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Article

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Two new polystomes (Monogenea: Polystomatidae) from the eyes of North American freshwater turtles

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Abstract

Neopolystoma moleri n. sp. and *Neopolystoma grossi* n. sp. are described as new polystome species on the eyes of *Apalone ferox* and *Pseudemys concinna floridana*, respectively from Florida, USA. Eleven other polystome species are currently known from chelonian hosts in the USA, but only *Neopolystoma elizabethae* and *Neopolystoma fentoni* were described from the eye. Ocular polystomes are characterized as having spindle-shaped eggs; an exceptionally firm grip on the host; as well as the ability to stretch, which gives them the advantage of being stationary while extending to feed, reducing the risk of being dislodged. The two new species can be distinguished from known *Neopolystoma* species by a combination of characteristics including marginal hooklet morphology.

Key words: attachment, *Apalone ferox*, eye, Florida, freshwater turtle, *Pseudemys concinna floridana*, North America, *Neopolystoma*, *Neopolystoma moleri* n. sp., *Neopolystoma grossi* n. sp., Polystomatidae, USA

Introduction

Polystomes (Monogenea, Polystomatidae) are currently known to be represented by 24 genera (Raharivololoniaina *et al*. 2011). The Polystomoidinae are parasites of caecilians and freshwater turtles and are characterised by undiverticulated intestinal caeca of equal length that do not form prehaptoral nor haptoral anastomoses; distribution of vitelline follicles in two lateral fields; a prominent compact medial spherical testis; skeletal elements present in the haptoral suckers, and a genital bulb with genital spines that may be arranged in two rings. Chelonian polystomatids are represented by three genera: *Polystomoides* Ward, 1917, with two pairs of hamuli; *Polystomoidella* Price, 1939, with one pair, and *Neopolystoma* Price, 1939, with none. Currently 54 turtle polystome species are known from 55 host species around the world (Morrison & Du Preez 2011). Eleven species are known from North America, namely two *Polystomoidella*: *Polystomoidella oblongum* (Wright, 1879) and *Polystomoidella whartoni* (Wright, 1879); four *Polystomoides*: *Polystomoides coronatum* (Leidy, 1888), *Polystomoides multifalx* (Stunkard, 1924a), *Polystomoides oris* Paul, 1938, and *Polystomoides pauli* Timmers & Lewis, 1979; and five *Neopolystoma*: *Neopolystoma elizabethae* Platt, 2000, *Neopolystoma fentoni* Platt, 2000, *Neopolystoma orbiculare* (Stunkard, 1916), *Neopolystoma rugosa* (MacCallum, 1918), and *Neopolystoma terrapenis* (Harwood, 1932). Of these, only *N. elizabethae* and *N. fentoni* are known from the conjunctival sack of the eye. America has a rich freshwater turtle diversity, with 46 species occurring in North America alone (Bonin *et al*. 2006), which explains the rich polystome biodiversity. It is probable that several unnamed USA turtle polystomes are yet to be discovered.

Material and methods

Turtles were obtained by trapping as well as examining frozen road kills obtained from the Fish & Wildlife Division in Gainesville, Florida. Baited crayfish traps were set in several ponds on the premises of the U.S. Geological Survey (USGS) research facility in Gainesville as well as in ponds in the Gainesville surroundings. Captured turtles

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were placed individually in 20 liter plastic buckets containing dechlorinated tap water to the depth of about 50 mm. After 24 hours, turtles were transferred to clean buckets and the water in which the turtles were kept was screened for the presence of polystome eggs. Water was poured through a pair of plankton sieves with respective mesh sizes of 500 μm and 100 μm. The first sieve removed the coarse debris while polystome eggs were retained on the second. Hereafter, the contents of both sieves were rinsed into glass Petri dishes and examined using a dissecting microscope. The contents from the coarse sieve were screened for parasites that may have dislodged and dropped out. The contents of the fine sieve were washed into a Petri dish. Recovered eggs were removed and incubated at room temperature in Petri dishes containing pond water. Turtles for which no eggs were detected were screened a second and third day and, if no polystome eggs were found, the animals were released at the point of collection.

Oncomiracidia that hatched were collected and mounted in ammonium-picrate (Malmberg 1956) for further studies. Infected turtles were killed by means of an injection of 0.5ml Uthapent (sodium pentabarbitone) diluted with 4.5 ml water, and dissected for examination of the bladder, cloaca, eyes, and oral, pharyngeal, and nasal cavities using a stereo microscope. Frozen turtles obtained from Fish & Wildlife were thawed, dissected and examined using the same procedure as for fresh material. Coverslips of ammonium-picrate mounts were secured and sealed using clear nail varnish.

Parasites were fixed for 24 hours in 70% EtOH under coverslip pressure. Parasites allocated for permanent mounts were hydrated to 30% EtOH, stained with alum carmine, gradually dehydrated to absolute EtOH, cleared in a 1:1 solution of 100% EtOH and xylene and then pure xylene, and mounted in Canada balsam. Live parasites selected for DNA extraction were maintained overnight in a 0.06% saline solution. After a 24 hour period, the gut contents were removed by applying pressure to the parasite by rolling a camel hair brush laterally from the haptor towards the mouth to force out the gut contents and minimise contamination. Parasites were then fixed in 96% EtOH. DNA extractions and molecular analyses were conducted at the University of Perpignan, France and forms part of a bigger study (Verneau *et al*. 2011).

Since no viable eggs could be retrieved from the frozen *Apalone ferox* Schneider and since parasite eggs harvested from live *Pseudemys concinna floridana* LeConte failed to incubate, marginal hooklets were located in and measured from the mature specimens. Only hooklets in a flat orientation were measured according to the marginal hooklet protocol suggested by Du Preez & Maritz (2006).

Results

Turtles screened and polystomes retrieved

In total, 47 turtles representing six species were collected. These included a single live *Pseudemys concinna floridana* (Florida Cooter) and one roadkill of *Apalone ferox* (Florida Softshell Turtle). These were the hosts of the new polystomes we describe in this paper.

