Neopolystoma fentoni n. sp. (Monogenea: Polystomatidae) a Parasite of the Conjunctival Sac of Freshwater Turtles in Costa Rica

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Neopolystoma fentoni n. sp. is described from the conjunctival sac of *Kinosternon leucostomum* (Duméril, Bibron, and Duméril 1851) and *Rhinoclemmys pulcherrima* (Gray 1855) from the Guanacaste Conservation Area in Costa Rica. The new species differs from all other species of *Neopolystoma*, except *N. elizabethae* Platt 2000 in possessing a circle of eight genital spines that are recurved and possess a crescent-shaped base. *N. fentoni* n. sp. differs from *N. elizabethae* in lacking cecal diverticula and in a number of morphometric criteria.

Key words: Monogenea - freshwater turtle parasite - *Neopolystoma* - conjunctival sac - Costa Rica

The monogene family Polystomatidae contains three genera parasitic exclusively in turtles. The majority of species of polystomatids described from turtles inhabit either the oral cavity or the urinary bladder as adults (du Preez & Lim 2000). A small number of species have been reported from the conjunctival sacs of freshwater turtles (Platt 2000). Examination of specimens of two species of turtles, *Kinosternon leucostomum* (Duméril, Bibron, and Duméril 1851) and *Rhinoclemmys pulcherrima* (Gray 1855), from the Guanacaste Conservation Area, Costa Rica, yielded specimens of *Neopolystoma* from the conjunctival sac that are herein described as a new species.

MATERIALS AND METHODS

Turtles were collected by hand from permanent pools in dry stream beds in the vicinity of the operations center of the Guanacaste Conservation Area during June 1998. Turtles were returned to the laboratory and examined within 48 h of capture. The animals were decapitated and necropsies were conducted following standard procedures. The lower eyelids were resected and worms were removed from the conjunctival sac with fine forceps and a camel hair brush.

Worms were killed, without flattening, in hot (steaming) 5% formalin. Specimens were placed in a vial with enough saline to cover them. Hot formalin was added to the vial, the vial; was quickly capped and gently shaken. The worms were stored in the same medium, stained in Mayer's haematoxylin (Pritchard & Kruse 1982), following standard procedures, and mounted in Canada balsam for examination as whole mounts. Definitions of prevalence and mean intensity follow the usage of Bush et al. (1997).

Specimens were observed with a Nikon Optiphot light microscope using bright field and phase contrast illumination. Drawings were made with the aid of a camera lucida and measurements were made using an ocular micrometer. All measurements are in micrometers with the mean followed by the range in parentheses, and n refers to the number of specimens measured.

DESCRIPTION

Neopolystoma fentoni n. sp.
Morphometrics: based on ten specimens
(Figs 1-3; Table I)

With the characteristics of the genus. Body pyriform, maximum width near midbody (Fig. 1). Tegument aspinose. Haptor slightly wider than long; six type 2 haptoral suckers (Pichelin 1995). Marginal hooks 16, 12.5 (11.8-12.7; n = 4) with blade oriented toward haptoral margin; hook pairs VI-VIII between anterior pair of suckers; hook pairs III-V in base of each sucker; hook pairs I-II between posterior-most pair of suckers. Marginal hooks rarely present, however, hoods typically present. Oral sucker large, wider than long. Pharynx large, wider than long; PW/OSW = 0.81:1. Genital bulb (Fig. 2) round to slightly oval; genital spines 8, recurved from base to tip with crescent-shaped handle at right angle to blade; eight genital spines 11 long (n = 8). Seminal vesicle small, oval. Testis located near midbody (excluding haptor).

