

CHAPTER 3:

DESCRIPTION OF A POLYSTOMATID

A new polystomatid: *Polystomoides* sp. 1 (Monogenea: Polystomatidae) from the mouth of *Pseudemys nelsoni* in North America

Higher taxon: Platyhelminthes

New taxa described: 1 species

Number of references cited: 21

Number of figures and tables: 1 figure and 1 table

Abstract

Polystomoides sp. 1 is described as new species of the Polystomatidae parasitic in the oral and pharyngeal region of *Pseudemys* sp. from Gainesville, Florida, USA. A survey of freshwater turtles collected in baited crayfish traps in Florida was undertaken. One specimen of *Pseudemys* sp. was found to release polystome eggs and this specimen was found to be infected with a yet unknown species of *Polystomoides*. This new species is only the fifth *Polystomoides* from the Nearctic Realm. It can be distinguished from known *Polystomoides* species by a combination of characteristics including marginal hooklet morphology.

Key words: Monogenea, Polystomatidae, *Polystomoides*, freshwater turtle, Florida, USA

3.1. Introduction

Polystomatid flatworms (Monogenea, Polystomatidae) are classified into 24 genera and are known from a diverse range of hosts including the Australian lungfish, amphibians, freshwater turtles and the hippopotamus (Raharivololoniaina, *et al.* 2011). Three polystome genera that exclusively parasitize turtles are *Polystomoides* Ward, 1917, *Polystomoidella* Price, 1939 and *Neopolystoma* Price, 1939 (see Du Preez & Moeng 2004; Du Preez, *et al.* 2007; Du Preez *et al.* 2008; Morrison & Du Preez, 2012; Price, 1939; Wright 1879 & Yamaguti 1963). Chelonian polystomes are characterised by undiverticulated intestinal gut caeca of equal length and subsequent, absence of prehaptoral or haptoral anastomoses and a compact medial testis. *Polystomoides* is found in the oral cavity, nasal cavity, cloaca and urinary bladder of the host. It can be differentiated by the presence of two pairs of hamuli, with the outer pair being larger than the inner pair. *Polystomoidella* parasitises the urinary bladder of turtles and can be distinguished by the presence of a single pair of hamuli. *Neopolystoma* is found in the oral cavity, nasal cavity, ocular cavity, cloaca and urinary bladder. This genus is characterised by the absence of hamuli.

There are presently 54 turtle polystome species from 55 host species known. Chelonian polystomes have a cosmopolitan distribution. However, only seven *Neopolystoma* species, two *Polystomoidella* and four *Polystomoides* are found in the Nearctic Realm. The *Polystomoides* species currently known from this region includes *Polystomoides coronatum* (Leydi, 1888) Ozaki, 1935 from *Trachemys dorbigni*; *Polystomoides multifalx* (Stunkard, 1924) from *Pseudemys concinna* (LeConte, 1830); *Polystomoides oris* Paul, 1938 and *Polystomoides pauli* Timmers & Lewis, 1979, both from *Chrysemys picta*.

3.2. Material and methods

During the period April-June 2004 baited crayfish traps we set to capture terrapins in ponds in and around Gainesville, Florida. A number of traps were set in several ponds on the premises of the U.S. Geological Survey (USGS) research facility in Gainesville. Captured turtles were individually placed in 20L plastic buckets with dechlorinated tap water to a depth of about 50 mm. After a period of 24 hours the turtles were removed and the water screened for the presence of polystome eggs. The water was poured through two plankton sieves with respective mesh size of 500 and 100 micron. The first sieve removed the coarse debris in the water while the second retained finer debris and any polystome eggs. The contents of both sieves were then washed into separate glass petri-dishes and examined under a dissecting microscope. The Petri dish with contents from the coarse sieve was scanned for adult parasites that may have dislodged, and the fine sieve for the presence of polystome eggs. Recovered eggs were removed and incubated at room temperature. Oncomiracidia that hatched were collected and mounted semi-permanently using ammonium-picrate as mounting medium to clear the parasites and reveal the marginal hooklets. Turtles for which no eggs were detected, were screened a

second and third period of 24 hours. Turtles not infected with polystomes were released back into the environment where they were captured.