Levels of infection

Three polystomes were recovered from the eye of *P. c. floridana,* and six from the eye of *A. ferox*. They were identified as belonging to *Neopolystoma*. They did not conform to any of the 21 known *Neopolystoma* species and are thus herein described as new species.

Species descriptions

The proposed new species are *Neopolystoma moleri* n. sp. and *Neopolystoma grossi* n. sp. They differ from one another with regard to a number of key characteristics (Table 1): *Neopolystoma moleri* n. sp. is considerably larger than *N. grossi* n. sp., with a larger maximum length and width, and has a larger haptor. *N. grossi* n. sp.; however, has a larger oral diameter, although its pharynx is much smaller than that of *Neopolystoma moleri* n. sp. *Neopolystoma moleri* n. sp. has a larger testis than *N. grossi* n. sp., and although their genital bulbous sizes are similar, *N. moleri* n. sp. possesses 12–13 genital spines, while *N. grossi* n. sp. possesses six to eight. *Neopolystoma moleri* n. sp. has slightly larger haptoral suckers than *N. moleri* n. sp., but a smaller haptor length:body length ratio. Marginal hooklets were located in the whole mounts of type specimens of *Neopolystoma moleri* n. sp. and *N. grossi* n. sp.

Marginal hooklets 1 that were in a flat orientation were measured. Unsuccessful attempts were made to obtain the type material of *N. elizabethae* and *N. fentoni*, the other two ocular polystomes known from North-America. For these two species, measurements were calculated from published drawings of the hooklets. In a plot of the products of the total length (a in Figure 1) and the width at the level of the guard (c in Figure 1) 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 1) of marginal hooklet C1 (Du Preez & Maritz 2006), both *N. grossi* n. sp. and *Neopolystoma moleri* n. sp. occupy distinct positions separate from *N. elizabethae* and *N. fentoni* (Figure 1).

TABLE 1. Comparison between the diagnostic measurements of *Neopolystoma moleri* n. sp., *N. grossi* n. sp., *N. elizabethae* Platt, 2000 and *N. fentoni* Platt, 2000. Measurements in micrometers.

Characteristics	*Neopolystoma moleri* n. sp.	*Neopolystoma grossi* n. sp.	*N. elizabethae* Platt, 2000	*N. fentoni* Platt, 2000
Body length	5975 (3249–7944)	4018 (3298–4873)	3125 (2550–3675)	1985 (1500–2450)
Greatest width	1269 (926–1694)	988 (911–1130)	823 (640–990)	568 (426–760)
Haptor length	779 (601–983)	769 (707–819)	865 (790–970)	571 (449–690)
Haptor width	1027 (784–1522)	891 (709–989)	975 (880–1070)	683 (550–850)
Width at vagina	984 (599–1426)	983 (900–1125)	–	–
Right caecum length	3155 (1692–1426)	2453 (1877–3003)	–	–
Left caecum length	3054 (1894–4101)	2290 (1923–2804)	–	–
False oral sucker width	288 (180–358)	403 (369–436)	473 (449–540)	370 (240–496)
Pharynx length	309 (243–415)	222 (206–232)	255 (216–269)	216 (156–257)
Pharynx width	331 (258–392)	229 (209–246)	305 (268–320)	278 (185–367)
Ovary length	210 (124–312)	218 (210–222)	301 (218–350)	103 (80–245)
Ovary width	92 (73–138)	128 (118–135)	122 (100–146)	105 (55–169)
Testis length	633 (449–753)	235 (186–293)	208 (178–262)	225 (98–367)
Testis width	548 (328–719)	236 (149–245)	155 (140–192)	181 (78–251)
Genital bulbous width	65 (36–79)	59 (58–60)	58 (50–63) × 57 (45–63)	55 (43–70) × 60 (30–83)
Number of genital spines	12–13	6–8	8	8
Genital spine length	9 (8–10)	8 (8–9)	10	11
Number of eggs *in utero*	1	1	3	3–7
Egg length	358	293 (288–301)	348 (322–367)	286 (245–332)
Egg width	168	139 (137–140)	120 (117–122)	136 (122–146)
Haptoral sucker width	280 (225–351)	241 (208–244)	372 (344–408)	265 (210–326)
Haptor length:body length ratio	0.16 (0.11–0.21)	0.20 (0.15–0.24)	0.28	0.29

Class: Monogenea Carus, 1863

Order: Polystomatidea Lebedev, 1988

Family: Polystomatidae Gamble, 1896

***Neopolystoma moleri* n. sp.**
(Figs. 2–4)

Specimens studied: One specimen was used to extract DNA. Morphological description based on five sexually mature worms. Holotype (NMB P335) five paratypes (NMB P336 – P340) deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa.

Type host: *Apalone ferox* (Schneider, 1783).

Type locality: Gainesville, Florida. Exact locality unknown. Road killed specimen collected by a member of the public and deposited at the office of the Florida Fish & Wildlife Conservation Commission, 4005 South Main Street, Gainesville FL 32601, USA.

Site: Conjunctival cavity of the eye.

Etymology: This parasite is named after Dr. Paul Moler of the Florida Fish & Wildlife Conservation Commission who provided the specimens.

