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Classification of pleurodire polystomes (Platyhelminthes, Monogenea, Polystomatidae) revisited with the description of two new genera from the Australian and Neotropical Realms

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ABSTRACT

Polystomatids are platyhelminth parasites that infect mostly amphibian and chelonian hosts. Polystomatid of testudines were, for more than seven decades, classified in the three genera – *Neopolystoma* Price, 1939, *Polystomoides* Ward, 1917 and *Polystomoidella* Price, 1939. The genus delimitation was primarily based on the absence of hamuli in *Neopolystoma*, the presence of one pair of hamuli in *Polystomoidella*, and two pairs in *Polystomoides*. From 2016 to 2020, five new genera were erected - namely *Uropolystomoides* Tinsley and Tinsley, 2016, *Uteropolystomoides* Tinsley, 2017, *Apaloneotrema* Du Preez and Verneau, 2020, *Aussietrema* Du Preez and Verneau, 2020 and *Fornixitrema* Du Preez and Verneau, 2020. The generic diagnosis was based not only on the size and shape of morphological characters such as hamulus 1, uterus and eggs, but also on the site of infestation (*i.e.* urinary bladder, oral cavity or conjunctival sacs). Despite large advancements in polystome classification over the last decade, *Neopolystoma* was still polyphyletic with some species nested within *Polystomoides* and others being closely related to the Australian *Aussietrema*. Regarding the distribution of freshwater turtles of the two suborders Pleurodira (Southern continents) and Cryptodira (distributed worldwide except in Australia), one may wonder whether Australian chelonian polystomes of the genus *Neopolystoma* may have diverged from species infecting other pleurodires of South America. In the present study based on the analysis of several species selected among all genera, we reveal striking morphological differences within polystomes infecting pleurodiran turtles, which herein led to the proposal of two new chelonian polystome genera, *Pleurodirotrema* n. g. and *Manotrema* n. g. *Pleurodirotrema* n. g. is characterized by the absence of hamuli, presence of latero-ventral vaginae and includes species that infect either the oral region or the urinary bladder of Australian hosts of the Pleurodira. *Manotrema* n. g. is characterized by the presence of small hamuli, latero-ventral vaginae, deep incisions between suckers, a low genital spine number (<10) and includes species that infect the oral region of South American Pleurodira.

1. Introduction

The Polystomatidae Gamble, 1896 sensu Sinnappah et al. (2001) is the largest family of the Monogenea Van Beneden, 1858 that infects aquatic or semi aquatic vertebrates of the Rhipidistia. Although monogenean parasites are mostly fish ectoparasites, polystomatids infect all three extant orders of Amphibia, namely Anura (frogs), Caudata

(salamanders) and Gymnophiona (caecilians) where adult worms are generally found in the urinary bladder. Polystomatids are also found within the urinary bladder, pharyngeal cavities and/or conjunctival sacs of freshwater turtles of the two testudines suborders Pleurodira and Cryptodira, in the mouth and on the gills of the Australian lungfish *Neoceratodus forsteri* and in the conjunctival sacs of the common hippopotamus *Hippopotamus amphibius*. Since their discovery in the late

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1700s from the common frog *Rana temporaria*, about 200 species have been reported and described from all over of the world except Antarctica. They are classified in 30 genera, of which 20 are found exclusively within amphibians, eight within freshwater turtles and, one each, within the Australian lungfish and the common hippopotamus, respectively.

The proposal of phylogenetic hypotheses for polystomes based on morphological characters has always been a very difficult, to near impossible, task, due to limited morphological interspecies variation. With the advancement of molecular technique in the late 1900s, particularly with the PCR approach, more and more phylogenetic studies have been focused on taxonomic groups for which morphological characters were inadequate. As an outcome, the first molecular phylogenies on polystomes were published in the early 2000s with Sinnappah et al. (2001), Bentz et al. (2001, 2006) and Verneau et al. (2002, 2009), providing a phylogenetic framework for discussion of both their evolution since their origin in the Paleozoic period and dispersal in more recent times. Though other publications contributed later to a better understanding of their evolution through time and space (Badets et al., 2011; Héritier et al., 2015), molecular phylogenies also proved to be an essential tool to the taxonomy of amphibian polystomes (Berthier et al., 2014; Chaabane et al., 2019; Du Preez et al., 2007, 2010, 2014; Fan et al., 2020; Landman et al., 2018, 2021; Raharivoloniaina et al., 2011; Yildirirhan et al., 2012) as well as chelonian polystomes (Du Preez et al., 2017; Dutton et al., 2021; Héritier et al., 2018).

