*, *Macravestibulum*, *Choanophorus*, *Metacetabulum*, *Cetiosaccus*, *Charaxicephalus*, *Desmogonius*, *Diaschistorchis*, *Pyelosomum*, *Cricocephalus*, *Barisomum*, and *Pseudobarisomum* are identical

FIGURE 2. Diagrammatic representation of the components of the male genitalia of Pronocephalidae. mgp, Male genital pore; c, cirrus; ed, ejaculatory duct; dc, ductus Caballeri; cp, cirrus pouch; pv, prostatic vesicle; pg, prostatic glands; sv, seminal vesicle.

FIGURE 3. Consensus tree depicting the proposed phylogenetic relationships among 20 genera of pronocephalid digeneans (*Paradenogaster* added as sister group of *Adenogaster* for host and geographic comparisons). AD, *Adenogaster*; PD, *Paradenogaster*; PL, *Pleurogonius*; HI, *Himasomum*; IG, *Iguanacola*; RE, *Renigonius*; PP, *Parapleurogonius*; PY, *Pyelosomum*; CR, *Cricocephalus*; PS, *Pseudobarisomum*; BA, *Barisomum*; CH, *Charaxicephalus*; DE, *Desmogonius*; DI, *Diaschistorchis*; PR, *Pronocephalus*; RU, *Ruicephalus*; CE, *Cetiosaccus*; ME, *Metacetabulum*; NE, *Neopronocephalus*; MA, *Macravestibulum*; CP, *Choanophorus*. Numbers accompanying slash marks on tree indicate apomorphic traits (character number followed by state in parentheses; *, homoplasious state) supporting each branch as follows: 1: 3(1), 7(1), 8(1), 9(1), 18(1); 2: 4(2)*, 15(1)*, 46(2); 3: 11(1); 4: 6(1), 12(1); 5: 19(1); 6: 2(1), 28(0)*, 29(1); 7: 13(1)*, 21(1); 8: 18(0)*, 20(1), 25(1)*; 9: 22(2); 10: 23(1); 11: 15(1)*, 16(1), 17(1); 12: 13(1)*; 13: 27(1), 45(1); 14: 14(1), 24(1), 44(1)*; 15: 18(2), 46(1); 16: 4(2)*, 25(1)*, 26(1), 27(0)*; 17: 15(1)*; 18: 7(0)*; 19: 30(1); 20: 13(1)*, 30(1); 21: 33(1); 22: 4(2)*, 32(1)*; 23: 2(3), 3(0)*, 44(1)*; 24: 34(0)*; 25: 1(1), 35(1), 36(1), 47(1); 26: 39(1); 27: 42(1), 43(1); 28: 37(1); 29: 8(0)*, 32(1)*; 30: 40(1); 31: 41(1); 32: 34(2), 38(1).

in all 6 trees. In 4 of the 6 trees, *Himasomum* is the sister group of the *Pyelosomum* clade and *Pleurogonius* is the sister group of *Renigonius* + *Parapleurogonius*, *Iguanacola*, *Himasomum*, and the *Pyelosomum* clade. The phylogenetic tree in Figure 3 is the Adams and the 50% majority-rule consensus tree topology and reflects the majority placements of *Himasomum* as the sister group of the *Pyelosomum* clade, and *Pleurogonius* as the sister group of *Renigonius* + *Parapleurogonius*, *Iguanacola*, *Himasomum*, and the *Pyelosomum* clade.

DISCUSSION

The monophyly of the Pronocephalidae Looss, 1902, is supported based on the following synapomorphies identified in this study: intercecal testes, presence of a cephalic collar, pretesticular ovaries, 2 genital pores, and relatively short and spherical, but weakly muscled, cirrus sacs. Within the family, three major clades have been identified. Two of them are sister groups, and subfamilial names have been proposed for them previously. The *Charaxicephalinae* Price, 1931 is the oldest subfamilial name associated with the clade containing *Charaxicephalus* Looss,

1901 [= *Charaxicephaloides* Groschafft and Tenora, 1978], *Desmogonius* Stephens, 1911, *Diaschistorchis* Johnston, 1913 [= *Wilderia* Pratt, 1914, *Synechorchis* Braker, 1922], *Pleurogonius* Looss, 1901 [= *Mediporus* Ozaki, 1936, *Epibathra* Looss, 1902, *Glyphicephalus* Looss, 1901 in part], *Renigonius* Mehra, 1939, *Parapleurogonius* Sullivan, 1977, *Iguanacola* Gilbert, 1938, *Himasomum* Linton, 1910, *Pyelosomum* Looss, 1899 [= *Epibathra* Looss, 1902, *Astrorchis* Poche, 1926, and *Myosaccus* Gilbert, 1938], *Cricocephalus* Looss, 1899 [= *Neocricocephalus* Gupta, 1962], *Barisomum* Linton, 1910, and *Pseudobarisomum* Siddiqi and Cable, 1960. The monophyly of this clade is supported by the synapomorphies of posterior body projections and truncated posterior ends. The Pronocephalinae Looss, 1902 is the oldest subfamilial name associated with the clade containing *Pronocephalus* Looss, 1899, *Cetiosaccus* Gilbert, 1938, *Metacetabulum* Teixeira de Freitas and Lent, 1938, *Ruicephalus* Skrjabin, 1955, *Neopronocephalus* Mehra, 1932, *Macravestibulum* Mackin, 1930, and *Choanophorus* Caballero, 1942. The monophyly of this clade is supported by the synapomorphies of testes located in posterior 1/3 of body, but some distance from the posterior end, intercecal testes and very short