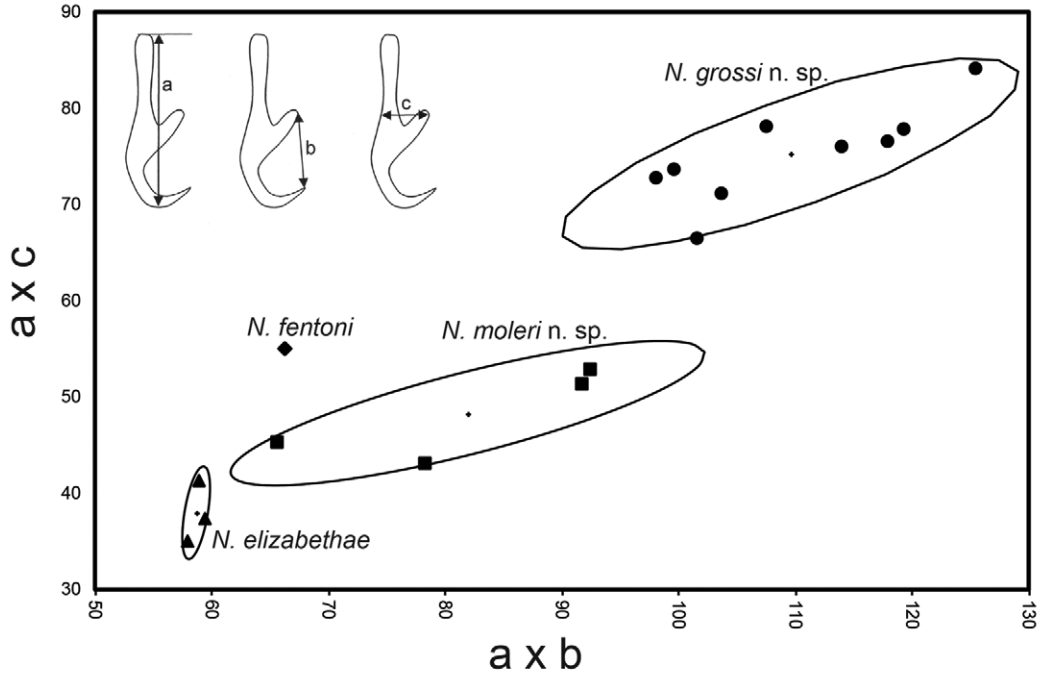

FIGURE 1. Scatter diagram of a × c plotted against b × c for the two known North-American eye polystomes as well as the new species *Neopolystoma grossi* n. sp. and *Neopolystoma moleri* n. sp. The ellipses represent 90% of the confidence interval about the mean.

Description: Based on five egg-producing adults, measurements are given in micrometers. The average measurement is followed by the range given in parentheses. No larval measurements or characters are given, as no oncomiracidia were available.

Adult: General characteristics given of mature, egg-producing parasite (Figure 2). Body elongated, some stretched out, total length 5975 (3249–7944), greatest width 1269 (926–1694), width at vagina 984 (599–1426), haptor length 779 (601–983), haptor width 1027 (784–1426); haptor length to body length ratio 0.16 (0.11–0.21); haptoral suckers 6, mean diameter 280 (225–351). Mouth sub-terminal, ventral. False oral sucker 288 (180–358) wide; pharynx length 309 (243–415), width 331 (258–392). Intestine bifurcate with no diverticula, right caecum 3155 (1692–4126) in length, left caecum 3054 (1894–4101) in length. No anastomoses; caeca do not join posteriorly and do not extend into the haptor.

Testis compact, mid-ventral, medial, and posterior to ovary (Figure 2). Vas deferens contains relatively low amounts of sperm. Genital atrium median, ventral, posterior to intestinal bifurcation: 28 (19–37) in length with 12–13 spines, 9 (8–10) long. Ovary dextral, 15% from anterior end, ovary length 210 (124–312), width 92 (73–138). Short tubular uterus anterior to ovary, one of the five paratypes containing a single egg, length 358, width 168. No intra-uterine development, egg operculate. Vitellarium organised in two broad lateral fields in the first 75% of the body. Genito-intestinal canal prominent, on the same side as ovary, joining intestinal caecum posterior to ovary (Figure 2).

Remarks: *Neopolystoma moleri* n. sp. differs from other members of the genus by a combination of characters (Table 1). *Neopolystoma moleri* n. sp. differs from *N. elizabethae* and *N. fentoni*: *N. moleri* n. sp. has an average body length of 5975 and a minimum length of 3249, significantly larger than *N. elizabethae* and *N. fentoni*, with body lengths of 3125 and 1985 respectively. Furthermore, *N. moleri* n. sp. is wider than *N. elizabethae* and *N. fentoni*, its minimum width, 926, being larger than the maximum width reported for *N. elizabethae* and *N. fentoni*. *Neopolystoma moleri* n. sp. has a wider haptor and a slightly larger pharynx, while its ovary, with an average length

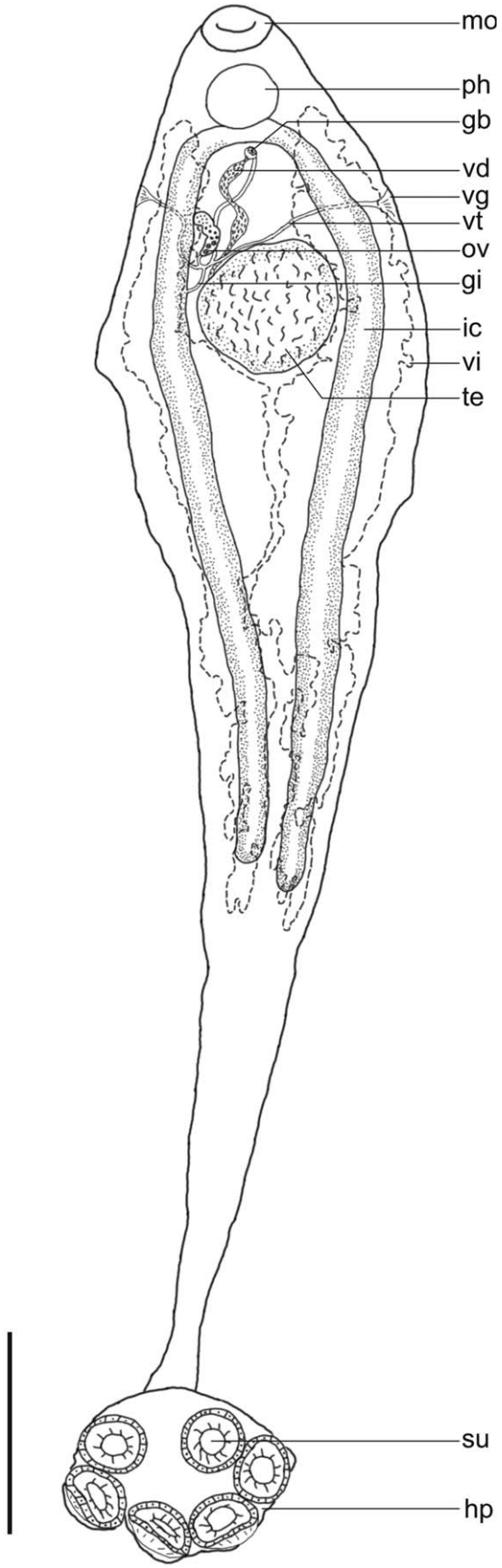

FIGURE 2. *Neopolystoma moleri* n. sp. Ventral view of holotype; the dotted line indicates the outline of the vitellarium. Abbreviations: gb, genital bulb; gi, genito-intestinal canal; hp, haptor; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; vd, vas deferens; vg, vagina; vi, vitellaria; vt, vitelline duct. Scale bar: 1 mm.

