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Polystoma floridana n. sp. (Monogenea: Polystomatidae) a parasite in the green tree frog, *Hyla cinerea* (Schneider), of North America

LOUIS H. DU PREEZ[1], OLIVIER VERNEAU[2] & TIMOTHY S. GROSS[3]

[1]*School of Environmental Sciences and Development, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa. E-mail: Louis.duPreez@nwu.ac.za*
[2]*UMR 5244 CNRS-EPHE-UPVD, Biologie et Ecologie Tropicale et Méditerranéenne, Parasitologie Fonctionnelle et Evolutive, Université Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France.*
[3]*6619 NW 52nd Terrace, Gainesville, FL 32653*

Abstract

Polystoma floridana is described as a new species of the Polystomatidae parasitic in the urinary bladder of *Hyla cinerea* in Gainesville, Florida, USA. This is the fifth polystome from an anuran host described from North America but only the third belonging to the genus *Polystoma*. Here we show that the parasite from *H. cinerea* is a separate species. It is distinguished from other American *Polystoma* species by a combination of characters including body size, haptor length, body rations, size and shape of hamuli and marginal hooklets. In a sample of 235 frogs, 13.6% were infected with up to four parasites per host (mean intensity 1.6).

Key words: Monogenea, Polystomatidae, *Polystoma, Hyla cinerea*, Florida, USA

Introduction

Polystomatids of anurans are represented in North America by the genera *Polystoma* Zeder, 1800, *Pseudodiplorchis* Yamaguti, 1963 and *Neodiplorchis* Yamaguti, 1963. The first record of an anuran polystome from North America was specimens from the urinary bladder of the grey tree frog, *Hyla versicolor* LeConte, 1825 (see Riley 1927). These parasites were originally identified as *Polystoma integerrimum* (Fröhlich, 1791), but were then redescribed and named as *Polystoma integerrimum nearcticum* by Paul (1935). This subspecific taxon was later elevated to species status (Price, 1939), based on morphological differences with *P. integerrimum*. Paul (1938) reported *Polystoma nearcticum* (Paul, 1935) from the urinary bladder of *H. versicolor* from Connecticut, USA and the green tree frog, *Hyla cinerea* Schneider, 1799 from Florida; Campbell (1967) reported a single *Polystoma* specimen from the rectum of *H. versicolor* from Chesterfield County, Virginia; and Campbell, (1969) found eight *P. nearcticum* specimens in the urinary bladder of five *H. versicolor* from Virginia Goucester County, Virginia. On one occasion a specimen of the invasive Cuban tree frog, *Osteopilus septentrionalis* Duméril & Bibron, 1841 collected in south Florida was found to be infected with a single specimen of *Polystoma stellai* Vigueras, 1955 (see Stunkard 1959).

Only two other anuran polystomes are currently known from North America. *Pseudodiplorchis americanus* Rodgers & Kuntz, 1940 is known from the urinary bladder of Couch's spadefoot toad, *Scaphiopus couchii* Baird, 1854 from Oklahoma, U.S.A. Tinsley and Earle (1983) reported this same species from *S. couchii* from Arizona, USA, while Lamothe-Argumedo (1985) reported this species from the same host from Mexico. *Neodiplorchis scaphiopi* Rodgers, 1941 infects the urinary bladder of the plains spadefoot toad, *Spea bombifrons* Cope, 1863 from Oklahoma, USA. Lamothe-Argumedo (1973) reported this species from the urinary bladder

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of Hurter's spadefoot toad, *Scaphiopus hurterii* Strecker, 1910 from Mexico, while Tinsley and Earle (1983) reported *N. scaphiopi* from the urinary bladder of the New Mexico spadefoot toad, *Spea multiplicata* Cope, 1863 and *S. bombifrons* from Arizona, USA.

Other polystomes described from South and Central American frogs include *Polystoma andinum* Combes & Laurent, 1978 in *Melanophryniscus rubriventris* (Vellard, 1947) from Argentina, *Polystoma borellii* Combes & Laurent, 1974 in *Pleurodema borellii* (Peracca, 1895) from Argentina, *Polystoma cuvieri* Vaucher, 1990 in *Physalaemus cuvieri* Fitzinger, 1826 from Paraguay, *Polystoma diptychi* Vaucher, 1986 in *Rhinella diptycha* (Cope, 1862) from Paraguay, *Polystoma guevarai* Combes & Laurent, 1979 in *Hypsiboas andinus* (Müller, 1924) from Argentina, *Polystoma lopezromani* Combes & Laurent, 1979 in *Trachycephalus venulosus* (Laurenti, 1768) from Argentina, *Polystoma naevius* Caballero & Cerecero, 1941 in *Smilisca baudinii* (Duméril & Bibron, 1841) from Mexico, *Polystoma napoensis* Vaucher, 1987 in *Osteocephalus taurinus* Steindachner, 1862 and *Osteocephalus leprieurii* (Duméril & Bibron, 1841) from Ecuador, *Polystoma praecox* Combes & Laurent, 1978 in *Telmatobius oxycephalus* Vellard, 1946 from Argentina, *Polystoma stellai* Vigueras, 1955 in *O. septentrionalis* from Cuba, *Polystoma touzeti* Vaucher, 1987 in *Gastrotheca riobambae* (Fowler, 1913) from Ecuador. Kohn *et al*. (1978) reported undescribed *Polystoma* species from *Trachycephalus mesophaeus* (Hensel, 1867), *Trachycephalus nigromaculatus* Tschudi, 1838 and *Leptodactylus pentadactylus* (Laurenti, 1768) from Rio and Para, Brazil.