Turtles infected with polystomes were euthanized by injecting them with 5 ml of sodium pentobarbitone (Uthapent) diluted with water (0.5 ml Uthapent and 4.5 ml water) in the heart and dissected. The cloaca, urinary bladder and accessory bladders as well as the oral cavities, nasal cavities, pharyngeal cavities, eye surface and cavity under the nictitating membrane were examined for any polystomes, with the aid of a stereo microscope.

Polystome whole mounts were prepared by individually fixating the mature polystome species collected from the host species, under coverslip pressure in 10% neutral buffered formalin (NBF). Representative specimens were fixed in 70% molecular grade ethanol for future molecular studies.

Parasites earmarked for permanent mounts were hydrated to 30% EtOH, stained with Alum Carmine, gradually dehydrated to absolute EtOH, cleared in a 1:1 ratio mix of absolute ethanol-xylene and then pure xylene, and mounted in Canada balsam. Preparations were studied using a Nikon E800 compound microscope fitted with a Nikon DXM1200 digital microscope camera connected to a PC. Measurements were taken using Eclipse network software (Nikon). Measurements for the marginal hooklets were obtained from the oncomiracidia that hatched from incubated eggs, following the protocol developed by Du Preez & Maritz (2006).

3.3. Results

Turtles screened and polystomes retrieved

Nine live *Pseudemys nelsoni* (Florida Red-Bellied Turtle) were collected and screened. Host specimens were collected from Lake Griffen, Lake Lochloosa, Lake Orange, and ponds in and around the U.S. Geological Survey (USGS) research facility in Gainesville.

Levels of infection

Of the nine *Polystomoides* sp. 1 host specimens three were found to be infected (prevalence 33%). Only one turtle was dissected and found to have nineteen polystomes in the oral region. These specimens were identified as belonging to *Polystomoides*, they did not conform to any of the 38 known *Polystomoides* species.

Molecular studies

Material collected was studied at the molecular level. Based on 18S and 28S the newly discovered polystome differ from all other known turtle polystomes for which molecular data is available and occupies a distinct position basal to other Nearctic chelonian polystomes (see Figures 2a and 2b in Verneau *et al.* 2011).

Species description (Figs. 3.1-3.2)

Class: Monogenea Carus, 1863

Order: Polystomatidea Lebedev, 1988

Family: Polystomatidae Gamble, 1896

Specimens studied: Morphological description based on ten sexually mature worms. Holotype (NMB____) nine paratypes (NMB____) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa.

Type host: *Pseudemys nelsoni* (Carr, 1938).

Type locality: Gainesville, Florida.

Site: Mouth.

Description: Based on ten egg-producing adults. The average measurement is followed by the range given in parentheses (text and Table 3.1). Measurements are given in micrometers. Larval (oncomiracidia) measurements are given for the marginal hooklets.

Adult: General characteristics given of mature, egg-producing parasite (Figure 3.1). Body elongated and ellipsoid, total length 5,707 (3,052–7,378), greatest width 2,278 (1,276–2,751), width at vagina 2,270 (1,276–2,739), haptor length 1,310 (912–1,616), haptor width 1,931 (1,232–2,182); haptor length to body length ratio 0.23; six haptoral suckers, mean diameter 564 (148–781), haptors internally supported by an elaborate skeletal structure. Mouth sub-terminal. False oral sucker 788 (398–1036) wide; pharynx length 539 (345–917), width 658 (391–881). Intestine bifurcates with no diverticula and no anastomoses present; caeca do not join posteriorly nor do they extend into the haptor but they extend to the end of the body proper.

