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Doodytrema carettochelydis n. gen., n. sp., (Digenea: Microscaphidiidae) from the Pig-Nosed Turtle, *Carettochelys insculpta*, (Cryptodira: Carettochelydidae) in Australia

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ABSTRACT: *Doodytrema carettochelydis* n. gen., n. sp. (Digenea: Microscaphidiidae) is described from the pig-nosed turtle, *Carettochelys insculpta* (Cryptodira: Carettochelydidae) from the Daly River, Northern Territory, Australia. *Doodytrema* n. gen. is differentiated from other microscaphidiid genera by the absence of pharyngeal diverticula and the organization of vitellarium, namely the absence of an intercecal vitelline field or vitelline fields that merge behind the ovary. The uterus of *Doodytrema carettochelydis* n. sp. differs dramatically from other microscaphidiids in that the uterus does not run between the testes, but instead passes dorsal to them. This is only the second microscaphidiid species from Australian freshwater turtles.

KEY WORDS: Platyhelminthes, Digenea, Microscaphidiidae, *Doodytrema carettochelydis*, *Carettochelys insculpta*, turtle, parasite, Northern Territory, Australia.

Microscaphidiid digeneans are common parasites of marine turtles in Australian waters (Blair, 1986, 1987; Pichelin et al., 1999) but until recently were not reported from the freshwater turtles of Australia. Snyder and Tkach (2006) described *Paradeuterobaris victoriae* from red-faced turtles, *Emydura victoriae*, taken from the Victoria River, Northern Territory. A single member of this genus, *Paradeuterobaris novaguineae* Blair and Rose, 1986 had been described previously from the pig-nosed turtle, *Carettochelys insculpta*, from New Guinea. In the course of investigation of the turtle parasite fauna in Northern Territory, Australia, we found numerous microscaphidiid specimens in the intestine of several *C. insculpta*. These parasites can not be circumscribed within *Paradeuterobaris* but represent a novel genus.

The pig-nosed turtle, *C. insculpta*, is unique among Australian freshwater turtles, representing a monotypic family (Carettochelydidae) that is now found only in Australia and New Guinea but was once a diverse group distributed over much of Laurasia (Meylan, 1987). Thus, *C. insculpta* is the only hidden-neck (Cryptodira) Australian freshwater turtle and the only Australian turtle not of Gondwanan origin. Pig-nosed turtles are currently restricted to the Daly, Victoria and Alligator drainages of Northern Territory, Australia (Cogger, 2000). No endoparasites have been reported from these turtles in Australia and only 1 ectoparasite, a leech, has been found on this turtle (Samure and Doody, 2000). The current report is the first record of an endohelminth from *C. insculpta* in Australia.

MATERIAL AND METHODS

Four *Carettochelys insculpta* were collected by hand from the Daly River, Northern Territory, Australia, in June 2005, under a collecting permit from the Northern Territory Parks and Wildlife Commission. Several hundred specimens of a new digenean species belonging to Microscaphidiidae were recovered from these turtles. Living worms were rinsed in saline, briefly examined prior to fixation, killed with hot water and fixed in 70% ethanol. Specimens were stained with aqueous alum carmine or Harris' haematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted permanently in Damar balsam. Xylene proved to be the best clearing medium to use with this particular microscaphidiid species. Clove oil and other organic oils traditionally used as clearing agents for digeneans rendered the parenchyma opaque and unsuitable for microscopic examination. Twenty eggs from one specimen destroyed in the process were removed and measured.

Measurements were taken from a compound microscope using an ocular micrometer. Mean, standard deviation and coefficient of variation (CV) were calculated according to Steel and Torrie (1980). The CV is a percentage value of the ratio of the standard deviation to the mean of a particular metric character. Characters with lower CV have values that are more stable around the mean than those with higher CV.

Specimens used for SEM were dehydrated in a graded series of ethanol and dried using hexamethyldisilazane (Ted Pella, Inc., Redding, California) as a transition fluid. The specimens were mounted on stubs, coated with gold and examined using a Hitachi 4700 scanning electron microscope (Hitachi USA, Mountain View, California) at an accelerating voltage of 5–10 kV.

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Doodytrema n. gen.