During present studies of American polystomatids, *H. cinerea* has been found infected with a new species of *Polystoma*. *Hyla cinerea* is a medium sized bright green, lime green, yellow green or olive green frog with a few spatters of gold or white. A prominent white or yellow stripe runs from the upper jaw to the groin (Conant & Collins 1998). It has a wide distribution in coastal lowlands ranging from the Delmarva Peninsula to the southern tip of Florida, westwards through the Gulf Coastal Plain to East and South-East Texas. This species is quite common and occurs throughout the state of Florida. Habitats include ponds, swamps, streams and borders of lakes. Green Treefrogs have an extended breeding season and calls can be heard from March to October. Eggs are laid underwater on submerged vegetation and tadpoles complete metamorphosis in two months (Conant & Collins 1998).

In the case of morphologically cryptic taxa such as polystomes, traditional approaches in studying and describing biological diversity have serious limitations. Sequencing and comparing variable portions of the genome of a taxon provides a valuable tool. Some scientists go as far as to say that biological identifications should be based on DNA barcodes (Hebert *et al*. 2003; Blaxter 2003). For this purpose, several different mitochondrial or nuclear sequences can be used as molecular markers, but it has been advised to use more than one sequence region for assigning taxonomic status (Tautz *et al*. 2003). Therefore, we generated partial nuclear 28S rDNA sequences, which include the first divergent domains in the 5'end region (Zardoya & Meyer 1996), and partial mitochondrial cytochrome c oxidase subunit I (COI) for African and American polystomes to investigate the taxonomic status of the new parasite.

This paper provides a formal description of this new polystome from *H. cinerea*.

Material and methods

Adult *Hyla cinerea* were collected on peripheral vegetation around a small pond in the Deer Run neighbourhood in Gainesville, Florida during June 2003 and May-June 2004. Frogs were placed individually in 500 ml plastic containers containing 50 ml water. Positive infection was determined after 24 hours by screening the water into which the frogs had urinated for the presence of polystome eggs using a plankton net with mesh size of 112 µm. Frogs that did not release any polystome eggs were screened a second and a third time after 24 hour intervals while polystome eggs were harvested from infected frogs for a period of four days and incubated in 50 mm Petri dishes at 23° C. A subset of 10 frogs that did not produce polystome eggs were dissected

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to verify that they did not contain any subadult parasites. Infected frogs were dissected to retrieve parasites. Frogs were anaesthetised with methane 3-aminomethanosulfonate (MS222) and, at dissection, the urinary bladder, urinary ducts, kidneys and alimentary canal were examined using a stereo binocular microscope. Oncomiracidia that hatched after 11 days were collected and mounted in ammonium-picrate (Malmberg 1956) for further studies of the sclerites. Coverslips were sealed with clear nail varnish.

Following fixation for 24 hours in 70% ethyl alcohol under coverslip pressure, mature parasites earmarked for permanent mounting were hydrated to 30% EtOH, stained in Alum Carmine, dehydrated, cleared, and mounted in Canada balsam. Material for molecular analysis was kept overnight in a 0.6 % saline solution. After a 24 hour period the gut contents were squeezed out by gently rolling a camel hair brush laterally over the parasite from the haptor towards the mouth forcing all gut contents out to limit contamination. Parasites were then fixed in 96% EtOH. A piece of host thigh muscle was also fixed for molecular studies to run concurrent with parasite extractions to verify that host contamination did not occur.

The molecular data set (Table 1) included 12 polystome species sampled from African and American neobatrachian frogs, including the new polystome species of *Hyla cinerea*. One specimen per species was analysed except for *Polystoma dawiekoki* Du Preez, Vaucher & Mariaux, 2002 and *Polystoma marmorati* Van Niekerk, Kok & Seaman, 1993, where two specimens were respectively examined and *Polystoma* sp of *H. cinerea* and *Wetapolystoma almae* Gray, 1993, where three specimens were respectively inspected.

TABLE 1. Parasite species investigated for DNA Barcoding, authorities, host species, geographical origin and GenBank accession numbers

Parasite species	Authority	Host species	Origin	Partial COI	Partial 28S
African polystomes					
Polystoma australis	Kok & van Wyk 1986	*Semnodactylus wealii*	South Africa	AM913854	AM913872
Polystoma sp (H.m.)	Murith 1981	*Hemisus marmoratus*	Ivory Coast	AM913855	AM913873
Polystoma dawiekoki	Du Preez, Vaucher & Mariaux 2002	*Ptychadena anchietae*	South Africa	AM913856	AM157204
Polystoma dawiekoki	Du Preez, Vaucher & Mariaux 2002	*Ptychadena anchietae*	Tanzania	AM913857	AM913875
Polystoma marmorati	Van Niekerk, Kok & Seaman 1993	*Hyperolius marmoratus*	South Africa	AM913858	AM157208
Polystoma marmorati	Van Niekerk, Kok & Seaman 1993	*Hyperolius marmoratus*	South Africa	AM913858	-
Polystoma testimagna	Du Preez & Kok 1993	*Strongylopus fasciatu*	South Africa	AM913860	AM157217
Polystoma umthakathi	Kok & Seaman 1987	*Natalobatrachus bonebergi*	South Africa	AM913861	AM913874
South, Central and North American polystomes					
Polystoma cuvieri	Vaucher 1990	*Physalaemus cuvieri*	Paraguay	AM913862	AM157203
Polystoma lopezromani	Combes & Laurent 1979	*Phrynohyas venulosa*	Paraguay	AM913863	AM157207
Polystoma naevius	Caballero & Cerecero 1941	*Smilisca baudenii*	Costa Rica	AM913864	AM157209
Polystoma nearcticum	(Paul 1935) Price 1939	*Hyla versicolor*	USA	AM913865	AM157210
Polystoma sp (H.c.)		*Hyla cinerea*	USA	AM913869	AM157211
Polystoma sp (H.c.)		*Hyla cinerea*	USA	AM913870	AM913878
Polystoma sp (H.c.)		*Hyla cinerea*	USA	AM913871	AM913879
Wetapolystoma almae	Gray 1993	*Bufo margaritifer*	French Guiana	AM913866	AM157220
Wetapolystoma almae	Gray 1993	*Bufo margaritifer*	French Guiana	AM913867	AM913876
Wetapolystoma almae	Gray 1993	*Bufo margaritifer*	French Guiana	AM913868	AM913877

Note. H.m. refers to *Hemisus marmoratus* and H.c. to *Hyla cinerea*.