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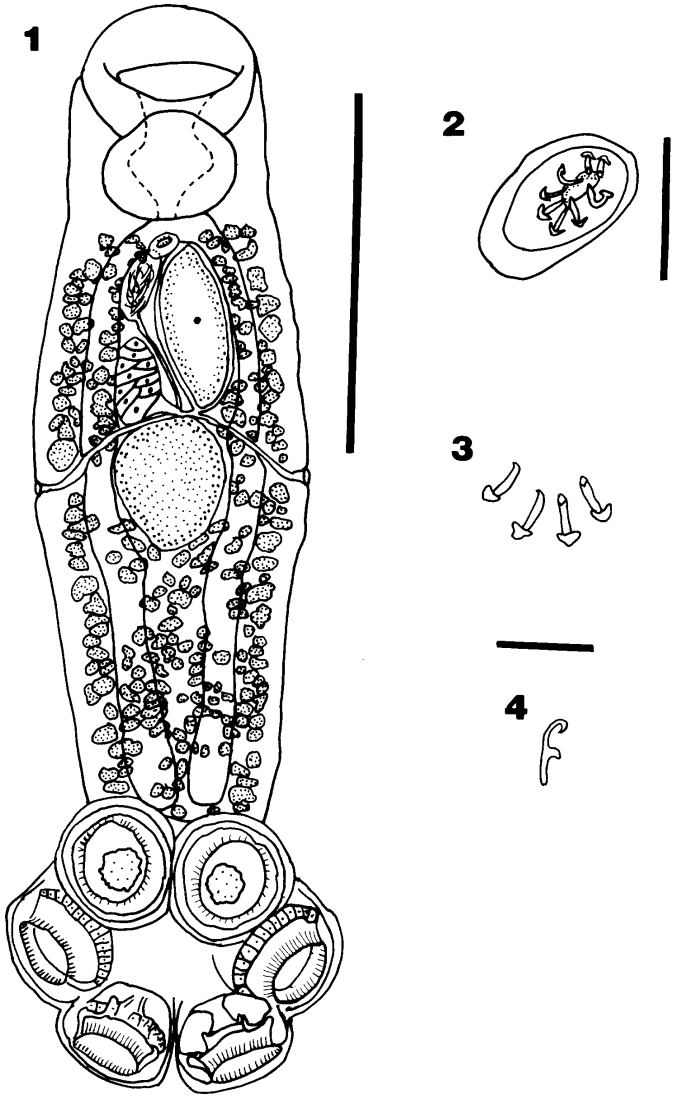
Genital system exhibits *situs inversus*. Ovary oval, anterior to testis. Genito-intestinal canal present. Uterus conspicuous, elliptical; ova elliptical. Vaginae lateral, uncapped, in second 1/4 of body. Vaginae from anterior end/total length as a percent = 39.97 (37.5-44.3). Vitellarium follicular from level of genital pore to anterior margin of haptor, filling intercecal space not occupied by genital structures; not extending into penduncle.

Taxonomic summary

Type host: *Kinosternon leucostomum* (Duméril, Bibron, and Duméril 1851): Kinosternidae
Other known host: *Rhinoclemmys pulcherrima* (Gray 1855): Bataguridae
Site of infection: conjunctival sac
Type locality: Quebrada Costa Rica (10º 49.666' N, 85º 38.216 W), Guanacaste Conservation Area, Santa Rosa, Guanacaste, Costa Rica
Other known localities: Quebrada el Duende (10º 50.236'N, 85º 36.724'W) and San Gerardo (10º 52.55'N, 85º 23.27'W), Guanacaste Conservation Area, Santa Rosa, Guanacaste, Costa Rica.
Type material: holotype and four paratypes: United States National Parasitological Collection, holotype (collection no. 89943); four paratypes (nos 89944-6): four paratypes Helminthological Collection of the Oswaldo Cruz Institute (collection nos 34292, 34293a-b, 34294).
Etymology: this species is named in memory of



Neopolystoma fentoni n. sp. in ventral view. Fig. 1: whole mount, ventral (holotype), bar = 500. Fig. 2: genital bulb and genital spines, ventral (paratype), bar = 50. Fig. 3: genital spines, ventral (paratype), bar = 20. Fig. 4: marginal spine, ventral (paratype), bar = 20.

my father-in-law, Mr William S Fenton, as a tribute to his friendship and counsel over the last quarter century.