Testis compact, mid-ventral, medial, and posterior to ovary (Figure 3.1). Genital atrium median, ventral, posterior to intestinal bifurcation: 586 (302–816) in length with 123 (108–132) spines, 101 (93–106) long. Ovary, dextral, 38% from anterior end, ovary length 251 (102–330), width 86 (27–124). Short tubular uterus anterior to ovary, containing up to eight eggs, length 227 (182–274), width 144 (118–194). No intra-uterine development, egg operculate. Vitellarium extends throughout most of the body proper posterior to the pharynx except the central area around the gonads (Figure 3.1).

Oncomiracidia: Marginal hooklets were observed and measured from slides prepared of incubated oncomiracidia (Figure 3.2). Marginal hooklet I 28 (25–30), hooklets II – VIII 27 (25–29).

Table 3.1: Measurements of *Polystomoides* sp. 1

Characteristics	Measurements
Body length	5,707 (3,052–7,378)
Greatest width	2,278 (1,276–2,751)
Width at vagina	2,270 (1,276–2,739)
Haptor length	1,310 (912–1,616)
Haptor width	1,931 (1,232–2,182)
Oral diameter	788 (398–1,036)
Pharynx length	539 (345–917)
Pharynx diameter	658 (391–881)
Testis length	401 (108–687)
Testis width	564 (148–781)
Ovary length	251 (102–330)
Ovary width	86 (27–124)
Egg length	227 (182–274)
Egg diameter	144 (118–194)
Intra–Uterine eggs (n)	3 (0–8)
Genital bulb width	586 (302–816)
G. bulb hooks 1(n)	123 (108–132)
G. bulb hook length	101 (93–106)
Haptoral sucker diameter	564 (148–781)
Hamulus length 1	138 (104–173)
Hamulus length 2	69 (48–95)
Hamulus hook length 1	22 (19–26)
Hamulus hook length 2	21 (17–26)
Marginal hooklet length	Hooklet I – 28 (25–30)
	Hooklets II–VIII – 27 (25–29)
Haptoral L/Body L	0.23
Nr of anastomosis	0

Remarks: *Polystomoides* sp. 1 differs from other *Polystomoides* species by a combination of characters. With a body length of 5,707 (3,052–7,378) *Polystomoides* sp. 1 is longer than *Polystomoides megaovum* (2,910), *Polystomoides asiaticus* (4,600), *Polystomoides siebenrockiella* (3,580) and *Polystomoides uruguayensis* (2,560–2,650). However *Polystomoides* sp. 1 has a smaller body length when compared to *Polystomoides australiensis* (6,193), *Polystomoides fuquesi* (7,480–7,550), *Polystomoides godavarii* (4,200–8,030) and *Polystomoides ludhianae* (6,640–10,060). *Polystomoides* sp.1 (1,310 x 1,931) also differs from *P. megaovum* (620 x 880), *P. asiaticus* (1,100 x 1,700), *P. siebenrockiella* (780 x 1,060), *P. australiensis* (1,353 x 2,190) and *P. godavarii* (1,120–1,620 x 1,250–1,710) in the length and width of the haptor.