DNA extraction, amplification and sequencing were done according to Bentz *et al*. (2006). The partial 28S rRNA gene corresponding to the 5' terminal end was amplified with primers forward LSU5', 5'-TAG-

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GTCGACCCGCTGAAYTTAAGCA-3' and reverse LSU3', 5'-TAGAAGCTTCCTGAGGGAAACTTCGG-3' (Snyder & Loker 2000), yielding a PCR product of about 1.4 kb that was subsequently sequenced with the same primers and also the following internal forward primers: IF13, 5'-AGCAAACAAGTACCGTGAGGG-3'; IF15, 5'-GTCTGTGGCGTAGTGGTAGAC-3'; and internal reverse primers: IR13, 5'-GTCGTGGCTTA-CACCCTGAGG-3' and IR14, 5'-CATGTTAAACTCCTTGGTCCG-3'. The portion of the COI gene was amplified with the primers forward L-CO1p, 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and reverse H-Cox1p2, 5'-TAAAGAAAGAACATAATGAAAATG-3' (Littlewood *et al*. 1997), yielding a PCR product of about 400 bp that was subsequently sequenced with the same primers.

28S and COI sequences were aligned manually using the MUST package (Philippe 1993) and gaps as well as ambiguous regions in the resulting 28S alignment were excluded for subsequent analyses. Mean and total character differences were estimated with PAUP* 4.0b9 (Swofford 2002), respectively from the nuclear 28S rRNA and mitochondrial COI gene alignments.

Results

Levels of infection

Nine of the 42 *H. cinerea* collected during June 2003 were infected with up to four parasites and a mean intensity of 2.1 while 22 of the 207 specimens (10.6 %) collected during May and June 2004 were infected with up to three parasites; mean intensity, 1.24 worms/host. For the total sample, the prevalence was 12.5 % and the mean intensity 1.6 worms/host. None of the hosts examined had parasites in the kidneys, intestine or rectum.

Molecular analyses

Both 28S and COI sequence alignments gave respectively 1419 and 313 characters among which 127 and 107 were variable. Mean and total character differences inferred from comparisons of 28S and COI sequences are given in Tables 2 and 3. 28S genetic differentiation between specimens of the same species varied from 0 to 0.070 % total character differences in *W. almae* and was 0.070 % in *P. dawiekoki*. No character differences were observed between the three polystome specimens from *Hyla cinerea*. Closely phylogenetically related African polystome species *P. testimagna* Du Preez & Kok, 1993 and *P. umthakathi* Kok & Seaman, 1987 (see Bentz *et al*. 2001) showed a total character difference of only 0.070%. When *P. australis* and *P. marmorati* were compared with each other and with *P. testimagna* and *P. umthakathi*, the total character differences varied between 0.282 % and 0.352 %. *Polystoma* sp. of *H. cinerea* differed from *P. nearcticum*, i.e. its most closely related species (Badets *et al*. unpublished data), by total character differences of 0.634 %.

COI genetic differentiation between specimens of the same species varied from 0.639 % to 1.278 % total character differences in *W. almae*, 0 % in *P. marmorati* and 1.917 % in *P. dawiekoki*. No character difference occurred between the three polystome specimens of *H. cinerea*. The total character difference between the most closely phylogenetically related African *Polystoma* species, i.e. *P. testimagna* and *P. umthakathi*, was 3.195 %. Variation of 5.112 % to 8.946 % occurred when *Polystoma australis* Kok & Van Wyk, 1986 and *P. marmorati* were compared with each other and with *P. testimagna* and *P. umthakathi*. *Polystoma sp.* of *H. cinerea* differed from *P. nearcticum* by total character differences of 4.153 %.

Class: Monogenea Carus, 1863

Oder: Polystomatidea Lebedev, 1988

Family: Polystomatidae Gamble, 1896

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***Polystoma floridana* n. sp.**
(Figs. 1–3)

Specimens studied: Twenty four sexually mature worms and 30 oncomiracidia. Holotype (USNPC 100411) and 6 paratypes (USNPC 100412-100417) deposited in the US National Parasite Collection, Beltsville, Maryland, USA; 6 paratypes (NMB P303-308) in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa; remaining specimens in the collection of first author.

Type host: *Hyla cinerea* Schneider, 1799 sexually mature male (NMB A 7517) deposited in the Amphibian Collection, National Museum, Bloemfontein 9300, South Africa.

Type locality: Small pond in Deer Run neighbourhood, Gainesville, Florida, USA (29° 42' 12" N 82° 23' 52" W).

Other records: Infected specimens of *H. cinerea* were found killed on the road at the United States Geological Survey USGS-BRD facility, 7920 N.W. 71st St., Gainesville, Florida, USA (29° 43' 31" N 82° 25' 04" W).

Site: Urinary bladder.

Etymology: The specific name *floridana* refers to the state Florida.