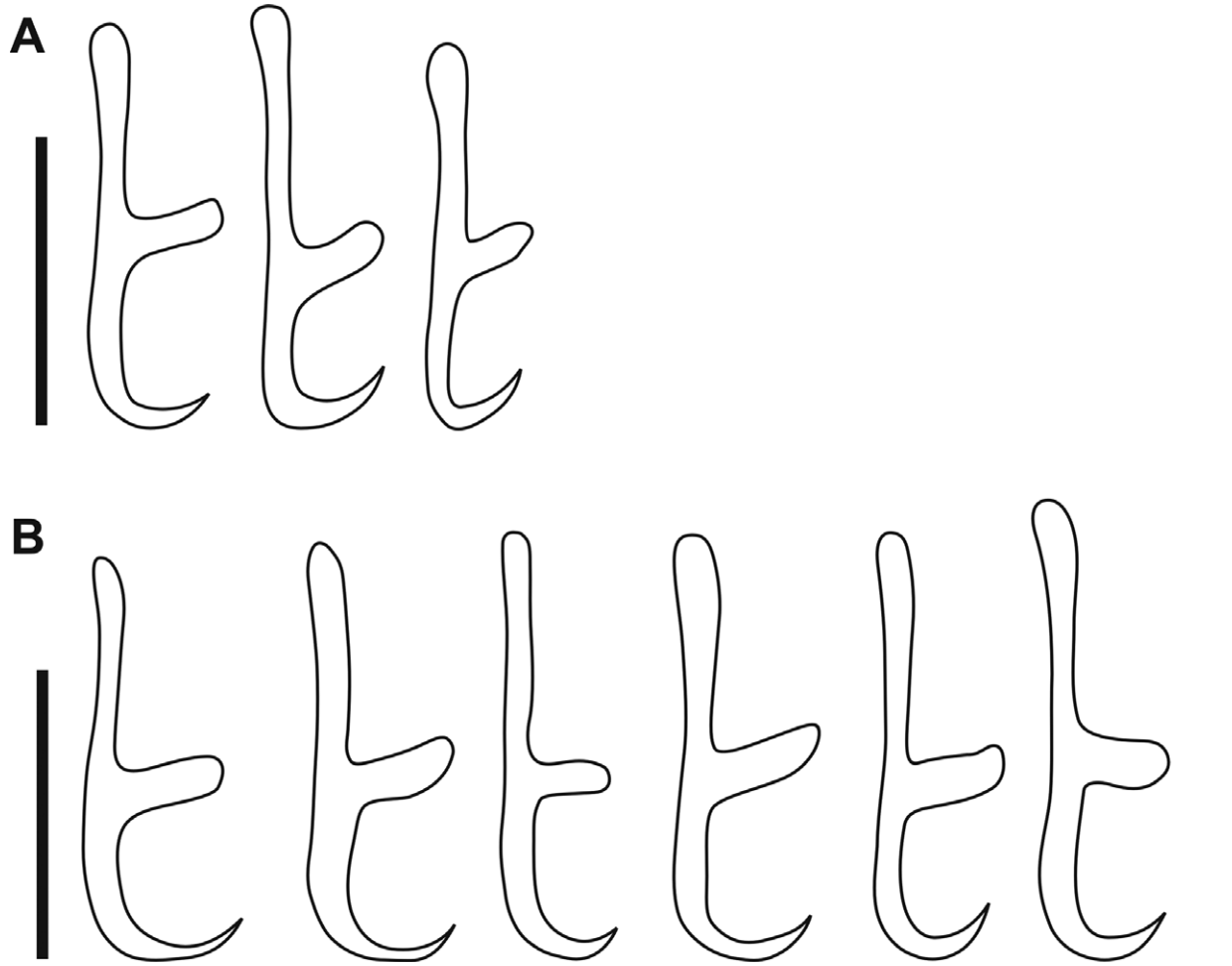

FIGURE 3. *Neopolystoma moleri* n. sp. **A**, marginal hooklets 1 from the holotype and paratypes; **B**, marginal hooklets 2-8 from the holotype and paratypes. Scale-bars: 10μm.

of 210, is slightly smaller than that of *N. elizabethae* (with an average length of 301), and slightly larger than *N. fentoni* (with an average length of 103). With an average size of 633 × 548, *N. moleri* n. sp. has a much larger testis than *N. elizabethae* (with an average size of 208 × 155), and *N. fentoni* (with an average size of 225 × 181). *Neopolystoma moleri* n. sp. can further be distinguished from *N. elizabethae* and *N. fentoni* in possessing more genital spines (12–13) compared to eight for *N. elizabethae* and eight for *N. fentoni*. *Neopolystoma moleri* n. sp. has larger eggs (358 × 168) compared to (332 × 146) for *N. fentoni* and (367 × 122) for *N. elizabethae.* Finally, *N. moleri* n. sp. has an average haptor length to body length ratio of 0.16 that distinguishes it further from *N. elizabethae* (0.28) and *N. fentoni* (0.29).

***Neopolystoma grossi* n. sp.**
(Figs. 5–7)

Specimens studied: Three sexually mature worms. The holotype (NMB P341) and two paratypes (NMB P342–P343) are deposited in the Parasitic Worm Collection, National Museum, Aliwal Street, Bloemfontein, South Africa.