Whereas phylogenetic and genetic studies provided an essential source of information in species delimitation of polystomes, it also helped in the revision of their systematics, more particularly for polystomes infecting freshwater turtles. Five new polystome genera have been described from freshwater turtles during the past ten years, namely *Uropolystomoides* Tinsley and Tinsley, 2016, *Uteropolystomoides* Tinsley, 2017, *Apaloneotrema* Du Preez and Verneau, 2020, *Aussietrema* Du Preez and Verneau, 2020 and *Fornixtrema* Du Preez and Verneau, 2020, however, none of them derived from the discovery of new species. *Uropolystomoides* was created to accommodate a particular clade of *Polystomoides* Ward (1917) occurring in the urinary bladder only and differing from all *Polystomoides* species of the pharyngeal cavity by the size of hamulus 1 - which is greater than the sucker diameter (Tinsley and Tinsley, 2016). *Uteropolystomoides* was created to accommodate *Polystomoides nelsoni* Du Preez and Van Rooyen, 2015 which was the single species of *Polystomoides* with a uterus holding numerous eggs and a massive genital bulb with more than 120 genital spines (Tinsley, 2017). Finally, *Apaloneotrema*, *Aussietrema* and *Fornixtrema* were created to accommodate three distinct lineages of *Neopolystoma* Price, 1939 species which all infect the conjunctival sacs of their host, but differ from each other mainly by the shape of the egg, i.e. a large fusiform egg with rounded tips for *Apaloneotrema*, a spherical egg for *Aussietrema* and a fusiform to diamond-shaped egg with acute tips for *Fornixtrema* which also has an egg-cell maturation chamber (Du Preez and Verneau, 2020).

According to Du Preez and Verneau (2020), *Neopolystoma* still represents a polyphyletic taxon with some species nested within *Polystomoides* and others being closely related to *Aussietrema*. With regard to the distribution of modern freshwater turtles, pleurodires are restricted to southern continents, i.e. across the Australian, Ethiopian and Neotropical Realms, while cryptodires are distributed worldwide with the exception of Australia (Rhodin et al., 2021). According to Pereira et al. (2017), the breakup of Pangaea drove the divergence between cryptodires and pleurodires. Whereas the biogeographic history of cryptodires is assumed to be complicated by the complex paleogeographic history of Laurasia, the biogeographic history of pleurodires was shown to be tightly related to the paleogeographical history of the Gondwana (Pereira et al., 2017). Though Ferreira et al. (2018) concluded that the current distribution of pleurodires could not be fully explained using vicariance or extinctions as sole explanations, they showed that the divergence time between South American and Australian Chelidae should be pushed back to the end of the Early Cretaceous

as some kind of barrier prevented any dispersal of chelids. While Australian pleurodires might have been isolated from all other freshwater turtles at least 100 Million years ago (Mya), ancestral trionychids (Cryptodira) would have dispersed to Australia in the Miocene (Pereira et al., 2017). This would have left ample time for polystomes of Australian pleurodires to diverge from all other polystome lineages and accumulate morphological changes. This agrees with the molecular dating proposed by Héritier et al. (2015) for chelonian polystomes that indicated a separation of about 98 Mya with a 95% confidence interval of 66–136 Mya between the Australian polystome lineage, including *Aussietrema* and species of Australian *Neopolystoma* infecting specifically pleurodires, and its sister polystome lineage including species of *Polystomoides* and *Neopolystoma*, both infecting cryptodires. By reanalyzing morphological types of several species of *Aussietrema*, *Neopolystoma* and *Uropolystomoides* of the Australian Realm on the one hand, followed by morphological comparisons with types of other pleurodiran and cryptodiran polystome species of the genera *Polystomoides*, *Neopolystoma* and *Uropolystomoides* on the other, we might expect to find morphological characters (synapomorphies) that delimitate a new genus within polystomes of pleurodires - at least in Australia.

The objectives of the present study were thus to focus on as many species as possible of the genera *Apaloneotrema*, *Aussietrema*, *Fornixtrema*, *Neopolystoma*, *Polystomoides*, *Polystomoidella*, *Uropolystomoides* and *Uteropolystomoides* in order to identify these characters if they exist.

2. Material and methods

2.1. Polystome sampling

Observations for the present study were based on: (i) a representative loan of several Australian polystome specimens from the Queensland Museum, Australia, including *Aussietrema cribbi* (Pichelin, 1995), *Aussietrema queenslandensis* (Pichelin, 1995), *Aussietrema spratti* (Pichelin, 1995), *Aussietrema tinsleyi* (Pichelin, 1995), *Neopolystoma chelodinae* (MacCallum, 1918), *Neopolystoma kreffti* Rohde, 1984, *Neopolystoma maclayi* Rohde, 1984, *Neopolystoma novaeguinae* Fairfax, 1990, *Uropolystomoides australiensis* (Rohde and Pearson, 1980) and *Uropolystomoides scottae* (Pichelin, 1995); (ii) specimens of *Polystomoides brasiliensis* Vieira et al., 2008 examined at the Oswaldo Cruz Institute in Rio de Janeiro, Brazil and a subsequent loan of material from this museum; (iii) *Uteropolystomoides nelsoni* (Du Preez and Van Rooyen, 2015) from the Parasitic Worm Collection, National Museum, Bloemfontein, South Africa; (iv) specimens of *Neopolystoma cayensis* Du Preez et al. (2017), *N. chelodinae*, *Neopolystoma orbiculare* (Stunkard, 1916), *Polystomoidella whartoni* (Wright, 1879), *Polystomoides asiaticus* Rohde, 1965, *Polystomoides multifalx* (Stunkard, 1924), *Polystomoides scriptanus* Héritier et al. (2018), *Polystomoides soredensis* Héritier et al. (2018), *Uropolystomoides chabaudi* (Euzet and Combes, 1965), *Uropolystomoides malayi* (Rohde, 1963) and *U. nelsoni* from the collection of the authors at the North-West University in Potchefstroom, South Africa.