Description. Based on egg-producing adults (n = 24); measurements given in micrometres. The average measurement is followed by the range in parenthesis. Larval sclerite characters based on oncomiracidia (n = 30) hatched from eggs released by the holotype, paratypes and other specimens.

Adult: General characteristics of mature, egg-producing parasite (Figure 1) typical of *Polystoma*. Body elongate, total length 6881 (4680–9080), greatest width 2415 (1480–2940), haptor length 917 (640–1300), width 1569 (640–1880); haptor length to body length ratio 0.14 (0.09 – 0.22); haptoral suckers 6, mean diameter 340 (241–420); hamuli 281 (202–350); hamulus hook or blade 40 (33–51). Mouth subterminal, ventral. Oral sucker 239 (155–303) wide; pharynx length 247 (163–311), width 209 (156–319). Intestine bifurcate with small lateral diverticula averaging 22 (10–29) and medial diverticula 9 (4–12). All specimens with 0 – 6 (2.9) or anastomoses; caeca confluent posteriorly, extending into haptor.

Testis follicular, ventral, medial and posterior to ovary (Figure 1). Seminal vesicle packed with sperm. Genital atrium median, ventral, posterior to intestinal bifurcation; genital bulb with (8 – 11) spines 22 (21–24) long. Ovary sinistral, 25 % from anterior end, ovary length 906 (606–1151), width 460 (326–591). Short tubular uterus only anterior to ovary, containing only one egg; egg capsule length 195 (185–210), width 163 (161–169). No indication of intrauterine development, eggs operculated. Vitellaria throughout most of body. Genito-intestinal canal prominent, on same side as ovary, joining intestinal caecum posterior to ovary (Figure 1).

Oncomiracidium: Ciliated larva with narrow cylindrical body and circular cup-shaped opisthaptor bearing 16 marginal hooklets. Marginal hooklet C1 24.4 (23.3–25.7); hamulus primordial 10.6 (9.0 – 10.6) (Figure 2).

Remarks. *Polystoma floridana* **n. sp.** differs from other members of the genus by a combination of characters. *Polystoma floridana* **n. sp.** with an average length of 6881 and a maximum of 9080 is twice the size of *P. nearcticum* with an average length of 3600. The smallest specimen recorded in the present study was longer than the largest specimen reported for *P. nearcticum*. In a plot of the products of the total length (a in Figure 3) and the width at the level of the guard (c in Figure 3) versus the product of the total length versus the length of a tangent between the tip of the blade to the guard (b in Figure 3) of marginal hooklet C1 (Du Preez and Maritz 2006), *P. floridana* **n. sp.** occupies a distinct position separate from *P. nearcticum* (Figure 3). *Polystoma floridana* **n. sp.** is distinguished from other nearctic and neotropical *Polystoma* species by a combination of characters. With its body length of (4680 – 9080) it is distinguished by size from *P. cuvieri* (2400 – 4200), *P. napoensis* (3120 – 3470) and *P. touzeti* (4180). *Polystoma praecox* has no anastomoses, while *P. floridana* **n. sp.** has up to six. Based on a hamulus length of 202 – 350 *P. floridana* **n. sp.** can be distinguished from *P. andinum* (370 – 480), *P. borelli* (350 – 530), *P. diptychi* (890 – 970) and *P. lopezromani* (544 – 606). The hap-

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tor length of (640–1300) for *P. floridana* **n. sp.** separates it from *P. naevius* (1460 – 1890) and the haptor length as percentage of body length of 13% separates *P. floridana* **n. sp.** from *P. stellai* (20%) .

TABLE 2. Mean character differences (above diagonal) and total character differences (below diagonal) inferred from comparisons of 28S rDNA sequences.

		1	2	3	4	5	6	7	8	9
1	*P. testimagna*	-	0.0007	0.00352	0.00282	0.02326	0.02396	0.02396	0.03946	0.04017
2	*P. umthakathi*	1	-	0.00282	0.00211	0.02255	0.02326	0.02326	0.03876	0.03946
3	*P. marmorati*	5	4	-	0.00352	0.02537	0.02607	0.02607	0.04017	0.04087
4	*P. australis*	4	3	5	-	0.02467	0.02537	0.02537	0.03946	0.04017
5	*P. dawiekoki*	33	32	36	35	-	0.0007	0.00423	0.04299	0.04369
6	*P. dawiekoki*	34	33	37	36	1	-	0.00493	0.04369	0.0444
7	*P.* sp(H.m.)	34	33	37	36	6	7	-	0.04087	0.04158
8	*W. almae*	56	55	57	56	61	62	58	-	0.0007
9	*W. almae*	57	56	58	57	62	63	59	1	-
10	*W. almae*	56	55	57	56	61	62	58	0	1
11	*P. cuvieri*	52	51	53	52	53	54	50	23	24
12	*P. lopezromani*	67	66	68	67	71	72	68	40	41
13	*P. naevius*	58	57	61	60	60	61	59	39	40
14	*P. nearcticum*	61	60	62	61	67	68	64	38	39
15	*P. floridana* n.sp.	61	60	62	61	66	67	65	41	42
16	*P. floridana* n.sp.	61	60	62	61	66	67	65	41	42
17	*P. floridana* n.sp.	61	60	62	61	66	67	65	41	42

continued.