Type host: *Pseudemys concinna floridana,* sexually mature individual deposited in the Florida Museum of Natural History, Division of Herpetology, Museum Road, Dickinson Hall, University of Florida, Gainesville, Florida 32611 as a type host. Museum number: UF 141647.

Type locality: Pond at the United States Geological Survey USGS-BRD facility, Florida Integrated Science Centres, 7920 NW 71st Street, Gainesville FL 32653, USA (29.725278N; 82.417778W).

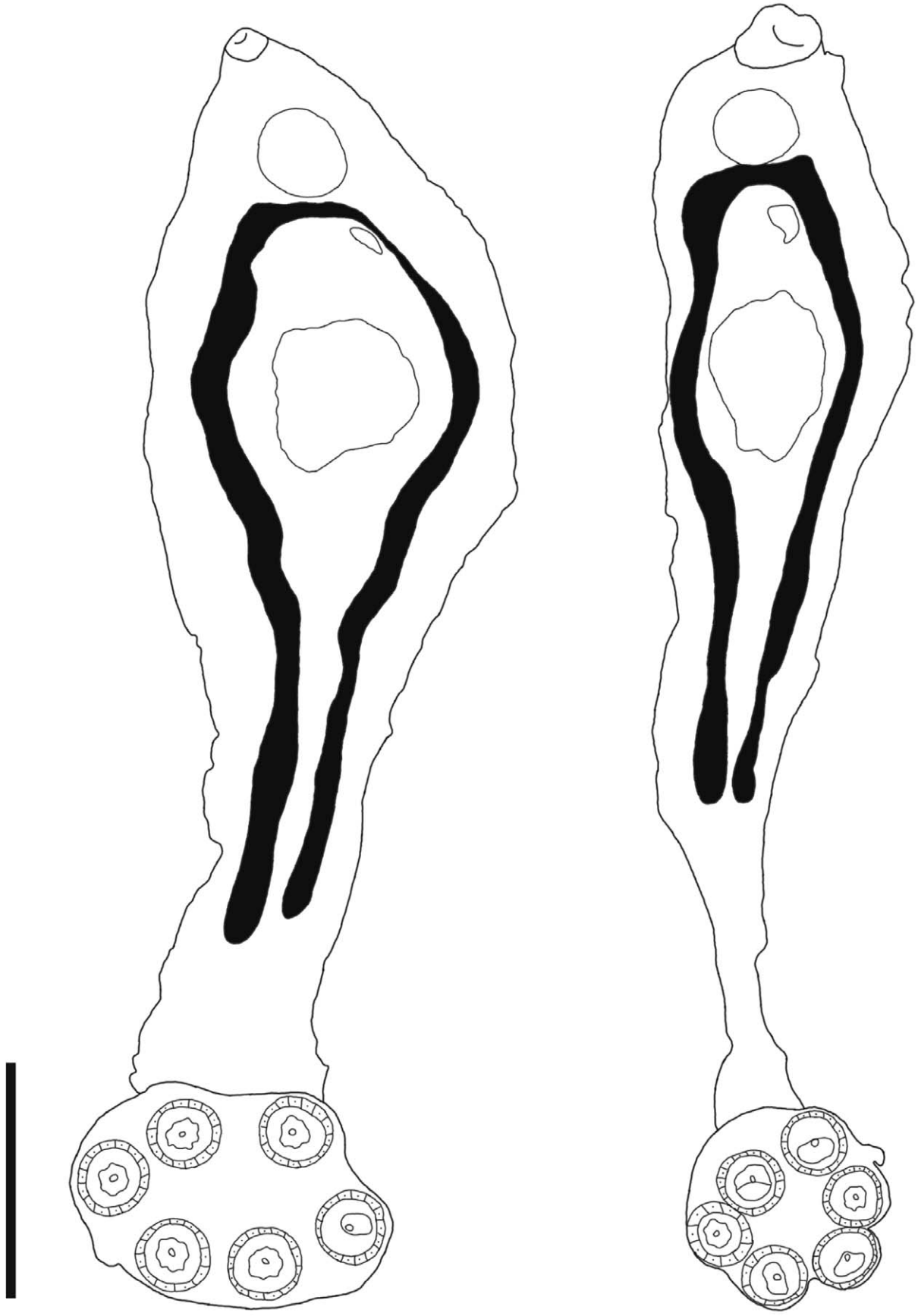

FIGURE 4. Paratypes of *Neopolystoma moleri* n. sp**.**, demonstrating its ability to stretch lengthwise in order to feed successfully without being dislodged. Scale bar: 1mm.