2.2. Specimen preparation and morphological structures of investigation

Fixed specimens from the authors' collection were rinsed in water, stained with acetocarmine, dehydrated, cleared in xylene and mounted in Canada balsam. Parasites were examined for morphological features including body size, position and size of the vaginae, genital spine number, shape of haptor and suckers and shape and size of hamuli, when present. Morphological features were photographed using a Nikon AZ100M microscope (Nikon, Netherlands) fitted with a 0.5X, 1X and 4X objectives as well as a Nikon U3 digital camera. Measurements were captured using the Nikon NIS software. Small structures were examined, measured and photographed using a Zeiss Imager Axio10 compound microscope (Zeiss, Germany) fitted with a Zeiss Axio cam 305 camera (Zeiss, Germany) and Zeiss Zen Blue elements (Zeiss, Germany) software program. Finally, type drawings for all recorded chelonian polystome

species (see Morrison and Du Preez, 2011) were also investigated for their morphology, genital spine number and intervaginal distances.

3. Results

3.1. Position and size of the vaginae

All polystomes infecting the oral region, urinary bladder or the conjunctival sacs of pleurodires have latero-ventral vaginae. These include species of the Australian Realm belonging to the genera *Aussietrema*, *Neopolystoma* (A in Fig. 1) and *Uropolystomoides* (B in Fig. 1), species of the Neotropical Realm of the genus *Polystomoides* infecting pleurodires (C in Fig. 1) and species of the Ethiopian Realm of the genus

Uropolystomoides infecting pleurodires. On the contrary, with a few exceptions - *Apaloneotrema moleri* (Du Preez and Morrison, 2012) and *Uropolystomoides* spp. - all other polystomes infecting the oral cavity, the urinary bladder or the conjunctival sacs of cryptodires, show peripheral vaginae. These include species of the Nearctic, Neotropical, Oriental and Palearctic Realms belonging to the genera *Fornixtrema*, *Neopolystoma* and *Polystomoides*, but also *U. nelsoni* from the Nearctic Realm. *Apaloneotrema moleri* and all species of the genus *Uropolystomoides* show opposite, near-peripheral vaginae. The vaginae for *A. moleri*, which were reported as latero-ventral in Du Preez and Verneau (2020), are actually near peripheral and lateral to the intestinal caeca, unlike all polystomes from pleurodires where the vaginae are more medial. Furthermore, the vaginae for *A. moleri* are pre-ovarian and located at a position about one third from the anterior tip (Du Preez and Verneau, 2020), whereas they are post-ovarian at the level of the anterior margin of the testis for all other chelonian polystomes.

When the inter-vaginal distance is expressed as a percentage of the body width at the level of the vagina, it was determined as 85.5 (76.4–96.6) for *Aussietrema* infecting pleurodires (A in Fig. 2); 62.1 (57.0–64.8) for *Neopolystoma* infecting pleurodires (B in Fig. 2); 70.8 (61.3–75.9) for *Polystomoides* infecting pleurodires (C in Fig. 2); 67.2 (61.5–72.9) for *Uropolystomoides* infecting pleurodires of the Australian Realm (D in Fig. 2); 73.3 (68.9–79.7) for *Uropolystomoides* infecting pleurodires of the Ethiopian Realm (E in Fig. 2); 98.3 (96.7–100) for *Fornixtrema* infecting cryptodires (F in Fig. 2); 97.8 (93.3–99.3) for *Neopolystoma* infecting cryptodires (G in Fig. 2); 94.7 (84.2–99.4) for *Polystomoides* infecting cryptodires (H in Fig. 2); 87.8 (75.0–98.4) for *Uropolystomoides* infecting cryptodires (I in Fig. 2); 96.0 for *A. moleri* (J in Figs. 2) and 99 for *U. nelsoni* (K in Fig. 2), both infecting cryptodire hosts.

3.2. Genital spine number

The genital spine number is 30 (23–50) for *Aussietrema* infecting pleurodires (A in Fig. 3); 21 (12–33) for *Neopolystoma* infecting pleurodires (B in Fig. 3); 6 (2–8) for *Polystomoides* infecting pleurodires (C in Fig. 3); 75 (73–78) for *Uropolystomoides* infecting pleurodires of the Australian Realm (D in Fig. 3); 33 (27–37) for *Uropolystomoides* infecting pleurodires of the Ethiopian Realm (E in Fig. 3); 9 (7–16) for *Fornixtrema*