Diagnosis

Platyhelminthes. Digenea. Microscaphidiidae. Body elongate, linguiform. Tegument unspined. Marginal bodies absent. Oral sucker absent, pharynx terminal, barrel-shaped with no posterior diverticula. Esophagus long, straight, with well developed muscular bulb at posterior end. Ceca long. Testes tandem, in middle third of body. Seminal vesicle extensively coiled, free in parenchyma posterior to intestinal bifurcation. Genital pore median, between pharynx and intestinal bifurcation, closer to pharynx. Ovary small, posttesticular, close to the end of ceca. Vitellarium in 2 lateral rows of large follicles overlapping ceca in posterior third of body. Intercecal vitelline follicles absent. Uterus intercecal, passing dorsal to testes. Excretory bladder small, excretory canals originate at lateral sides of bladder. Excretory opening dorsal, subterminal. Parasitic in the intestine of turtles.

Taxonomic summary

Type species: Doodytrema carettochelydis n. sp.

Etymology: The generic name refers to Dr. J. Sean Doody, an expert in the biology of pig-nosed turtles and the person who made collecting these turtles and parasites possible.

Doodytrema carettochelydis n. sp. (Figs. 1–7)

Description

Description based on 20 adult specimens. Measurements of holotype given in text; measurements of entire type series given in Table 1. All measurements in micrometers unless otherwise stated.

Body elongate, linguiform, nearly uniform in width, with narrow anterior end and somewhat more rounded posterior end. Body margins nearly parallel (Figs. 1–3). Body length 5.4 mm, body width at level of anterior testis 624. Body width 11.5% of body length. Tegument thin, unspined, densely covered with tiny papillae of apparently glandular nature (Figs. 4–7).

Oral sucker absent, pharynx terminal, barrel-shaped, 200 × 130, with deep cavity and without diverticula. Esophagus straight, 738 long, with muscular bulb, 75 × 80, at posterior end. Intestinal bifurcation 938 from anterior end of body. Ceca almost reach posterior end of body 610 (left), 610 (right) from posterior end and terminate at level of posterior margin of vitelline fields.

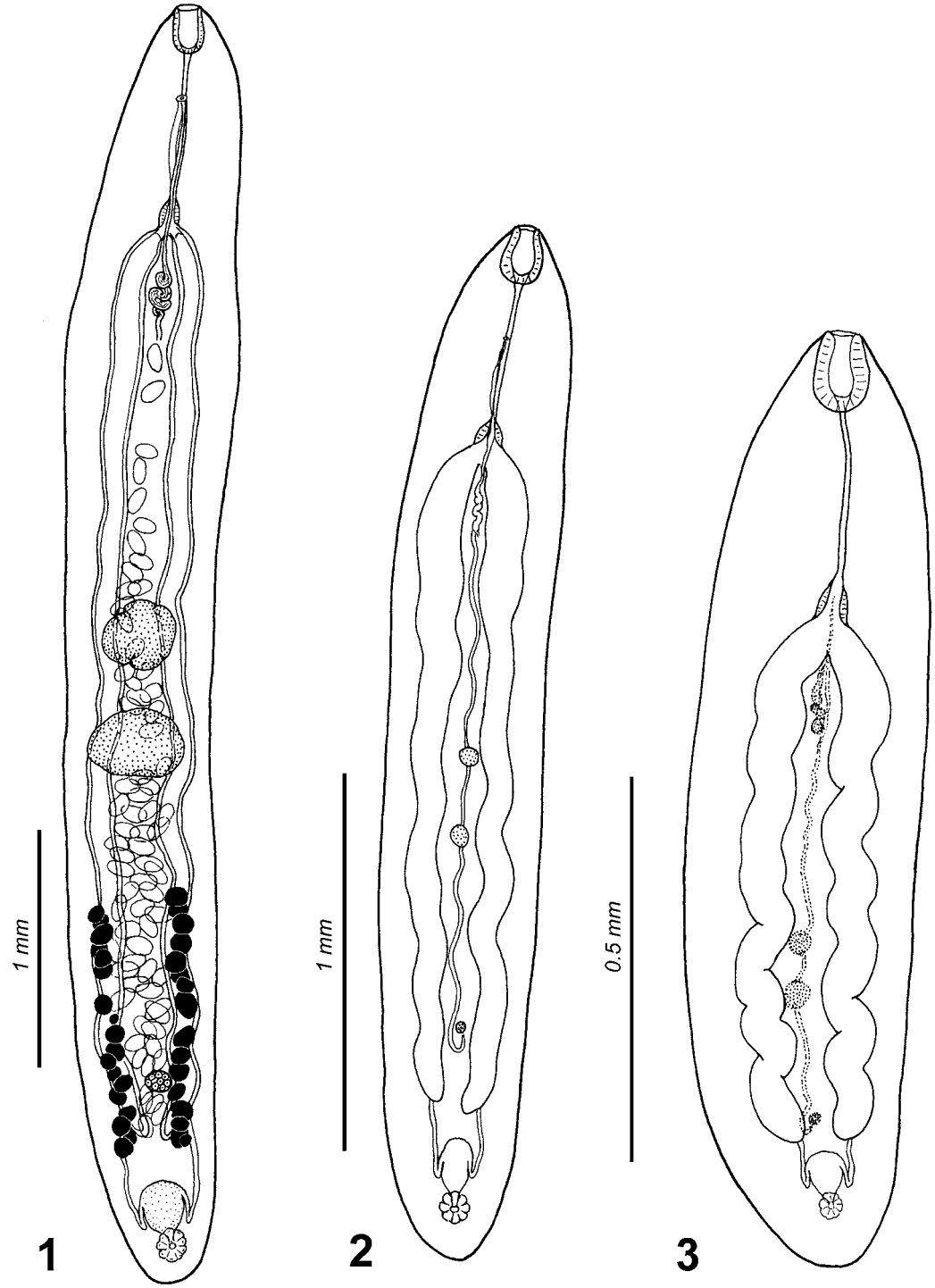
Testes 2, preovarian, in middle of body, tandem, usually slightly elongated transversally. Distance between testes 200. Anterior testis 260 × 330, posterior testis usually slightly larger, 270 × 430. Seminal vesicle long, extensively coiled, free in parenchyma posterior to intestinal bifurcation. Prostatic duct long, from approximately level of intestinal