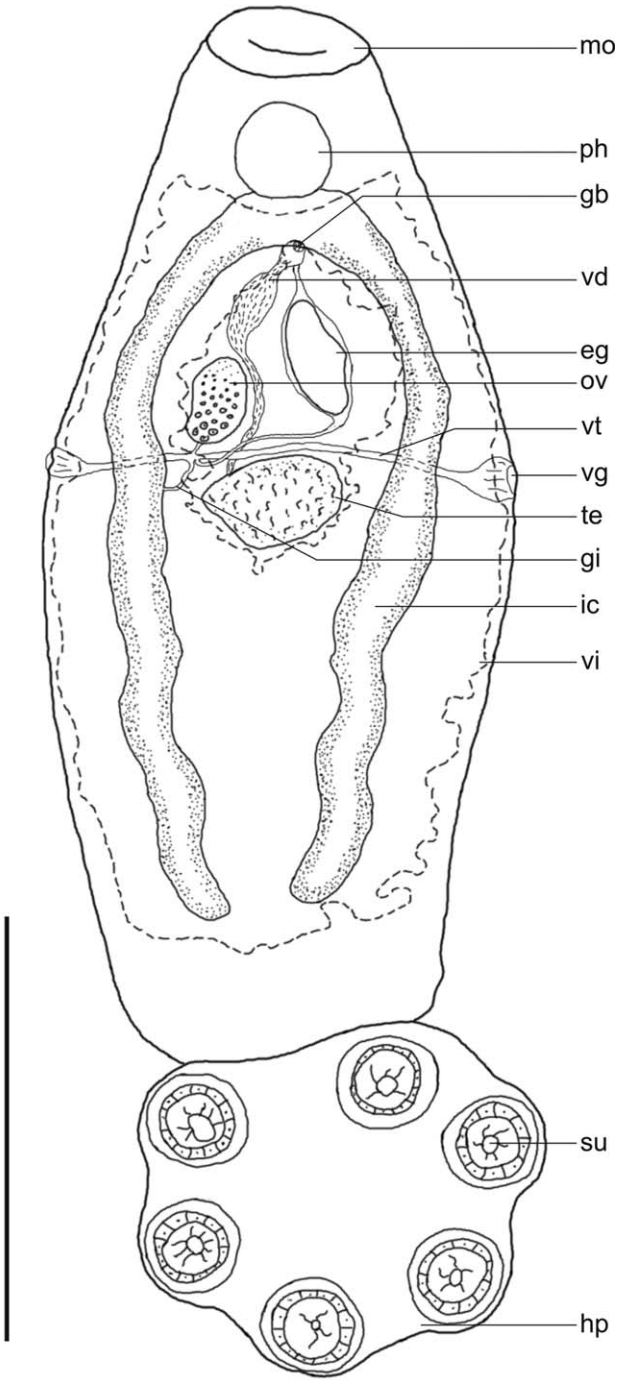

FIGURE 5. *Neopolystoma grossi* n. sp. Ventral view of the holotype; the dotted line indicates the outline of the vitelline system. Abbreviations: eg, egg; gb, genital bulb; gi, genito-intestinal canal; hp, haptor; ic, intestinal caecum; mo, mouth; ov, ovary; ph, pharynx; su, sucker; te, testis; vd, vas deferens; vg, vagina; vi, vitellarium; vt, vitelline duct. Scale bar: 1mm.

Site: Conjunctival cavity of the eye.

Etymology: This parasite is named after Dr. Timothy Gross who facilitated this study.

Description: Based on three egg-producing adults; measurements are given in micrometers. The average measurement is followed by the range given in parentheses. No larval measurements or characters are given, and although many eggs were harvested from the host, no development took place.

Adult: General characteristics given of mature, egg-producing parasite (Figure 5). Body elongate, some stretched out, total length 4018 (3298–4873), greatest width 988 (911–1130), width at vagina 983 (900–1125), haptor length 769 (707–819), haptor width 891 (709–989); haptor length to body length ratio 0.2 (0.15–0.24); haptoral suckers 6, mean diameter 241 (208–244). Mouth subterminal, ventral. False oral sucker 403 (369–436) wide; phar-

ynx length 222 (205–232), width 229 (209–246). Intestine bifurcate with no diverticula, right caecum 2453 (1877–3003) in length, left caecum 2290 (1923–2804) in length. No anastomoses; caeca do not join posteriorly and do not extend into the haptor. Testis compact, mid-ventral, medial, and posterior to ovary (Figure 5). Seminal vesicle packed with sperm. Genital atrium median, ventral, posterior to intestinal bifurcation, 21 (12–24) in length with 6–8 spines, 8 (8–9) long. Ovary dextral, 25% from anterior end, ovary length 218 (210–222), width 128 (118–135). Short tubular uterus anterior to ovary, containing only one egg; egg capsule length 293 (288–301), width 139 (137–140). No intrauterine development, eggs operculate. Vitellarium situated in the first 70% of the body, forming no prehaptoral or haptoral anastomoses. Genito-intestinal canal obscured by the testis, located on the same side as ovary, joining intestinal caecum posterior to ovary (Figure 5).