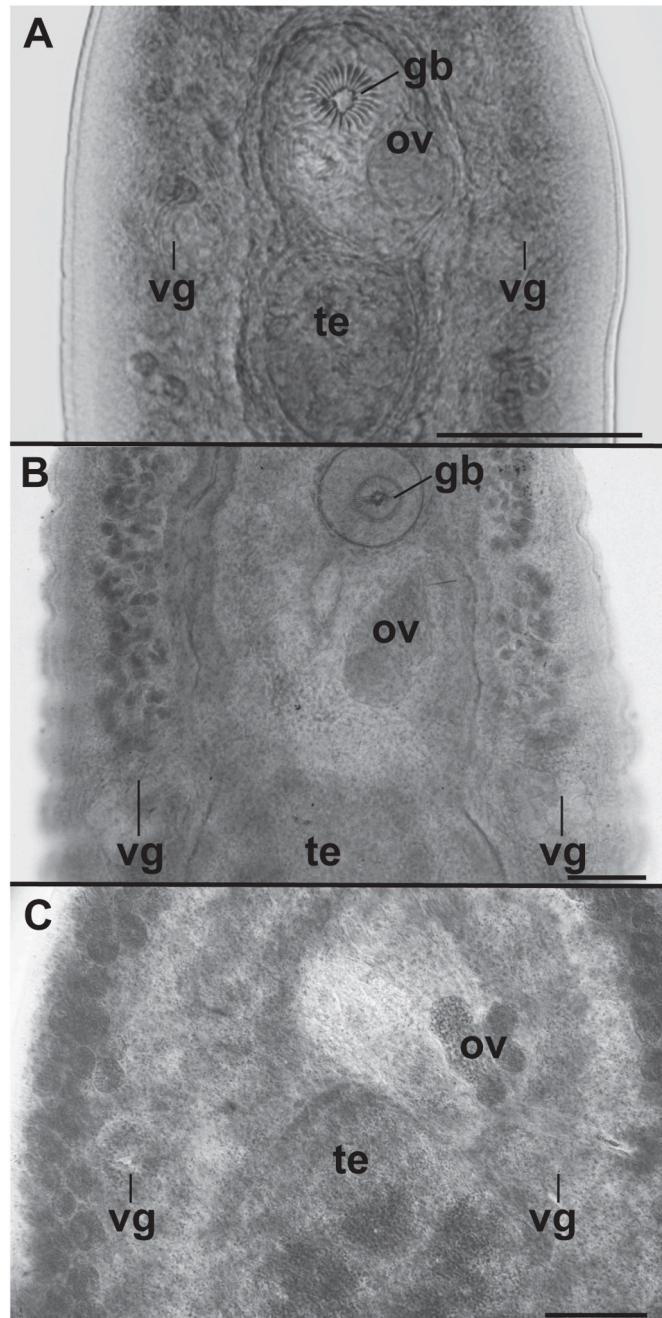


Fig. 1. Lateroventral vaginae as observed for *Neopolystoma kreffti* (A), *Uropolystomoides scottae* (B) and *Polystomoides brasiliensis* (C). Annotations: gb, genital bulb; ov, ovary; te, testis; vg, vaginae. Scale bar = 200 µm.

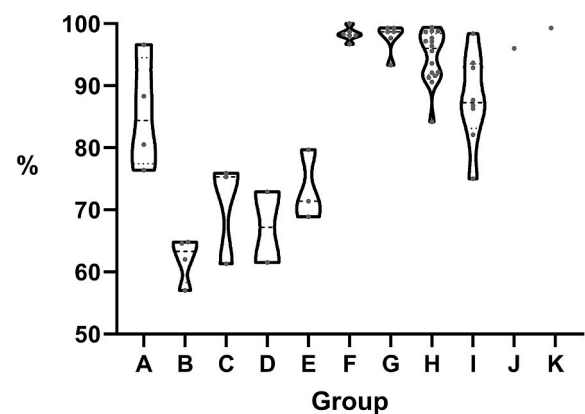


Fig. 2. Violin plot of inter-vaginal distance as % of the body width at the level of the vagina. The plot visualises the probability density of the data at different values. Within each violin is a marker indicating the median of the data as well as marker indicating the interquartile range. A = *Aussietrema* infecting pleurodires; B = *Neopolystoma* infecting pleurodires; C = *Polystomoides* infecting pleurodires; D = *Uropolystomoides* infecting pleurodires of the Australian Realm; E = *Uropolystomoides* infecting pleurodires of the Ethiopian Realm; F = *Fornixtrema* infecting cryptodires; G = *Neopolystoma* infecting cryptodires; H = *Polystomoides* infecting cryptodires; I = *Uropolystomoides* infecting cryptodires; J = *Apaloneotrema moleri* infecting a cryptodire host. K = *Uteropolystomoides nelsoni* infecting a cryptodire host.

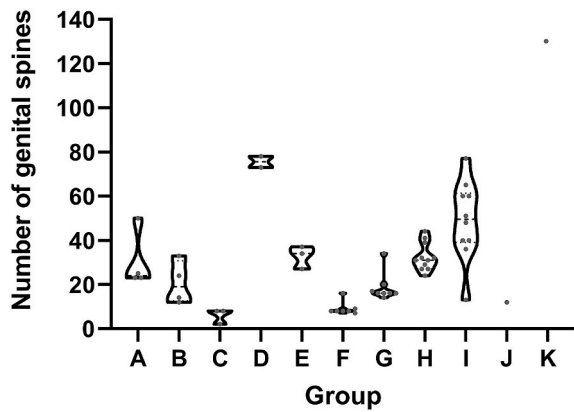


Fig. 3. Violin plot of the genital spines number.