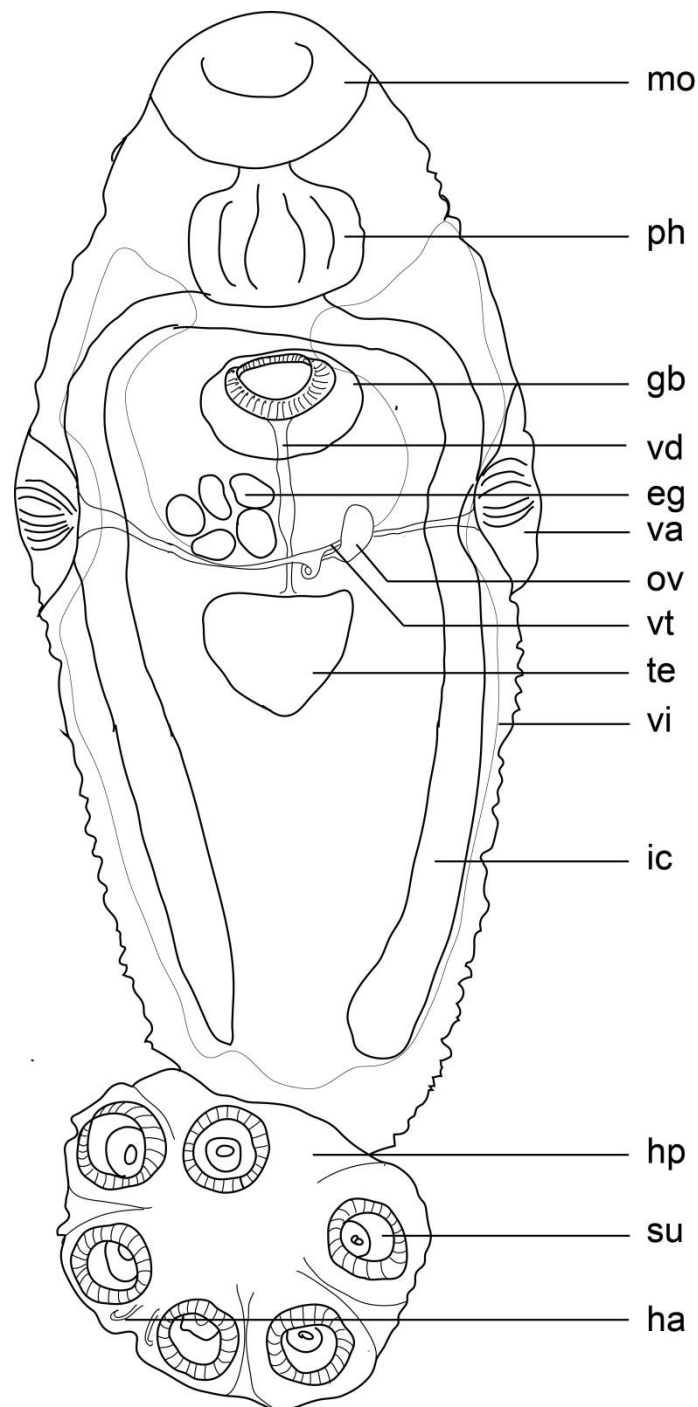


Figure 3.1: *Polystomoides* sp. 1 Ventral view of holotype; the dotted line indicates the outline of the vitellarium. Abbreviations: gb, genital bulb; eg, eggs; hp, haptor; ha, haptor hooks; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; vd, vas deferens; vg, vagina; vi, vitellaria; vt, vitelline duct. Scale bar: 1 mm.