		10	11	12	13	14	15	16	17
1	*P. testimagna*	0.03946	0.03665	0.04722	0.04087	0.04299	0.04299	0.04299	0.04299
2	*P. umthakathi*	0.03876	0.03594	0.04651	0.04017	0.04228	0.04228	0.04228	0.04228
3	*P. marmorati*	0.04017	0.03735	0.04792	0.04299	0.04369	0.04369	0.04369	0.04369
4	*P. australis*	0.03946	0.03665	0.04722	0.04228	0.04299	0.04299	0.04299	0.04299
5	*P. dawiekoki*	0.04299	0.03735	0.05004	0.04228	0.04722	0.04651	0.04651	0.04651
6	*P. dawiekoki*	0.04369	0.03805	0.05074	0.04299	0.04792	0.04722	0.04722	0.04722
7	*P.* sp(H.m.)	0.04087	0.03524	0.04792	0.04158	0.0451	0.04581	0.04581	0.04581
8	*W. almae*	0	0.01621	0.02819	0.02748	0.02678	0.02889	0.02889	0.02889
9	*W. almae*	0.0007	0.01691	0.02889	0.02819	0.02748	0.0296	0.0296	0.0296
10	*W. almae*	-	0.01621	0.02819	0.02748	0.02678	0.02889	0.02889	0.02889
11	*P. cuvieri*	23	-	0.02326	0.02396	0.02537	0.02607	0.02607	0.02607
12	*P. lopezromani*	40	33	-	0.03101	0.03383	0.03735	0.03735	0.03735
13	*P. naevius*	39	34	44	-	0.02467	0.02396	0.02396	0.02396
14	*P. nearcticum*	38	36	48	35	-	0.00634	0.00634	0.00634
15	*P. floridana* n.sp.	41	37	53	34	9	-	0	0
16	*P. floridana* n.sp.	41	37	53	34	9	0	-	0
17	*P. floridana* n.sp.	41	37	53	34	9	0	0	-

Note. H.m. refers to *Hemisus marmoratus*. *P. dawiekoki* specimens, i.e. numbers 5 and 6, are from South Africa and Tanzania respectively. All specimens of *W. almae*, i.e. numbers 8, 9 and 10, are from the same area in French Guiana but 9 was collected in 1994 and 8 and 10 were in 2003. All specimens of *P. floridana* n.sp, i.e. numbers 15, 16 and 17, are from the same area in USA (Florida), but 15 and 17 were collected in 2003 and 16 in 2004.

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TABLE 3. Mean character differences (above diagonal) and total character differences (below diagonal) inferred from comparisons of COI mitochondrial sequences.

		1	2	3	4	5	6	7	8	9
1	*P. testimagna*	-	0.03195	0.06709	0.06709	0.05112	0.13419	0.13419	0.11821	0.14696
2	*P. umthakathi*	10	-	0.08946	0.08946	0.07029	0.14058	0.14377	0.13419	0.15974
3	*P. marmorati*	21	28	-	0	0.08307	0.13419	0.1278	0.10863	0.16933
4	*P. marmorati*	21	28	0	-	0.08307	0.13419	0.1278	0.10863	0.16933
5	*P. australis*	16	22	26	26	-	0.13738	0.13099	0.13099	0.15335
6	*P. dawiekoki*	42	44	42	42	43	-	0.01917	0.09585	0.18211
7	*P. dawiekoki*	42	45	40	40	41	6	-	0.09585	0.18211
8	*P.* sp(H.m.)	37	42	34	34	41	30	30	-	0.15974
9	*W. almae*	46	50	53	53	48	57	57	50	-
10	*W. almae*	47	50	54	54	47	56	57	50	2
11	*W. almae*	46	51	52	52	49	59	58	49	3
12	*P. cuvieri*	43	44	43	43	48	52	53	54	44
13	*P. lopezromani*	40	42	38	38	41	46	44	42	48
14	*P. naevius*	42	48	40	40	50	40	41	42	52
15	*P. nearcticum*	49	51	48	48	50	50	50	49	58
16	*P.floridana* n.sp.	54	56	50	50	53	53	50	49	61
17	*P.floridana* n.sp.	54	56	50	50	53	53	50	49	61
18	*P.floridana*n.sp.	54	56	50	50	53	53	50	49	61

continued.

		10	11	12	13	14	15	16	17	18
1	*P. testimagna*	0.15016	0.14696	0.13738	0.1278	0.13419	0.15655	0.17252	0.17252	0.17252
2	*P. umthakathi*	0.15974	0.16294	0.14058	0.13419	0.15335	0.16294	0.17891	0.17891	0.17891
3	*P. marmorati*	0.17252	0.16613	0.13738	0.12141	0.1278	0.15335	0.15974	0.15974	0.15974
4	*P. marmorati*	0.17252	0.16613	0.13738	0.12141	0.1278	0.15335	0.15974	0.15974	0.15974
5	*P. australis*	0.15016	0.15655	0.15335	0.13099	0.15974	0.15974	0.16933	0.16933	0.16933
6	*P. dawiekoki*	0.17891	0.1885	0.16613	0.14696	0.1278	0.15974	0.16933	0.16933	0.16933
7	*P. dawiekoki*	0.18211	0.1853	0.16933	0.14058	0.13099	0.15974	0.15974	0.15974	0.15974
8	*P.* sp(H.m.)	0.15974	0.15655	0.17252	0.13419	0.13419	0.15655	0.15655	0.15655	0.15655
9	*W. almae*	0.00639	0.00958	0.14058	0.15335	0.16613	0.1853	0.19489	0.19489	0.19489
10	*W. almae*	-	0.01278	0.14058	0.15655	0.16613	0.1853	0.19489	0.19489	0.19489
11	*W. almae*	4	-	0.14058	0.15655	0.17252	0.1885	0.19808	0.19808	0.19808
12	*P. cuvieri*	44	44	-	0.14696	0.13419	0.15016	0.15974	0.15974	0.15974
13	*P. lopezromani*	49	49	46	-	0.13419	0.15016	0.15016	0.15016	0.15016
14	*P. naevius*	52	54	42	42	-	0.11182	0.12141	0.12141	0.12141
15	*P. nearcticum*	58	59	47	47	35	-	0.04153	0.04153	0.04153
16	*P.floridana* n.sp.	61	62	50	47	38	13	-	0	0
17	*P.floridana* n.sp.	61	62	50	47	38	13	0	-	0
18	*P.floridana* n.sp.	61	62	50	47	38	13	0	0	-