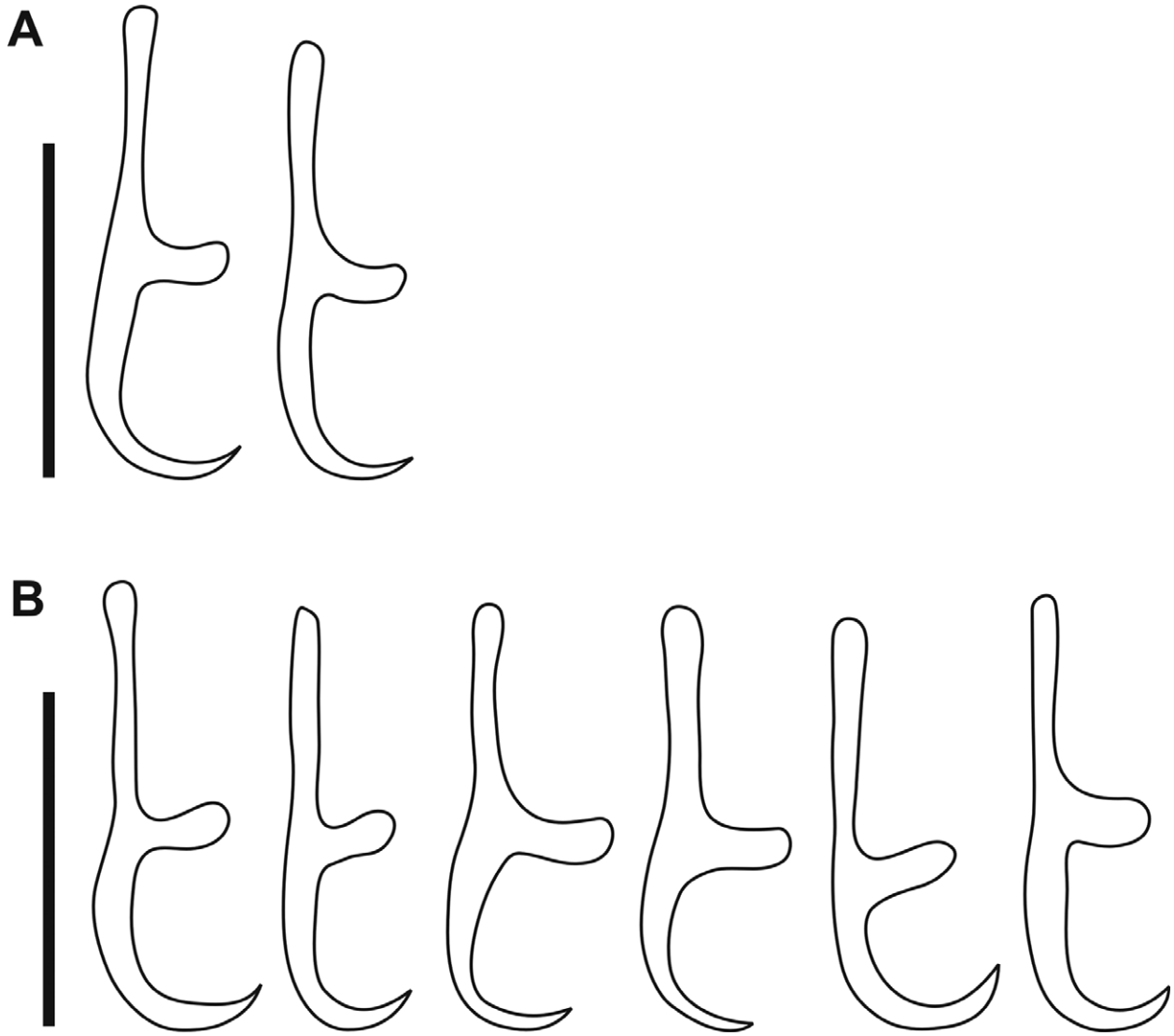

FIGURE 6. *Neopolystoma grossi* n. sp. **A**, marginal hooklets 1 from holotype and paratypes; **B**, marginal hooklets 2-8 from holotype and paratypes. Scale-bars: 10µm.

Remarks: *Neopolystoma grossi* n. sp. differs from *N. elizabethae* and *N. fentoni* by a combination of characteristics (Table 1). With an average length of 4018 and a minimum length of 3298, *N. grossi* n. sp. is significantly longer than *N. fentoni* (with a maximum length of 2450), and even also *N. elizabethae* (with a maximum length of 3675). *Neopolystoma grossi* n. sp., with an average width of 988, is wider than *N. fentoni*, which has a maximum width of 760, and also possesses a slightly larger haptor. *Neopolystoma grossi* n. sp., with an average ovary size of 218 × 128, also differs from *N. fentoni* (with an average ovary size of 103 × 105), which is significantly smaller. *N. grossi* n. sp. can also be distinguished from *N. elizabethae* and *N. fentoni* by slightly smaller genital spines. *Neopolystoma elizabethae*, with a length of 322–367, possesses more elongated eggs than those of *N. grossi* n. sp. (with

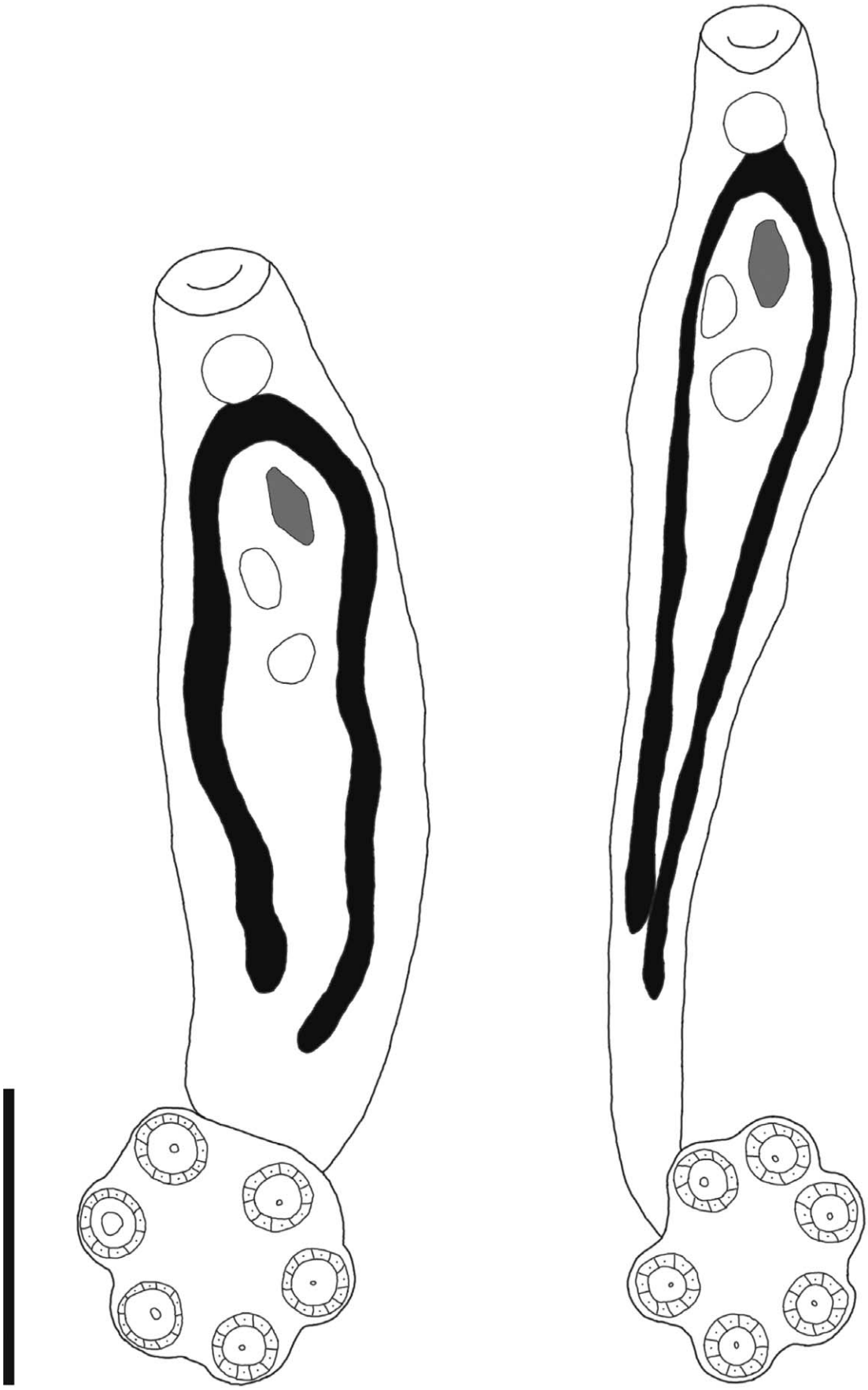

FIGURE 7. Paratypes of *Neopolystoma grossi* n. sp., demonstrating its ability to stretch. Scale bar: 1mm.

a length of 288–301). *Neopolystoma grossi* n. sp. can also be distinguished from *N. elizabethae* by a smaller haptoral sucker size of 241, as opposed to 372. Finally, *N. grossi* n. sp. has a smaller maximum haptor length to body length ratio of 0.20 that distinguishes it further from *N. elizabethae* (0.28) and *N. fentoni* (0.29).