Table 1. Metric data (measurements in micrometers) for *Doodytrema carettochelydis* n. sp.

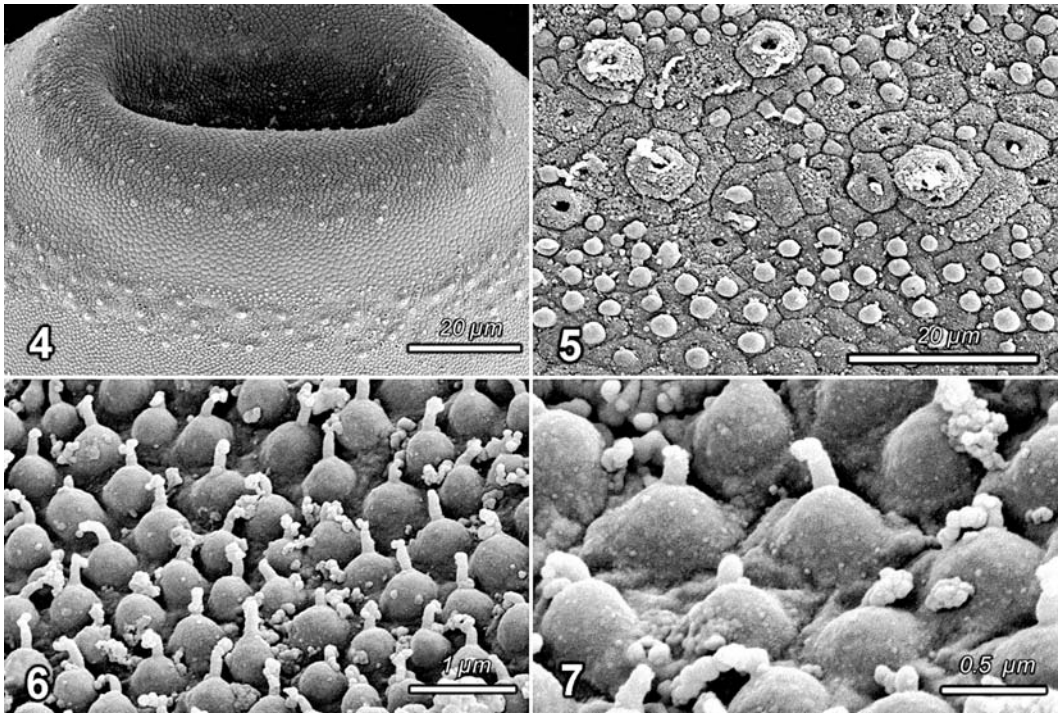
Characters	n	Min-Max	Mean	StD	CV*
Body length	20	4630–6490	5566.8	460.2	8.3
Body width†	20	550–870	674.2	89.5	13.3
Body width (as % of body length)	20	10–16	12.0	1.4	11.3
Pharynx length	20	180–230	201.0	14.1	7.0
Pharynx width	20	130–180	145.9	14.2	9.8
Esophagus length	20	570–830	741.9	62.2	8.4
Esophageal bulb length	20	70–95	80.8	8.0	9.9
Esophageal bulb width	20	65–85	74.0	6.6	8.9
Anterior end to intestinal bifurcation	20	750–1060	942.9	70.8	7.5
Left cecum from posterior end	20	580–980	733.5	110.4	15.0
Right cecum from posterior end	20	560–1020	750.5	125.6	16.7
Distance between testes	20	170–380	261	58.4	22.4
Anterior testis length	20	170–340	247.5	48.1	19.4
Anterior testis width	20	240–400	305.5	42.0	13.7
Posterior testis length	20	180–320	249.0	38.9	15.6
Posterior testis width	20	260–430	343.5	43.0	12.5
Anterior end to genital pore	20	375–470	422.5	19.4	4.6
Anterior end to genital pore as % body length	20	6.5–8.5	7.6	0.5	6.0
Ovary length	20	60–130	89.0	17.0	19.1
Ovary width	20	75–115	92.8	9.8	10.6
Ovary to posterior end	20	800–1280	1029.0	125.0	12.2
Ovary to posterior end as % of body length	20	15–21	18.0	1.4	7.8
Ovary to posterior testis	20	930–1420	1123.5	143.2	12.7
Left vitellarium to posterior end	20	550–1040	742.8	134.9	18.2
Left vitellarium length	20	760–1260	1027.4	134.4	13.1
Left vitellarium length as % of body length	20	15–23	18.5	2.1	11.1
Right vitellarium to posterior end	20	530–1210	767.0	177.5	23.1
Right vitellarium length	20	660–1370	1060.0	149.0	14.1
Number of excretory pore diverticula	19	7–9	7.8	0.9	8.1
Excretory pore to posterior end	20	140–250	184.5	26.1	14.1
Egg length (in situ)	19	100–130	117.0	6.1	5.2
Egg width (in situ)	19	55–75	63.0	6.4	10.2