Note. H.m. refers to *Hemisus marmoratus P. marmorati* specimens, i.e. numbers 3 and 4, are from South Africa. *P. dawiekoki* specimens, i.e. numbers 6 and 7, are from South Africa and Tanzania respectively. All specimens of *W. almae*, i.e. numbers 9, 10 and 11, are from the same area in French Guiana but 10 was collected in 1994 and 9 and 11 were in 2003. All specimens of P. sp (H.c.), i.e. numbers 16, 17 and 18, are from the same area in USA (Florida), but 16 and 18 were collected in 2003 and 17 in 2004.

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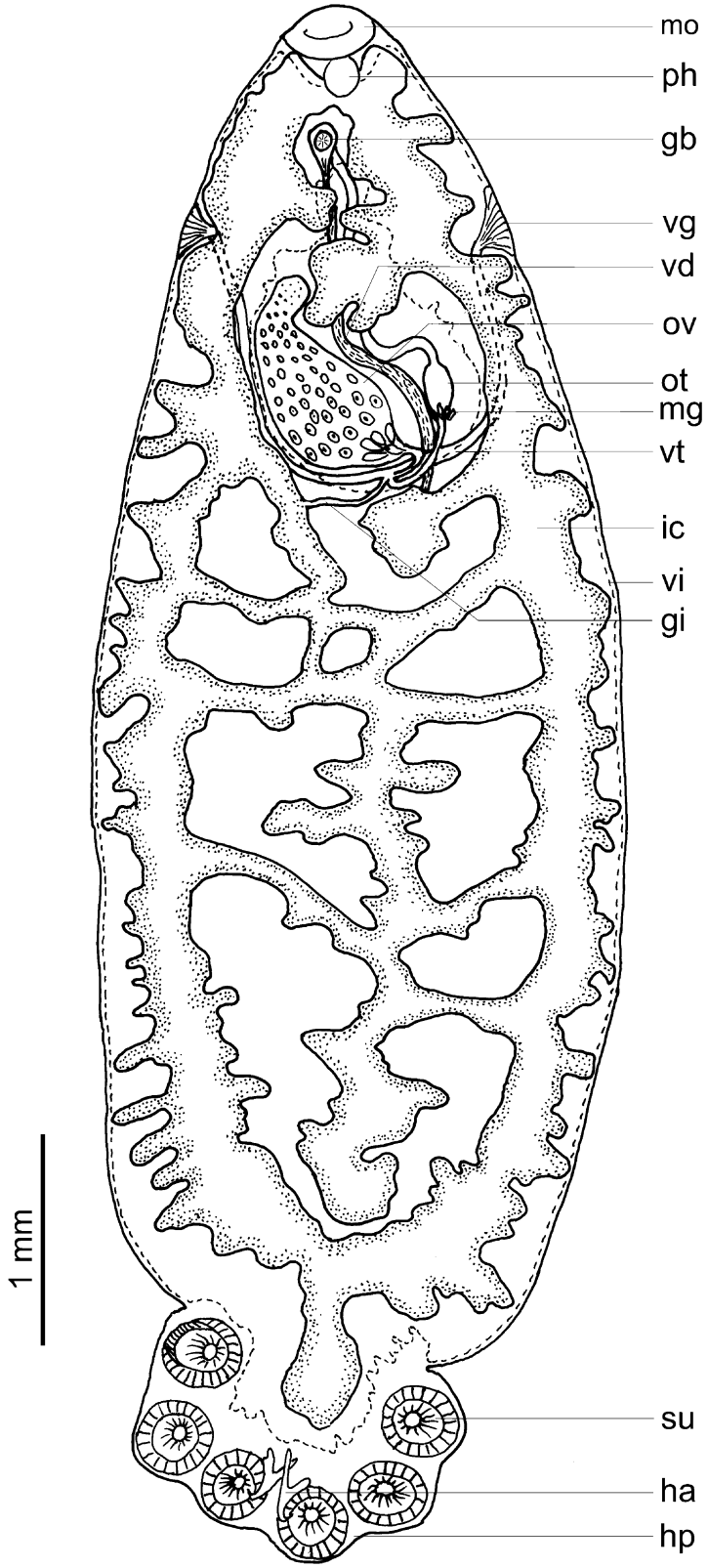

FIGURE 1. *Polystoma floridana* **n. sp.** Ventral view of holotype; dotted line indicates the outline of the vitelline system. Abbreviations: gb, genital bulb; gi, genito-intestinal canal; ha, hamulus; hp, haptor; ic, intestinal caecum; mg, Mehlis' gland; mo, mouth; ot, ootype; ov, ovary; ph, pharynx; su, sucker; vg, vagina; vd, vas deferens; vi, vitellaria; vt, vitelline duct. Scale bar: 1 mm.