REMARKS

The family Polystomatidae contains three genera that exclusively parasitize turtles. The genus *Neopolystoma* Price, 1939 is differentiated from *Polystomoides* Ward, 1917 and *Polystomoidella* Price, 1939, by the absence of anchors between the posterior-most pair of suckers on the haptor. The new species clearly belongs to the genus *Neopolystoma*. Specimens collected from *K. leucostomum* and *R. pulcherrima* overlapped morphometrically in all attributes and are treated as a single species, *N. fentoni*, which is considered new to science. Prevalence and intensity of *N. fentoni* were low in both host species (Table II).

Seven species of *Neopolystoma* have been described from the conjunctival sac of freshwater turtles (Table III). *N. fentoni* n. sp. differs from all other species in the genus, except *N. palpabrae* Strelkov, 1950, and *N. elizabethae* Platt, 2000, in having genital spines with a crescent-shaped base. *N. palpabrae* was described by Strelkov (1950) from the soft-shelled turtle, *Pelodiscus sinensis* (Wiegmann 1835), from Lake Chanka, north of Vladivostok, in the former Soviet Union. The genital spines in *N. palpabrae* are identical in shape to those of the new species; however, they numbered 16 and are arranged in a circle of alternating large and small spines. Strelkov (1950) did not give separate measurements for the large and small spines for *N. palpabrae*, however, the range of spine length was given as 10-16 µm, while the lengths of the spines of *N. fentoni* and *N. elizabethae* are 11 and 10, respectively.

TABLE II

Prevalence and mean intensity of *Neopolystoma fentoni* n. sp. in freshwater turtles from the Guanacaste Conservation Area, Santa Rosa, Costa Rica

Host	% prevalence	X intensity (range)
Kinosternon leucostomum	12.5 (2/16)	2.0 (1-3)
Rhinoclemmys pulcherrima	28.6 (2/7)	3.5 (3-4)

Number infected/number examined; total number of parasites/number of hosts infected

TABLE I

Morphometric comparison of *Neopolystoma fentoni* n. sp. and *Neopolystoma elizabethae* Platt, 2000 (mean followed by the range in parentheses)

Character		*N. fentoni* (n = 10)	*N. elizabethae* (n = 4)
Body	L	1985 (1500-2450)	3125 (2550-3675)
	W	568 (426-760)	823 (640-990)
Haptor	L	571 (449-690)	865 (790-970)
	W	683 (550-850)	975 (880-1070)
Marginal hooks (n = 4;8)	L	12.5 (11.8-12.7)	12.3 (11.8-12.7)
Haptor sucker (n = 60;24)	D	265 (210-326)	372 (344-408)
Oral sucker	L	230 (150-303)	271 (251-292)
	W	370 (240-496)	473 (449-540)
Pharynx	L	216 (156-257)	255 (216-268)
	W	278 (185-367)	305 (268-320)
Ph/OS		0.82:1 (0.75-0.93:1)	0.65:1 (0.61-0.71)
Genital bulb	L	55 (43-70)	58 (50-63)
	W	60 (30-83)	57 (45-63)
Seminal vesicle	L	79 (50-100)	151 (140-175)
	W	50 (35-65)	63 (50-68)
Testis	L	225 (98-367)	208 (178-262)
	W	181 (78-251)	155 (140-192)
Ovary	L	103 (80-245)	301 (218-350)
	W	105 (55-169)	122 (100-146)
Ova (n = 7;3)	L	286 (245-332)	348 (332-367)
	W	136 (122-146)	120 (117-122)
Vaginae to anterior end		788 (510-920)	1071 (880-1250)
VAE/TL (%)		40.0 (37.5-44.3)	34.3 (33.2-35.4)
Uterus (empty; n = 3)	L	99 (50-133)	330 (188-402)
	W	35 (33-38)	151 (140-192)

Ph/OS: pharynx width-oral sucker width ratio; VAE/TL (%): vaginae to anterior end as a percent of total length

Pichelin (1995) described four species of *Neopolystoma* from the conjuntival sacs of freshwater turtles in Australia. Three species had 20 or more genital spines which would clearly separate them from the American forms. Only *N. cribbi* Pichelin, 1995 possesses a smaller number of genital spines (range 6-10), however, they possess typical fan-shaped roots (Pichelin 1995) and lack the crescent-shaped base present in *N. fentoni* and *N. elizabethae*. du Preez and Lim (2000) described *N. liewi* from the conjunctival sac of the batagurid turtle, *Curora amboinensis*, from Malaysia. This species also possesses a small number of genital spines (8-11); however, they also have typical "fan-shaped" roots and lack the recurved base present in *N. fentoni* and *N. elizabethae*.