Egg length (removed)	20	115–130	124.5	3.9	3.3
Egg width (removed)	20	65–80	73.5	3.2	4.5

* Coefficient of variation.

† Body width was measured at the level of the anterior testis.



Figures 1–3. *Doodytrema carettochelydis* n. gen., n. sp.: **1.** Holotype. **2, 3.** Juvenile specimens. Note that excretory canals originate at the lateral margins of the excretory bladder.



Figures 4–7. *Doodytrema carettochelydis* n. gen., n. sp.: **4, 5.** Anterior end. Note numerous papillae arranged in two wide circles around the oral opening (antero-ventral view). **6, 7.** Arrangement of tegumental papillae on ventral surface.

bifurcation to genital pore. Genital pore at 410 from anterior end of body, 7.6% of body length.

Ovary small, spherical or subspherical, 90 × 100, situated somewhat anterior to posterior ends of ceca, 800 from posterior end of body (14.8% of body length) and 1280 posterior of posterior testis. Mehlis' gland and ootype medial and slightly posterior to ovary. Uterus intercecal, first forming coils between ovary and posterior testis, then passing dorsal to testes and forming several coils between anterior testis and seminal vesicle. Weak, thin-walled metraterm begins posterior to seminal vesicle. Vitellarium consisting of large follicles aligned in 2 lateral rows usually overlapping ceca. Posterior ends of vitelline rows approximately at level of ends of ceca. Posterior end of left row at 550 from posterior body end and extending 1110 anteriorly. Left vitellarium 20.5% of body length. Posterior end of right row at 560 from posterior body end and extending 1040 anteriorly. Left vitellarium 19.3% of body length. Vitelline fields do not merge at any point. Uterine seminal receptacle present. Vitelline reservoir just posterior to ootype. Eggs (in situ) 100 × 55.