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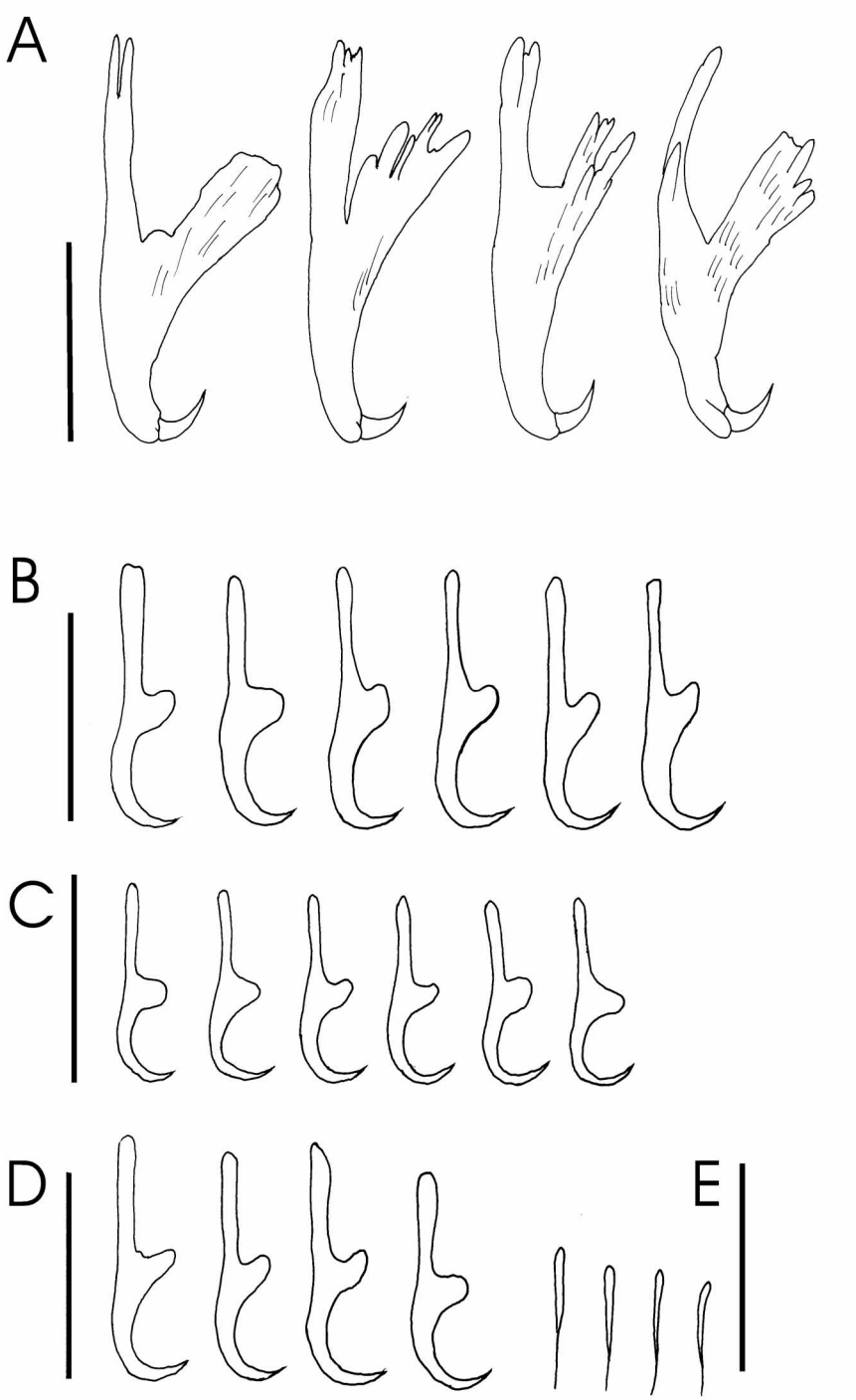

FIGURE 2: *Polystoma floridana* **n. sp. A**, hamuli; **B**, marginal hooklets 1 from holotype and paratypes; **C**, marginal hooklets 2 from holotype and paratypes ; **D**, marginal hooklets 8 from holotype and paratypes; **E**, hamulus primordia. Scale-bars: A, 500 µm; B–E, 20 µm.

Discussion

Of the 21 currently described polystome genera, the genus *Polystoma* outnumbers all others by far and is known from all continents except Australia and Antarctica. This genus is represented in Africa by 33 described and several undescribed species, Asia and Europe by 21 described and several undescribed species and South America by 11 described and four undescribed species (Du Preez unpublished data). In the light of

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this it is therefore surprising that in spite of the rich anuran diversity in North America there is currently only one *Polystoma* species widely known from the Palearctic region, namely *P. nearcticum*. This is even more surprising if one takes into account that North America has, with 10 described and several undescribed species, one of the richest diversities of chelonian polystomes. It is however, quite possible that the focus of previous studies was more on chelonian polystomes, neglecting the anuran polystomes, and that many more anuran polystomes await discovery.