A = *Aussietrema* infecting pleurodires; B = *Neopolystoma* infecting pleurodires; C = *Polystomoides* infecting pleurodires; D = *Uropolystomoides* infecting pleurodires of the Australian Realm; E = *Uropolystomoides* infecting pleurodires of the Ethiopian Realm; F = *Fornixtrema* infecting cryptodires; G = *Neopolystoma* infecting cryptodires; G = *Polystomoides* infecting cryptodires; I = *Uropolystomoides* infecting cryptodires; J = *Apaloneotrema moleri* infecting a cryptodire host. K = *Uteropolystomoides nelsoni* infecting a cryptodire host.

infecting cryptodires (F in Fig. 3); 19 (14–34) for *Neopolystoma* infecting cryptodires (G in Fig. 3); 33 (24–44) for *Polystomoides* infecting cryptodires (H in Fig. 3); 49 (13–77) for *Uropolystomoides* infecting cryptodires (I in Fig. 3); 12–13 for *A. moleri* (J in Figs. 3) and 130 (123–136) for *U. nelsoni* (K in Fig. 3), both infecting cryptodire hosts.

3.3. Shape of haptor and suckers

The three *Polystomoides* species known to infect South American pleurodires are the sole polystomes with deep incisions between suckers, giving the impression of a hand with fingers or that of a colony of polyps on narrow stalks. The incisions stretch about halfway to the centre of the hamulus in *P. brasiliensis* with suckers that are directed ventro-laterally to laterally (Fig. 4). Within the suckers of *P. brasiliensis* are some skeletal elements which are different from all other known *Polystomoides* species and polystomes in general. The ring of skeletal blocks, present in *Polystomoides*, appears to be absent and the skeletal funnel is small (Fig. 5). The walls of the suckers appear to have elongated spatulate sclerites

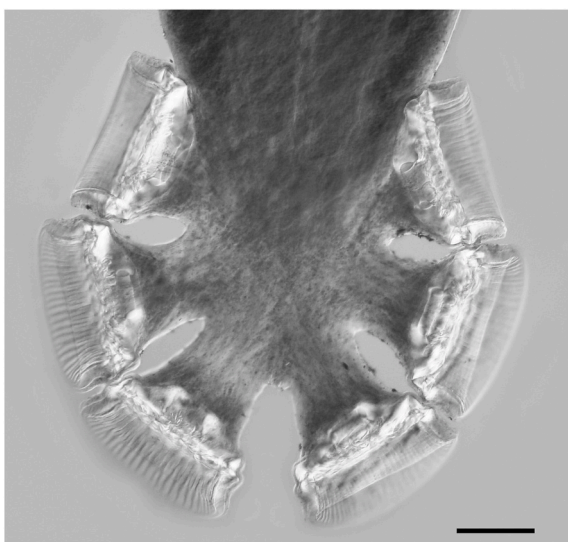


Fig. 4. Haptor of *Polystomoides brasiliensis* showing the deep incisions between suckers. Scale bar = 200 μ m.

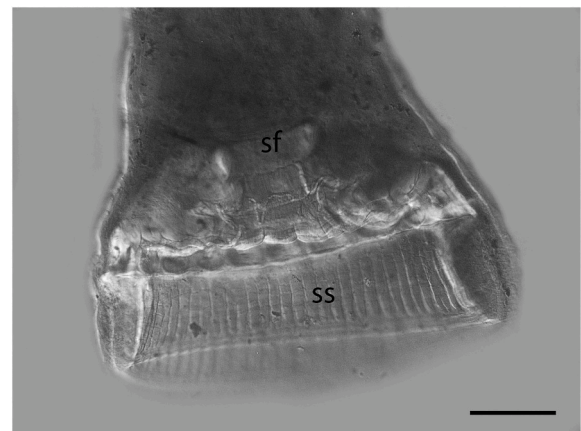


Fig. 5. Skeletal elements inside the sucker of *Polystomoides brasiliensis*. Annotations: sf, skeletal funnel; ss, spatulate sclerites. Scale bar = 100 μ m.

(Fig. 5).

3.4. Shape and size of hamuli

Whereas the majority of *Polystomoides* spp. infecting cryptodires have hamuli in excess of 100 μ m long (Fig. 6A–F), those infecting pleurodires have small hamuli ranging from 52 μ m in *Polystomoides uruguayensis* Mané-Garzon and Gil, 1961 to 72 μ m in *P. brasiliensis* (Fig. 6G–I). Furthermore, *Polystomoides* spp. of cryptodires mostly have broad hamuli with either no cut or a very small cut, between handle and blade, while *Polystomoides* spp. of pleurodires have a very deep cut leaving both the handle and the guard as long thin structures (Fig. 6G–I).