Discussion

Chelonian polystomes have been fairly well studied in the USA and 11 species are known from the continent. Polystomes of the eye, however, have been overlooked for decades and only recently been included in studies, with the first ocular polystome from a turtle, namely *Neopolystoma palpebrae* Strelkov, 1950, being described only 62 years ago. Since then, new species were described sporadically. Only eight out of 21 *Neopolystoma* have been described from the eye, seven of which were described after 1994. Apart from the eye, *Neopolystoma* can be found in the cloaca, urinary and accessory bladders, oral, pharyngeal and nasal cavities (Pichelin 1995). Chelonian polystomes are known to be site-specific, allowing for speciation and thus more than one species can be found in a single species of turtle (Du Preez & Lim 2000). Littlewood *et al*. (1997) found that chelonian polystomes in different host species, but in a specific site, are evolutionary closer together than parasites from different sites within a single host individual. The conjuctival sac of the turtle eye provides the parasites with easy access as well as favourable living conditions (Stunkard 1924b).

Chelonian polystomes are often semi-transparent since they do not feed on blood and are thus difficult to detect. This is especially true for ocular parasites as they tend to hide under the nictitating membrane and often quite deep in the conjunctival sac. The characteristic yellow spindle-shaped intrauterine egg often gives away the presence of a parasite. Screening the water in which turtles were maintained will reveal the spindle-shaped eggs that are characteristic to turtle eye polystomes. Spindle-shaped eggs have been reported for *N. liewi* (see Du Preez & Lim 2000), *N. elizabethae* (see Platt 2000a), *N. fentoni* (see Platt 2000b), and *N. tinsleyi* (see Pichelin 1995). The elongated shape of the eggs renders them stronger so that they can better tolerate the pressure of sliding membranes under the eyelids. *Oculotrema hippopotami* Stunkard, 1924b, is found under the nictitating membrane of the hippopotamus eye. Unlike chelonian polystomes, the eggs of *O. hippopotami* are oval, however the egg wall is quite thick, which protects the eggs from getting colloquially damaged (Du Preez & Moeng 2004). Increasingly larger numbers of polystomes are discovered occupying the eye of freshwater turtles, and it is highly likely that many new polystome species await discovery.

Both *N. moleri* n. sp. and *N. grossi* n. sp. share quite a number of other characteristics with the mammalian eye polystome *O. hippopotami*. Ocular polystomes have an exceptionally firm grip on the host's tissue and often form clusters on the eye with their haptors tightly arranged (Du Preez & Moeng 2004). Their haptoral suckers have rigid skeletal elements that facilitate the firm grip on the host. *Neopolystoma* and *Oculotrema* do not possess hamuli, but both have a compact round testis and a small ovary. No intra-uterine development takes place, and while the eggs do not develop in saline conditions associated with the eye (Thurston 1968), the eye of the hippopotamus and freshwater turtles are not constantly saline, because the hosts spend most of their time in the water and are also known to open their eyes when submerged. Eggs trapped under the eyelids may thus develop which may lead to re-infection.

A characteristic that *O. hippopotami* and *N. moleri* n. sp. and *N. grossi* n. sp. share is the ability to stretch. Du Preez & Moeng (2004) indicated that *O. hippopotami* has a flexible peduncle between the anterior body and haptor that enables the parasite to stretch out and double its length. In this region, they have well developed longitudinal and circular muscle fibres that enhance the flexibility and elasticity of the parasite (Moeng *et al*. 1998). Concomitantly with this, the parasites' reproductive and digestive organs are confined to the anterior third of the body. This same phenomenon was observed for both *N. moleri* n. sp. and *N. grossi* n. sp. and most likely enables the parasite to stretch out and feed on a larger area without detaching (Du Preez & Moeng 2004). An added advantage of this mechanism is that not needing to move while feeding minimizes the risk of being dislodged when the host blinks. The parasites also have an exceptionally firm grip on the host's tissue, and after removal prominent bud-shaped attachment marks often remain on the host tissue.

The specimens of *N. moleri* n. sp. used in this species description were retrieved from a host that was run over by a car and stored in a freezer for months. The polystomes on the eyes were frozen in a completely relaxed and extended state, explaining the elongated body size. This stresses the flexibility of the polystome body which means

that body length and width measurements are of limited taxonomic value. This was pointed out by Platt *et al*. (2011) who advocated that polystomes should not be fixed under cover slip pressure as this does increase body measurements. This is absolutely true and because of this flexibility we place much bigger emphasis on sclerite measurements. In order to obtain reliable measurements of especially the marginal hooklets it is important to have the hooklets in a flat orientation and therefore we recommend to fix at least some specimens flat under coverslip pressure. In the species description it would also be important to report whether a specimen was flattened when fixed and also whether it was under coverslip pressure or whether the coverslip was weighted down.

Neopolystoma, *Polystomoides*, *Polystomoidella* and *Oculotrema* share some characteristics pointing to a possible common ancestry. They all abandoned a sanguiniforic diet and adopted a possible diet of epithelial cells and mucus, supported by the increased thickness and toughness found in the epithelial lining of the parasites. Because their hosts share similar habits and habitats and may come into contact with each other, Du Preez and Moeng (2004) pointed out the possibility of a transfer of a freshwater turtle polystome to the hippopotamus, which could have evolved and adapted in isolation to the environment of the hippopotamus eye.

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