Excretory pore dorsal, subterminal, at 150 from posterior end of body, surrounded by excretory rosette of 8 diverticula. Excretory bladder small, sac-like. Main excretory canals originate at approximately mid-point of lateral sides of bladder. Canals first directed backward and then turn and run anteriorly giving lateral branches. Complete organization of excretory system impossible to determine in detail based on total mounts.

Taxonomic summary

Type host: Pig-nosed turtle *Carettochelys insculpta* (Ramsay, 1886) (Chelonia: Cryptodira: Carettochelydidae).

Type locality: Daly River, near Oolloo Crossing, Northern Territory, Australia, 14°00.31′S, 131°14.46′E.

Site of infection: Intestine.

Prevalence and intensity of infection: Four of 4 *C. insculpta* were infected with hundreds to thousands of worms each.

Specimens deposited: The type series consists of 20 fully mature specimens. Holotype: Queensland

Museum (QM) no. G 225664. Paratypes: QM nos. G 225665 to G 225673, Harold W. Manter Laboratory (HWML) nos. HWML48315 to HWML48324. Two juvenile worms deposited (HWML48325). Slides labeled identically: ex. *Carettochelys insculpta*, Daly River, Northern Territory, Australia, June 2005.

Etymology: The specific epithet refers to the turtle genus which seems to be a specific host for this parasite.

Remarks

Immature worms showed the general morphological characteristics of the adult parasites, including the position of developing gonads and uterine primordium (Figs. 2–3).

Examination of a large number of specimens reveals that the majority of metric characters such as body size and organ size are quite variable and, although informative, may not be adequate to differentiate additional species in this genus. However, qualitative features such as the relative position of organs were stable and may be more appropriate for future differentiation. The distance from the anterior end to the genital pore and the pharynx length were the most stable among metric characters, as revealed by the coefficient of variation (Table 1).

Based on general morphology the new species belongs to the Microscaphidiidae but cannot be placed confidently into any recognized genus or subfamily. Although *Doodytrema carettochelydis* n. sp. resembles some members of Deuterobariinae, particularly members of *Deuterobaris* and *Paradeuterobaris*, it lacks the distinctive ventral glands of Deuterobariinae. The overall body shape and position of the internal organs of the new species most closely resemble those of some Microscaphidiinae, in particular representatives of *Angiodictyum*. However, the new species differs from *Angiodictyum* in the absence of pharyngeal diverticula and an intracecal vitelline field. The new species also lacks the marginal bodies typical of many *Angiodictyum* species. A rather unique feature of the new species is that the uterus does not pass between the testes as it does in *Angiodictyum* and most other microscaphidiids; instead it passes dorsal to both testes. Additionally, the ovary in *Doodytrema carettochelydis* n. sp. is shifted considerably to the posterior when compared to members of *Angiodictyum*.

Based on these numerous differences, we establish a new genus *Doodytrema* with *Doodytrema carettochelydis* n. sp. as the type and only species.

DISCUSSION

Doodytrema carettochelydis n. sp. is only the second species of digenean reported from the pig-nosed turtle *C. insculpta*. We did not find any other digeneans in 4 *C. insculpta* examined as part of this study and a few Aspidogastrea were the only other helminths recovered. Several other turtle species examined from the Daly River harbored multiple helminth species, although none were infected with *Doodytrema carettochelydis* n. sp. During the dry season *C. insculpta* is herbivorous, primarily feeding on ribbon weed (*Vallisneria spiralis*) (Heaphy, 1990: unpublished dissertation, University of New South Wales, Sydney, Australia). This report is supported by our observations; all 4 *C. insculpta* had intestines filled with partially digested ribbon weed. Within this ribbon weed were thousands of *Doodytrema carettochelydis* n. sp., free in the intestinal lumen, and the shells of numerous unidentified prosobranch snails. A North American freshwater microscaphidiid, *Dictyangium chelydrae*, uses prosobranch snails as a first intermediate host with cercariae that leave the snail to encyst on nearby substrate (Lotz and Corkum, 1984). Approximately 100 snails were taken from ribbon weed in the Daly River and crushed but no larval digeneans were found. Future efforts to determine the life cycle of *Doodytrema carettochelydis* n. sp. should focus on a more exhaustive examination of snails and ribbon weed in the Daly River. *Elseya dentata*, the northern snapping turtle, also feeds heavily on ribbon weed in the Daly River (J. S. Doody, personal communication) and were infected with numerous helminths, but *Doodytrema carettochelydis* n. sp. were not recovered from 6 turtles examined.