4. Discussion

4.1. Value of the morphological characters under investigation for the systematic revision of chelonian polystomes

All polystomes infecting pleurodires show latero-ventral vaginae whereas polystomes infecting cryptodires, have peripheral or near-peripheral vaginae. *Apaloneotrema moleri* is unique in turtle polystomes in that its vaginae are in pre-ovarian positions. Concerning the inter-vaginal distance, when expressed as a percentage of the body width at the level of the vagina, there is no overlap between polystome species infecting pleurodires and those from cryptodires (Fig. 2), except for *Aussietrema*, but, also for species of *Uropolystomoides* from the Ethiopian Realm whose inter-vaginal distance overlaps with that of *Uropolystomoides* spp. infecting pleurodires of the Australian Realm on the one hand and with that of *Uropolystomoides* spp. infecting cryptodires on the other. Though the position of the vaginae is of limited interest for species delimitation, it provides a valuable and consistent character to separate species of *Neopolystoma* and *Polystomoides* infecting pleurodires of the Australian and Neotropical Realms, respectively, from all others infecting cryptodires.

Although the number of genital spines may be a useful character for distinguishing distinct species, it is not often of much use for genus delimitation (Fig. 3) except for *Uteropolystomoides* that shows 100 or more genital spines (Tinsley, 2017). However, the fewer than 10 genital spines reported for these species, separates this group of species from all other chelonian polystome genera. The reporting of only two genital spines for *Polystomoides fuquesi* Mané-Garzon and Gil (1962) was surprising, but has been noticed from a sample of 28 specimens (Mané-Garzon and Gil, 1962). However, the fact that both *P. fuquesi* and *P. uruguayensis* were described from the same host species in the same geographical area requires further investigation to validate the syntopic occurrence of *P. fuquesi* and *P. uruguayensis*. As a consequence, the