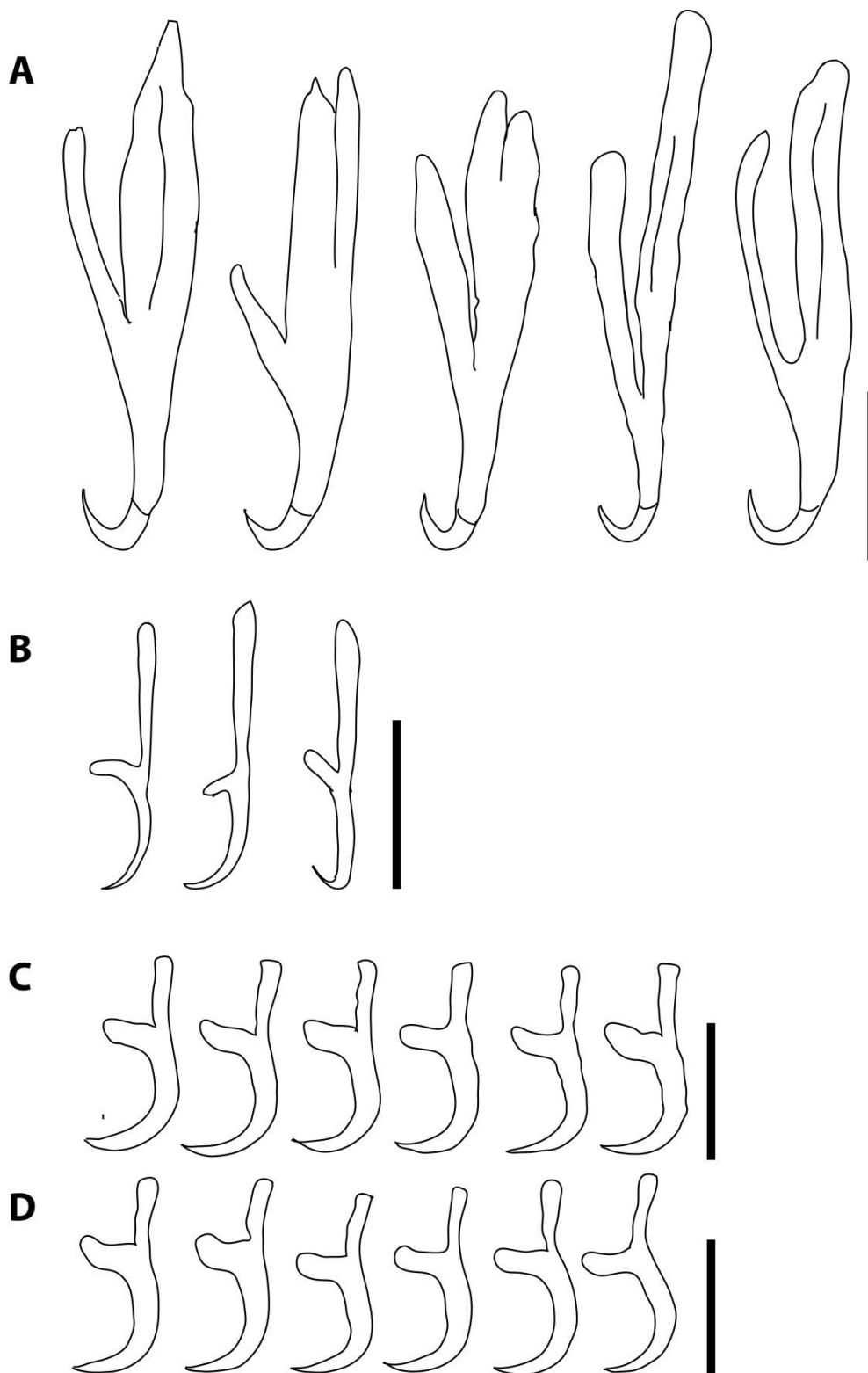


Figure 3.2: *Polystomoides* sp. 1 A, Large hamuli from the holotype and paratypes; B, Small hamuli from holotype and paratype; C, marginal hooklets 1; D, marginal hooklets 2-8. Scale-bars: A, 50 µm; B, 50 µm; C, 20 µm; D, 20 µm.

3.4. Discussion

All polystome species are host specific, with chelonian polystomes being strictly site-specific. As a result of this strict site-specificity a single host could be infected by more than one polystome species. Chelonian polystomes have been fairly well studied in the USA, with 11 polystomes known from various freshwater turtle hosts (Du Preez & Lim, 2000 & Du Preez & Morrison, 2012).

The two *Polystomoidella* species known from North America are *Polystomoidella oblongum* Wright, 1879 and *Polystomoidella whartoni* Wright, 1879. The seven *Neopolystoma* species from the USA includes *Neopolystoma elizabethae* Platt, 2000, *Neopolystoma fentoni* Platt, 2000, *Neopolystoma grossi* Du Preez & Morrison, 2012, *Neopolystoma moleri* Du Preez & Morrison, 2012, *Neopolystoma orbiculare* Stunkard, 1916, *Neopolystoma rugosa* MacCallum, 1918 and *Neopolystoma terrapenis* Harwood, 1932. The four *Polystomoides* species include *P. coronatum* Leidy, 1888, *P. multifalx* Stunkard, 1924, *P. oris* Paul, 1938, and *P. pauli* Timmers & Lewis, 1979 (Du Preez & Morrison, 2012).