FIGURE 4. Definitive host group (turtles, marine fish, marine iguanas) and primary habitat (marine/estuarine, freshwater) optimized onto phylogenetic tree for pronocephalid digeneans.

cirrus sacs. The third, containing only *Adenogaster*, is the sister group of the other 2. Its monophyly is supported by the synapomorphies of Mehlis' gland located laterally to the ovary, separate anterior arms of excretory system and having a long and straight ductus Caballeri. To our knowledge, no subfamilial name has ever been proposed for *Adenogaster*, so we propose the following:

Adenogasterinae n. subfam.

Diagnosis: Digenea; Paramphistomiformes; Pronocephalidae. Body slender, elongate. Posterior end rounded, projections lacking. Glands present on ventral surface. Oral sucker relatively small; esophagus relatively short, bulb lacking; ceca smooth-walled throughout their length or with regular diverticula found only on medial surface of, extending posttesticularly to near posterior end of body. Male genital pore intercecal, sinistral, in anterior $\frac{1}{3}$ of body, immediately posterior to cecal bifurcation; cirrus sac relatively long and spherical, weakly muscled, containing prostatic complex, long and straight ductus Caballeri, ejaculatory duct, and relatively short cirrus; external seminal vesicle short, winding. Testes extracecal, symmetrical, near posterior end of body, dorsal to cecal plane, with irregular margins. Ovary immediately pretesticular, intercecal, dextral, spherical, or with irregular margins; Mehlis' gland located laterally to ovary. Uterus with transverse intercecal coils between level of anterior margin of testes and midbody; metraterm approximately half length of cirrus sac, surrounded by gland cells; female genital pore intercecal, immediately posterior to male genital pore. Vitellaria follicular, preovarian and pretesticular, in 2 lateral extracecal bands extending from the level of the anterior margin of the testes to midbody or more restricted; follicles relatively large or very small. Eggs with single filament at each pole or with single knob at each pole. Excretory vesicle V-shaped with separate arms; pore opening dorsally near posterior end of body. Intestinal parasites of marine and freshwater turtles.

Cosmopolitan. Type genus: *Adenogaster* Looss, 1901 [= *Paradenogaster* Fischthal and Kuntz, 1975, *Corinasoma* Oshmarin and Zharikova, 1984].

Our study also indicates that *H. candidulum* Linton, 1910 and *G. lobatus* Looss, 1901 form a group diagnosably distinct from *Pleurogonius* and *Barisomum*. In recognizing *Himasomum*, we propose the following amended diagnosis:

Himasomum Linton, 1910

Amended diagnosis: Digenea; Paramphistomiformes; Pronocephalidae; Charaxicephalinae. Body slender, elongate. Posterior end rounded, projections lacking. Oral sucker relatively small, bulb lacking; esophagus relatively short; ceca irregularly diverticulate near cecal bifurcation, extending posttesticularly to near posterior end of body. Male genital pore extracecal, sinistral, immediately posterior to level of cecal bifurcation; cirrus sac relatively large, containing prostatic complex, ejaculatory duct, and relatively short cirrus; external seminal vesicle winding; testes extracecal, near posterior end of body, dorsal to cecal plane, with irregular margins. Ovary immediately pretesticular, intercecal, dextral, spherical, or with irregular margins; Mehlis' gland postovarian; uterus with transverse cecal and intercecal coils between level of anterior margin of the testes and midbody; metraterm approximately half length of cirrus sac, surrounded by gland cells; female genital pore extracecal, immediately posterior to male genital pore. Vitellaria follicular, pretesticular and preovarian, in 2 lateral extracecal bands extending from the level of the anterior margin of the testes to midbody. Eggs with single filament at each pole. Excretory vesicle V-shaped with anterior arms united at mid-esophageal level; pore opening dorsally near posterior end of body. Intestinal parasites of marine turtles and fishes. Cosmopolitan. Type species: *H. candidulum* Linton, 1910 [= *B. candidulum*: Price, 1937; *P. candidulum*: Ruiz, 1946; *P. candidulum*: Manter, 1947; *G. candidulum*: Siddiqi and Cable, 1960; *Glyphicephalus macintoshi* Siddiqi and Cable,

FIGURE 5. General geographic distributions (cosmopolitan or more restricted) depicted on phylogenetic tree for pronocephalid digeneans.