Doodytrema carettochelydis n. sp. and *Paradeuterobaris victoriae* Snyder and Tkach, 2006 are the only 2 microscaphidiid species known from freshwater turtles in Australia. Microscaphidiids primarily are parasites of marine fishes and turtles (Yamaguti, 1971; Blair, 1986, 1987, 2005) with reports of freshwater representatives scattered about the globe. In freshwater turtles, *Paradeuterobaris novaguineae* Blair and Rose, 1986, was described from *Carettochelys insculpta* from New Guinea (Blair and Rose, 1986) and *Dictyangium chelydrae* Stunkard, 1943, was recovered from *Chelydra serpentina* in Louisiana (Stunkard, 1943). Two additional North American species, *Octangioides skrjabini* Price, 1937 and *Octangioides tlacotalpensis* Caballero, 1942, were reported from Mexico (Yamaguti, 1971). The remaining freshwater turtle microscaphidiids come from

South American turtles: *Podocnemitrema papillosus* Alho and Vicente, 1964 from *Podocnemis expansa* in Brazil (Thatcher, 1993) and *Neodeuterobaris pritchardae* Brooks, 1976, from *Podocnemis lewyana* in Colombia (Brooks, 1976).

The relatively high diversity of microscaphidiids in marine hosts when compared to freshwater hosts and the diverse morphologies and subfamilial affiliations of freshwater microscaphidiids (Stunkard, 1943; Yamaguti, 1971; Brooks, 1976; Blair and Rose, 1986; Thatcher, 1993; Blair, 2005) suggest that microscaphidiids have been acquired independently by freshwater fishes and turtles from ancestral microscaphidiids of marine fishes and turtles on numerous occasions. *Carettochelys insculpta* inhabits estuarine environments in New Guinea (Rose et al., 1982) and numerous other extant freshwater turtle species can be found occasionally in estuarine or other brackish habitats, habitats sporadically inhabited by various species of sea turtles (Ernst and Barbour, 1989). This proximity creates the potential for host switching to occur over evolutionary time.

One of the prominent morphological features of *Doodytrema carettochelydis* n. sp. is presence of numerous papillae on the surface of the tegument (Figs. 4–7). These structures are not readily observed using light microscopy and have not been reported previously in microscaphidiids. These structures might be misinterpreted as tiny spines, although SEM images suggest that they are glandular in nature because they produce some sort of secretion. Species in numerous microscaphidiid genera (*Angiodictyum*, *Deuterobaris*, *Hexangium*, *Microscaphidium*, *Neoctangium*, *Octangium*, *Polyangium*, *Polygorgyra*, *Pseudohexangium*) are reported to possess small tegumental spines (Blair, 2005). It is possible that upon examination with SEM some or all of these spines may be revealed to be papillae similar to those found in *Doodytrema carettochelydis* n. sp. In the new species both large and small papillae were present at the anterior end of the body (Figs. 4, 5). These papillae may have different functions from one another, however examination using transmission electron microscopy and/or cytochemical studies may be necessary to determine such functions.

As mentioned previously *Doodytrema carettochelydis* n. sp. does not conform to the diagnoses of any of the existing subfamilies of the Microscaphidiidae (Yamaguti, 1971; Blair, 1986, 1987). Among the features that distinguish *Doodytrema carettochelydis* n. sp. from typical Microscaphidiinae are the absence of pharyngeal diverticula and the organization of vitellarium, namely the absence of an intercecal vitelline field. The new species differs from Deuterobariinae in absence of ventral glands and arrangement of vitelline follicles in 2 straight rows that do not merge posteriorly. One of the most striking features that differentiates *Doodytrema carettochelydis* n. sp. from most microscaphidiids is that the uterus does not run between the testes, but instead passes dorsal to them. The new genus does share many features of both Microscaphidiinae and Deuterobariinae and probably merits the erection of a new subfamily. However, we consider establishing a new subfamily premature because interrelationships among microscaphidiid genera and the status of subfamilies are presently unclear (Blair, 2005). Molecular and morphological data should be used to construct a robust phylogeny that will clarify subfamilial affiliation within this interesting and diverse group of digeneans.

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