N. fentoni appears most closely related to *N. elizabethae* as the genital and marginal spines are nearly identical in shape and size; however, the new species differs from the North American form in lacking intestinal diverticula present in the posterior half of the cecae. The two forms also differ in number of morphometric criteria (Table I). *N. fentoni* is significantly smaller, the ratio of pharyngeal and oral suck width is greater, the diameter of the haptoral suckers is larger, the testis is larger, the seminal vesicle is smaller, and the eggs are, on average, 60 shorter. These differences are sufficient to warrant recognizing a new species.

The conjunctival sac has been overlooked as a site for the presence of polystomatids. The discovery of *N. fentoni* is only the seventh species recorded from this location since the first report of *N. palpabrae* from *Pelodiscus* (=*Amyda*) *sinensis* (Wiegmann, 1835) by Strelkov (1950). Pichelin (1995) described four species of *Neopolystoma* from the side-necked turtles (Chelidae) in Australia and Platt (2000) described *N. elizabethae* from the western painted turtle, *Chrysemys picta belli* (Gray, 1831) from northern Michigan in the United States.

The range of turtle taxa harboring *Neopolystoma* spp. in the conjunctival sac is shown in Table III. In addition to the fact that species in both major lineages of extant turtles harbor these worms, the five host families represent nearly 70% of genera and species of living turtles (Iverson 1992). Nor are these parasites rare in the individual host populations examined. Strelkov (1950) reported a prevalence of 35% for *N. palpabrae*, while Platt (2000) reported 80% prevalence for *N. elizabethae* in a very small sample (n = 5). While published data are not available for the Australian polystomatids, the author found over 20% prevalence in a sample of 70 chelids in Australia (Platt, unpublished observations). Any parasitologist examining turtles should examine the conjunctival sac for polystomes.

Turtles and turtle helminths may provide a valuable, if overlooked, resource for the study of host-parasite coevolution, biogeographic analysis, and speciation. The distribution of polystomatids in Australian and southeast Asian turtles (Rohde & Pearson 1980) and blood flukes (Family Spirorchiidae) (Platt 1992) suggest that these associations date to the breakup of Pangea (~200 mya). Littlewood et al. (1997) demonstrated, using molecular techniques, that polystomatid species from the same location (e.g., urinary bladder) but geographically disjunct localities, are more closely related to each other than congeners from the same host but a different location (e.g., oral cavity). Turtle populations in the midwestern United States and Australia typically harbor polystomatids in the mouth, urinary bladder and conjunctival sac (Platt, unpublished observations). Therefore, these helminths offer the opportunity to provide three independent tests of coevolution-

TABLE III

Classification of extant turtle species (Order Testudines) known to harbor *Neopolystoma* spp. in the conjunctival sac (turtle classification based on various sources)

Classification		Locality	Author
Infraorder Pleurodira			
Family Chelidae	*Emydura* spp. *Chelodina* spp. *Elseya latisternum*	Australia	Pichelin (1995)
Infraorder Cryptodira			
Family Trionychidae	*Pelodiscus sinensis*	Russia	Strelkov (1950)
Family Kinosternidae	*Kinosternon leucostomum*	Costa Rica	Current study
Family Emydidae	*Chrysemys picta belli*	United States	Platt (2000)
Family Bataguridae	*Rhinoclemmys pulcherrima*	Costa Rica	Current study
	Curora amboinensis	Malaysia	du Preez & Lim (2000)

ary, biogeographic and speciation hypotheses if sufficient material is made available. South America and Asia are two areas of high turtle diversity, yet recent checklists of monogenes from these areas identify only four and seven species of polystomes reported in the literature, respectively (Kohn & Cohen 1998, Lim 1998). Judicious examination of turtles for helminths from these areas, preserving specimens for both morphological and molecular analysis, has the potential to yield significant information about the history of life.

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