1960] in *Pomacanthus arcuatus* (type host) and *Angelichthys isabelita* from the United States (Florida) (type locality), Puerto Rico, and Jamaica. Other species: *H. lobatus* (Looss, 1901) comb. n. [= *G. lobatus* Looss, 1901; *G. lobatus*: Looss, 1901; *G. lobatus*: Oguro, 1936; *P. lobatus*: Ruiz, 1946; *P. lobatus*: Caballero et al., 1955] in *C. mydas* (type host), *Eretmochelys squamosa*, and *Lepidochelys olivacea* from Egypt (type locality), Panama, Philippines (Palao Islands), Mexico (Oaxaca), United States (Florida), Puerto Rico, and Jamaica.

Brooks and McLennan (1993a, 1993b) presented evidence that parasitic platyhelminths did not exhibit unusually high levels of character loss and homoplasy, indicative of unusual degrees of evolutionary simplification or plasticity. Our study supports their findings. The consistency index value of 70.1% for the entire tree compares favorably with the values reported for phylogenetic analysis of morphological data for other digenean groups (71.7% for the overall data base: see Brooks and McLennan, 1993a, 1993b). In addition, of 63 character changes for Pronocephalidae, 5 (8%) are evolutionary losses; Brooks and McLennan (1993a, 1993b) reported 12% for digeneans as a whole. Characters that show evolutionary loss in this study are adult nonreproductive characters (loss of the cephalic collar, of the ventral glands, and of the connection between the anterior arms of the excretory vesicle). Among digeneans, Brooks and McLennan (1993a, 1993b) reported 23% of the male reproductive characters, 3% of the female reproductive characters, 14% of the adult nonreproductive characters, and 13% of the larval

characters were lost at least once. Three of the 5 losses (60%) in our study are homoplasious; Brooks and McLennan (1993a, 1993b) listed 55%. Brooks and McLennan (1993a, 1993b) reported that 30% of male reproductive characters, 35% of female reproductive characters, 34% of adult nonreproductive characters, and 9% of larval characters exhibited homoplasy. Of the 63 character changes for Pronocephalidae, 14 are homoplasious. These include 4–7 changes in male reproductive characters (30–50%), 3–6 changes in female reproductive characters (20–40%), and 4 changes in adult nonreproductive characters (30%). The range in numbers for male and female characters is due to homoplasy in characters involving the genital pores, which include both male and female components.

Optimizing-definitive host type and general habitat (marine/estuarine or freshwater) onto the phylogenetic tree (Fig. 4) suggests that pronocephalids are primitively, as well as predominantly, parasites of marine turtles. The evidence supports 4 shifts from marine to freshwater turtles (*Cortinasoma*, *Renigonius* + *Parapleurogonius*, *Neopronocephalus*, *Macravestibulum* + *Choanophorus*) or 3 shifts from marine to freshwater turtles (*Cortinasoma*, *Renigonius* + *Parapleurogonius*, *Neopronocephalus* + *Ruicephalus* + *Macravestibulum* + *Choanophorus*) and 1 secondary return to marine turtles (*Ruicephalus*). The habitat shifts also involve host switching, as there is no close phylogenetic relationship among the marine and fresh-

water chelonian hosts for pronocephalids; aside from being turtles, what the hosts have in common is herbivorous feeding habits. Published host lists for pronocephalids suggest extensive host switching among marine turtles as well, but we require species level phylogenetic trees to make better estimates of the nature and extent of those switches. There is evidence of 3 separate host shifts from marine turtles to the Galapagos marine iguana *Amblyrhynchus cristatus* (*Iguanacola*, *Pyelosomum amblyrhynchi*, and *Cetiosaccus*), and 3 shifts from marine turtles to marine fish, primarily the French angelfish *Pomacanthus arcuatus* (*H. candidulum* [also found in *Angelichthys isabelita*], *C. albus*, and the common ancestor of *Barisomum* + *Pseudobarisomum*). In no case have we found evidence supporting a switch from marine to freshwater (or freshwater to marine) coinciding with a host switch from chelonian to non-chelonian definitive hosts.

Figure 5 depicts general geographic distribution patterns for the pronocephalid taxa considered herein. Without a species level phylogenetic tree, we cannot draw many specific conclusions. It is evident, however, that the biogeographic history of the pronocephalids is complex, suggesting a mixture of dispersal and vicariance played out over a significant period of time.

This study represents an important preliminary step in the quest to understand the evolution of the helminth communities of marine turtles. The glimpse provided by this study indicates that history is likely to be complex and fascinating. In the future, we need better resolution of the pronocephalid genera that contain more than 2 species, such as *Pyelosomum* (see Pérez Ponce de León and Brooks, 1995), *Cricocephalus*, *Barisomum*, *Diaschistorchis*, *Neopronocephalus*, *Macravestibulum*, and, in particular, *Pleurogonius*. We also need phylogenetic hypotheses for other species-rich helminth groups inhabiting marine turtles, such as the microscaphidiid and spirorchid digeneans.

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