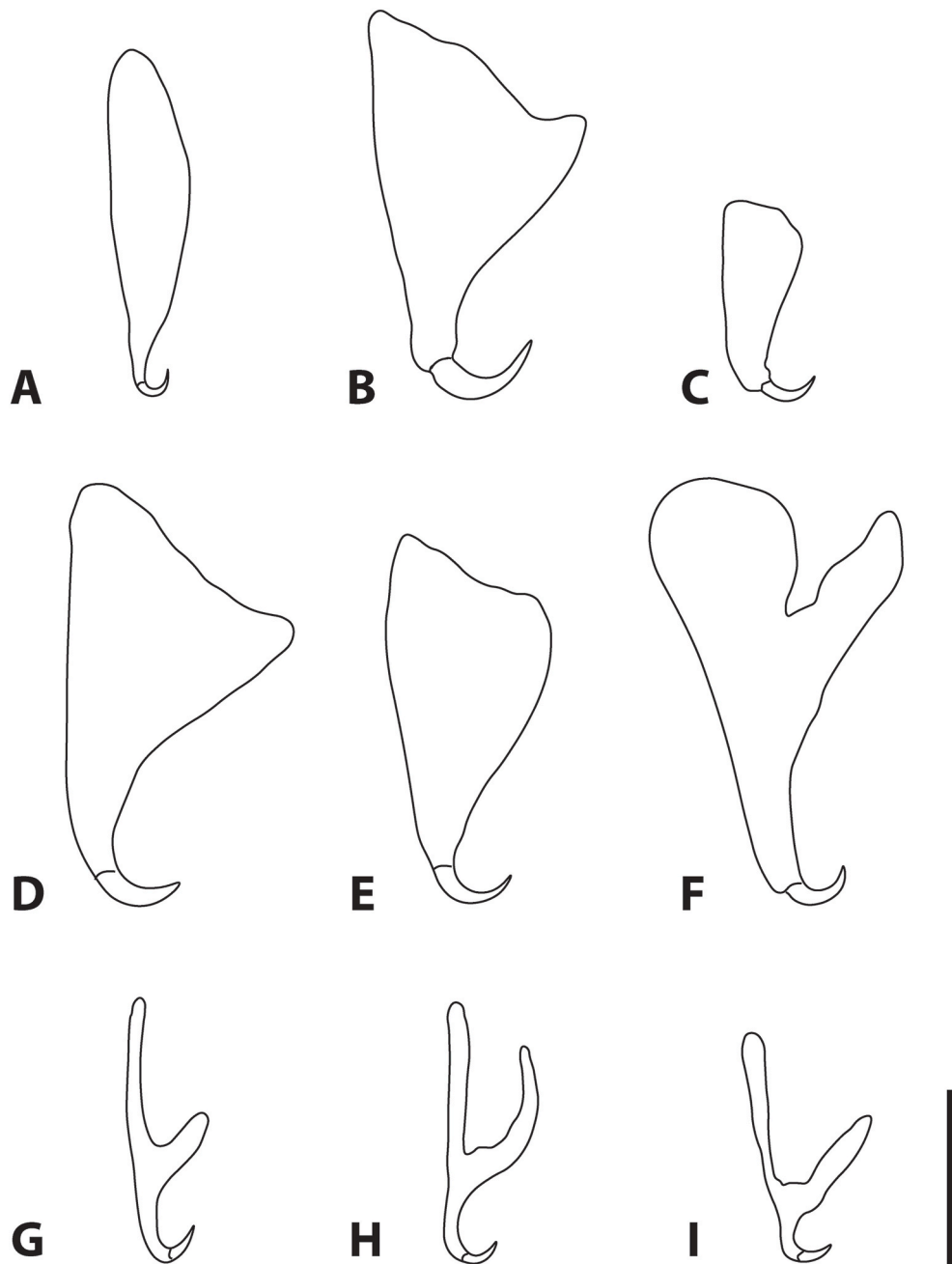


Fig. 6. Hamuli of *Polystomoides* spp. A = *Polystomoides japonicum* Ozaki, 1935; B = *Polystomoides magdalenensis* Lenis and Garcia-Prieto, 2009; C = *Polystomoides ocellatum* (Rudolphi, 1819); D = *Polystomoides pauli* (Timmers and Lewis, 1979); E = *Polystomoides platynotae* Combes and Rohde, 1979; F = *Polystomoides rohdei*; G = *Polystomoides brasiliensis*; H = *Polystomoides fuquesi* Mané-Garzon and Gil, 1962; I = *Polystomoides uruguayensis* Mané-Garzon and Gil, 1961. Scale bar = 50 μ m.

genital spine number appears to be a valuable taxonomic character to separate *Polystomoides* spp. infecting South American pleurodires from all other polystomes.

In mature polystomes, the haptor is, as a rule, a rigid discoid structure which is usually dorso-ventrally flattened with a greater width than length. The deep incisions between the suckers of *Polystomoides* spp. infecting South American pleurodires thus provides a reliable character for these species, distinguishing them from all other polystomes. However, *Polystomoides digitatum* (MacCallum, 1918), described at the New York Aquarium from the softshell turtle *Apalone spinifera* (Lesueur, 1827) is currently considered as a junior synonym of *Polystomoides coronatum* (Leydi, 1888) (Price, 1939). This species has a similar haptor with deep cuts between the suckers judging from the species drawing of the syntype. However, two syntypes examined and photographed in

2015 by one of us at the US Parasite Collection in Baltimore, did not show deep cuts between suckers. The fact that this species has been described from an aquarium where turtles of different species and origins are often kept together in confined spaces, suggests a possibility of a lateral transfer from another host (see Verneau et al., 2011). However, this species shows peripheral vaginae in line with polystomes of cryptodires. Therefore, polystome specimens of the oral and nasal cavities of *A. spinifera* need to be located in the wild to resolve the systematic status of *P. digitatum*.

The shape and morphology of polystome suckers vary from soft, simple cups to complex structures with elaborate skeletal elements. Du Preez and Theunissen (2021) studied sucker morphology and proposed a classification system with four types of suckers. All polystomes of chelonian hosts have Type III suckers sensu Du Preez and Theunissen

(2021) which entail an elaborate system of skeletal elements aiding in securing a firm grip on the host tissue. While *Neopolystoma* spp. of the Australian Realm have typical Type III suckers with a skeletal ring and a prominent skeletal funnel, the suckers of South American *Polystomoides* spp. infecting pleurodires are more delicate and with their spatulate sclerites resembling Type IV suckers sensu Du Preez and Theunissen (2021) reported from *Concinocotyla* Pichelin, Whittington and Pearson, 1991 (see Du Preez and Theunissen, 2021).

The delicate hamuli, with very deep cuts between the handle and the guard, characterize only *Polystomoides* spp. of South American pleurodires (Fig. 6G–I). Although, a small cut or incision exists between the handle and the guard within hamuli of *Polystomoides rohdei* Mané-Garzon and Holman-Spector, 1968, which infects a cryptodire host from the Neotropical Realm, its hamuli are clearly distinct from polystomes infecting pleurodires (Fig. 6F). In the remaining *Polystomoides* spp. infecting cryptodires (Fig. 6A–E), the hamulus blade is a solid structure without any incision, enabling this character to also be used to separate *Polystomoides* spp. of pleurodires from all other polystomes.

4.2. Taxonomy

In the light of the morphological evidence presented above, we propose two new genera for the polystomes infecting pleurodires of the Australian and Neotropical Realms, respectively.

4.2.1. Genus: *Pleurodirotrema* n. g

4.2.1.1. Generic characteristics. Polystomatidae. Polystomoidinae. Mouth with false oral sucker subterminal. Muscular pharynx. Intestinal caeca extending full length of body proper, not confluent posteriorly. Testis single, compact equatorial. Ovary pretesticular, small. Vitellaria throughout most of body proper, not extending into haptor. Vaginae latero-ventral in line with anterior margin of testis. Uterus absent. Haptor without hamuli. Skeletal elements in suckers arranged as ring of blocks. Sucker type III. Parasitic in urinary bladder and oral region of pleurodires of Australian Realm.

4.2.1.2. Taxonomic summary. *Ethymology:* Refers to the turtle sub-order Pleurodira.

Type species: *Pleurodirotrema chelodinae* (MacCallum, 1918) n. comb.

Synonyms: *Polystoma chelodinae* MacCallum, 1918; *Neopolystoma chelodinae* Price, 1939.

Site of infection: urinary bladder.

Other species: *Pleurodirotrema krefftii* (Rohde, 1984) n. comb. *Synonym:* *Neopolystoma krefftii* Rohde, 1984. *Site of infection:* oral cavity; *Pleurodirotrema macleayi* (Rohde, 1984) n. comb. *Synonym:* *Neopolystoma macleayi* Rohde, 1984. *Site of infection:* urinary bladder; *Pleurodirotrema novaeguineae* (Fairfax, 1990) n. comb. *Synonym:* *Neopolystoma novaeguineae* Fairfax, 1990. *Site of infection:* oral cavity.