The main feature distinguishing *Polystomoides* from other turtle polystomes is the presence of two unequal pairs of hamuli. The other genera that parasitize turtles either have a single pair of hamuli as for *Polystomoidella* or the hamuli are lacking altogether as in *Neopolystoma*. *Polystomoides* and *Neopolystoma* species can also occasionally be distinguished from *Polystomoidella* in the additional sites (the cavity of the eye and nose, pharynx, cloaca, and mouth) that these species parasitize, as *Polystomoidella* parasites are only found to infect the urinary bladder of their host species.

Polystomoides sp. 1 can be distinguished from the other *Polystomoides* species in the number of genital spines. *Polystomoides* sp. 1 has 123 (108–132) genital spines compared to *P. fuquesi* with 2, *Polystomoides brasiliensis* with 8–9, *Polystomoides bourgati* with 26–29, *P. asiaticus* with 34–40, *P. ludhiana* with 54–64, *P. godavarii* with 64–66 and *P. australiensis* with 74–95. However *P. multifalx* (120–124) and *Polystomoides stunkardi* (92–109) are two species that also has a large number of genital spines. Compared to *Neopolystoma* species *Polystomoides* sp. 1 also has a larger number of genital spines. *Neopolystoma chelodinae* has 14 (12–16), *N. elizabethae* 8 and *Neopolystoma euzeti* 34 (33–36). *P. oblongum* and *P. whartoni* both have 16 genital spines.

The total length of the genital spines of *Polystomoides* sp. 1 101 (93–106) are larger compared to other *Polystomoides* species. The length of the genital spines for other species includes *P. siebenrockiella* 58 (54–60), *Polystomoides rohdei* 34–52, *Polystomoides platynota*, 60–70, *Polystomoides nabedei* 42–46, *Polystomoides microrchis* 75–88 and *Polystomoides chabaudi* 27 (22–31). The genital spines for *Polystomoides* sp. 1 are in the same size range as those of *P. australiensis* 93 (78–105). *Polystomoides* sp. 1 also has larger genital spines compared to *Neopolystoma* species, such as *N. chelodinae* 23.6 (20.8–27.2), *N. euzeti* 57 and *N. elizabethae* 10 as well as compared to *Polystomoidella* species, such as *P. oblongum* 18–22 and *P. whartoni* 15–18.

Unlike most other polystomes, those parasitising chelonians have a cosmopolitan distribution. *Neopolystoma* and *Polystomoides* both have been reported from the realms around the globe known to be inhabited by freshwater turtles. *Polystomoidella* on the other hand is mainly known from the

Nearctic Realm where it is represented by five species. Richardson & Brooks (1987) described *Polystomoidella mayesi* from Malaysia. The presence of *Polystomoidella* in the Oriental Realm raises questions of possible misidentifications or a possible parasite transfer. According to Du Preez & Lim (2000) the possibility of transfer from an introduced American turtle can only be confirmed or refuted if and when *P. mayesi* is found in this chelonian.

Part of the evolutionary success of chelonian polystomes is the fact that they are site specific and occupies various sites including the oral and nasal cavities, eye cavity and the cloaca and urinary bladder. Littlewood *et al.* (1997) stated that congeneric species infecting the same site in different hosts are more closely related than congeneric species infecting different sites in the same host individuals. The high degree of site specificity allows for speciation and could explain the polystome diversity found in freshwater turtles. With the huge diversity of freshwater turtles globally it is likely that a vast number of chelonian polystomes remain to be discovered.