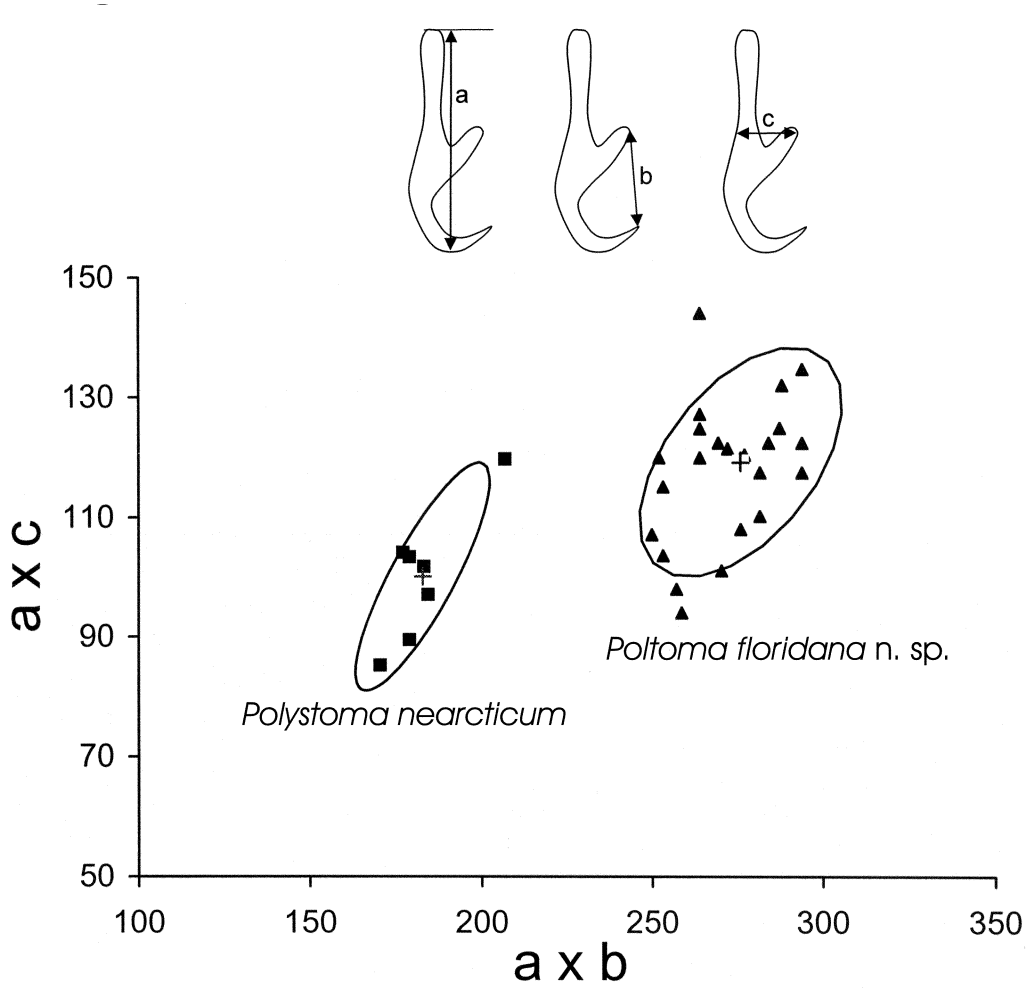

FIGURE 3: Scatter diagram of a x b plotted against a x c for *P. floridana* **n. sp.** and *P. nearcticum*. Measurements for oncomiracidia of *P. nearcticum* were obtained from specimens in the collection of Prof. R.C. Tinsley and the US National Parasite Collection in Beltsville, Maryland, USA.

Host-specificity in the Monogenea is well documented and various authors have commented on host-specificity in the genus *Polystoma* (see Combes 1966, 1968, Euzet *et al.* 1974a, b, Tinsley 1973, 1974, Bourgat & Salami-Cadoux 1976, Murith 1981, 1982, Combes & Channing 1979, Kok & Van Wyk 1986, Kok & Du Preez 1987, Du Preez & Kok 1992, 1993, 1997). A similar high level of host-specificity will most likely apply for North American *Polystoma* but this remains to be investigated. Our hypothesis that *Polystoma nearcticum* does not infect *Hyla cinerea* but that *H. cinerea* is infected by its own species specific polystome, *P. floridana* n.sp., is supported by Campbell (1969) who collected 12 *H. versicolor* and 14 *H. cinerea* in Gloucester County, VA. He concluded that *H. cinerea* was not infected while 41% of the *H. versicolor* were infected with *P. nearcticum*. Bolek and Coggins (1998) conducted a survey of endoparasites of *Hyla chrysoscelis* Cope in Wisconsin and found that 10% were infected with a polystome which they identified as *P. nearcticum*. Brooks (1976) conducted a study of amphibians in Nebraska and did not find polystomes in *H.*

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chrysoscelis. It is possible that molecular studies and detailed morphological studies of the sclerites of the polystome from *H. chrysoscelis* will reveal that this parasite belongs to a yet undescribed species, but this remains to be investigated.

According to our molecular results, polystome specimens that are classified in the same species show no more than 0.070 % and 1.917 % total character differences in the 28S and in the COI respectively. This is primarily based on data for sympatric South American *Wetapolystoma almae* specimens and for allopatric African *P. dawiekoki* specimens. In comparison, the most closely related African polystome species, i.e. *P. testimagna* and *P. umthakathi*, differ by total character differences of 0.070 % in the 28S and 3.195 % in the COI. Although the 28S genetic differentiation observed between two different valid species may, in some cases, be similar to that observed between conspecifics, the COI genetic differentiation is higher between sympatric closely related species than between allopatric individuals of the same species. This suggests that the molecular level of species delineation within polystomes can be fixed approximately to about 0.07 % in the 28S and to about 2.0 % in the COI. Because the three analyzed polystome specimens from *H. cinerea* do not show any difference, as well in the 28S as in the COI, they probably belong to the same species. On the other hand, the level of genetic differentiation estimated between *P. nearcticum* and the polystomes from *Hyla cinera*, i.e. total character differences of 0.634 % in the 28S and 4.153 % in the COI, suggests that *Polystoma* sp of *H. cinerea* is a different species.

Although molecular studies have been shown to be of great assistance in resolving taxonomic complexes, general body features and morphometrics remain the fundamental basis of polystome species identification and description. The analytical protocol for marginal hooklet measurements proposed by Du Preez and Maritz (2006) was developed on South African species of *Polystoma* but turned out to be effective in highlighting morphological differences between *P. floridana* **n. sp.** and *P. nearcticum.* This protocol will most likely be of great value in the description of most new polystomes in the America's.

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