4.2.2. Genus: *Manotrema* n. g

4.2.2.1. Generic characteristics. Polystomatidae. Polystomoidinae. Mouth with false oral sucker subterminal. Muscular pharynx. Intestinal caeca extending full length of body proper, not confluent posteriorly. Testis single, compact equatorial. Ovary pretesticular, small. Vitellaria throughout most of body proper, not extending into haptor. Vaginae latero-ventral in line with anterior margin of testis. Uterus absent. Haptor with deep incisions between suckers, which appear fingerlike. Skeletal elements in suckers not arranged as a ring of blocks but rather thin spatulate outward bending needles. Sucker type IV. Two small pairs of hamuli present with very deep cuts between handle and guard. Parasitic in urinary bladder of pleurodires of the Neotropical Realm.

4.2.2.2. Taxonomic summary. *Ethymology:* The haptor with deep cuts

between the suckers resembles a hand with fingers.

Type species: *Manotrema uruguayensis* (Mané-Garzon and Gil, 1961) n. comb. *Synonym:* *Polystomoides uruguayensis* Mané-Garzon and Gil, 1961. *Site of infection:* urinary bladder.

Other species: *Manotrema fuquesi* (Mané-Garzon and Gil, 1962) n. comb. *Synonym:* *Polystomoides fuquesi* Mané-Garzon and Gil, 1962. *Site of infection:* urinary bladder; *Manotrema brasiliensis* (Viera, Novelli, Sousa and SouzaLima, 2008) n. comb. *Synonym:* *Polystomoides brasiliensis* Viera, Novelli, Sousa and SouzaLima, 2008. *Site of infection:* urinary bladder.

4.3. What does sucker morphology within *Manotrema* n. g. suggest about functionality?

The deep cuts and the outwards directed suckers resemble the branchial generation of two polystome genera infecting anuran hosts, namely *Polystoma* Zeder, 1800 and *Metapolystoma* Combes, 1976. When an oncomiracidium of these polystomes establishes on the gills of a young tadpole, well before metamorphosis, it develops rapidly and produces eggs in a matter of 16 days (Kok and Du Preez, 1989; Du Preez and Kok, 1998). This parasite, which is usually considered as a neotenic form, attaches to the branchial filaments inside the branchial chamber of the tadpole. Its haptor shows deep incisions between suckers, increasing flexibility of the haptor. Furthermore, suckers are not directed ventrally like in mature parasites found in the bladder of their host, but ventro-laterally, making attaching to gill filaments within a tightly packed gill chamber challenging. Therefore, one could argue that the deep cuts between the suckers in *Manotrema* n. g. may also provide additional flexibility to the suckers. However, their functional adaptations require further investigation.

4.4. The impact of chelonian evolution on polystome systematics

Following revision of the chelonian polystome classification, we can now consider 10 genera, among which *Apaloneotrema*, *Fornixtrema*, *Neopolystoma*, *Polystomoidella*, *Polystomoides* and *Uteropolystomoides* that infect cryptodires, *Aussietrema*, *Manotrema* n. g. and *Pleurodirotrema* n. g. that specifically infect pleurodires and *Uropolystomoides* that infects both cryptodires and pleurodires. With *Aussietrema* and *Pleurodirotrema* n. g. - both infecting chelids of the Australian Realm - and *Manotrema* n. g. infecting chelids of the Neotropical Realm, one may wonder about the origin of these genera. Is there any relationship between parasite speciation and plate tectonics, as was shown for their hosts following biogeographic vicariance (Pereira et al., 2017)? Though it was previously documented that *Aussietrema* and *Pleurodirotrema* n. g. (initially considered as *Neopolystoma*) form a solid monophyletic group (See Du Preez and Verneau, 2020), a hypothesis that results from biogeographic vicariance holds true only if that clade is a sister group of *Manotrema* n. g., which at this stage cannot be formally demonstrated.

Uropolystomoides currently includes polystomes of both cryptodire and pleurodire turtles. Those infecting pleurodires are found in the Pelomedusidae of the Ethiopian Realm as well as the Chelidae of the Australian Realm. Although the size of hamulus 1 for all species of *Uropolystomoides* is greater than the sucker diameter, i.e. a unique characteristic for this genus (Tinsley and Tinsley, 2016), all *Uropolystomoides* species infecting pleurodires differ from *Uropolystomoides* spp. infecting cryptodires in shape and size of their vaginae. Moreover, *Uropolystomoides* spp. of the Australian Realm also differ from their congeners of the Ethiopian Realm by several characteristics such as the body size, the sucker diameter and the genital spine number (see Morrison and Du Preez, 2011). As a result, a more thorough analysis of the phylogenetic relationships of the Polystomatidae, including several representatives of the genera *Aussietrema*, *Pleurodirotrema* n. g. and *Uropolystomoides* from the Australian Realm as well as species of *Manotrema* n. g., will assist in establishing the origins of polystomes infecting pleurodires of the Australian realm.

Declaration of interest

None.

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