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VERSITA

Choanocotyle platti sp. nov. from the northern long-necked turtle, *Chelodina rugosa* (Pleurodira, Chelidae) in Australia

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Abstract

Choanocotyle platti sp. nov. (Digenea, Choanocotylidae) is described from the northern long-necked turtle, *Chelodina rugosa* (Pleurodira, Chelidae) from the Daly and Mary Rivers, Northern Territory, Australia. This is the fifth known member of *Choanocotyle*. *Choanocotyle platti* sp. nov. differs from *Choanocotyle nematoides* Jue Sue et Platt, 1998 and *Choanocotyle hobbsi* Platt et Tkach, 2003 by smaller body length, larger oral sucker, relatively greater distance between testes, and prepharynx with an infolded posterior region. In addition the new species does not have the looped cirrus sac characteristic of *Choanocotyle nematoides*. Comparison of sequences of 18S, ITS (ITS1, 5.8S, ITS2) and partial 28S regions of nuclear rDNA among all 3 species strongly supports the status of *Choanocotyle platti* sp. nov. as a new species.

Keywords

Digenea, Plagiorchiida, Choanocotylidae, *Choanocotyle platti* sp. nov., DNA sequences, 18S gene, ITS region, 28S gene, *Chelodina rugosa*, northern long-necked turtle, Northern Territory, Australia

Introduction

Choanocotylidae was erected by Jue Sue and Platt (1998) and currently circumscribes 5 species of elongated plagiorchiid digeneans of Australian freshwater turtles characterized by intriguingly complex oral suckers. *Choanocotyle elegans* and *C. nematoides* were reported from short-necked turtles (*Emydura macquarrii*) in southeast Queensland (Jue Sue and Platt 1998), *Auriculotrema lechneri* was described from short-necked turtles (*Emydura krefftii* and *Elseya latisternum*) in northeastern Queensland (Platt 2003), and *C. hobbsi* and *C. jusuei* were reported from long-necked turtles (*Chelodina oblonga*) in southwest Western Australia (Platt and Tkach 2003).

In 2004 and 2005 we collected a number of digenean species from 35 individuals of 5 species of turtles in Northern Territory, Australia as part of an examination of turtle parasite biodiversity. Several of these worms proved new to science (Snyder and Tkach 2006, 2007; Tkach and Snyder 2006, 2007; our unpublished data) and among them was a new species of *Choanocotyle*, described herein. This parasite represents the second fluke found in the northern long-necked turtle, *Chelodina rugosa*, and the first Choanocotylidae reported from Northern Territory.

Materials and methods

Five *Chelodina rugosa* were collected by hand from the Daly River, 1 *C. rugosa* was collected in a baited crab trap from a billabong on the Daly River Mango Farm near Nauiyu Community, and 2 *C. rugosa* were collected in a baited crab trap from the Mary River, Northern Territory, Australia. All collections proceeded under a permit from the Northern Territory Parks and Wildlife Commission. Approximately 15 specimens of a new digenean species belonging to *Choanocotyle* were recovered from 3 of these turtles. Living worms were rinsed in saline, briefly examined prior to fixation, killed with hot water and fixed in 70% ethanol. For morphological examination, specimens were stained with aqueous alum carmine, dehydrated in a graded ethanol series, cleared in clove oil, and mounted permanently in Damar balsam. Representative specimens have been deposited in the Queensland Museum (QM), Brisbane, Queensland, Australia and the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, USA. All measurements are in micrometers unless otherwise stated.

Measurements were taken from a compound microscope using an ocular micrometer. Mean, standard deviation and

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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.

Type and voucher specimens of all 4 previously described *Choanocotyle* species were examined from the collection of the Queensland Museum (QM), Brisbane, Queensland, Australia: *Choanocotyle nematoides* (G213991-G213993), *Choanocotyle elegans* (G213983-G213987), *Choanocotyle juesuei* (G219528-G219536) and *Choanocotyle hobbsi* (G219520-G219527).

Specimens used for scanning electron microscopy (SEM) were fixed in 70% ethanol, dehydrated in a graded series of ethanol and dried using hexamethyldisilazane (HMDS) (Ted Pella, Inc., Redding, California) as 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.

Genomic DNA for molecular analysis was isolated from single specimens of *C. platti* sp. nov. (*Chelodina rugosa*, Daly River, Northern Territories), *C. nematoides* (1 specimen from *Emydura macquarii*, MacCleay River, New South Wales and 1 specimen from *Emydura krefftii*, West Barratta Creek, Northern Queensland), *C. hobbsi* (*Chelodina oblonga*, Melaleuca Swamp on the campus of Murdoch University, Perth, WA) according to Tkach and Pawlowski (1999) or using Qiagen DNAeasy tissue kit (Qiagen Inc., Valencia, California) following the manufacturer's instructions. Three different fragments of the nuclear ribosomal ribonucleic acid (rRNA) were amplified by polymerase chain reaction (PCR) and sequenced: the nearly complete 18S gene, the fragment containing internal transcribed spacer 1 (ITS1), 5.8S gene, and ITS2, and an approximately 1,350 bp long fragment at the 5' end of the 28S gene. Primers Worm A (5'-GCGAATGGCTCATTAAATCAG-3') and Worm B (5'-ACGGAAACCTTGTTACGACT-3') (Littlewood and Olson 2001), were used to amplify 18S gene fragment. Primers ITSF (5'-CGCCCGTCGCTACTACCGATTG-3') and 1500R (5'-GCTATCCTGAGGAAACTTCG-3') were used to amplify a fragment spanning the whole internal transcribed spacer (ITS1 + 5.8S + ITS2) region and the 5' end of the 28S gene. PCR reactions were performed according to protocols described by Tkach *et al*. (2003). PCR primers and several internal primers were used in sequencing reactions (for sequences of internal primers see Littlewood and Olson 2001, Platt and Tkach 2003, Tkach and Snyder 2007).

PCR products were purified directly using Qiagen Qiaquick™ columns, cycle-sequenced using ABI BigDye™ chemistry, alcohol-precipitated, and run on an ABI Prism 3100™ automated capillary sequencer. Contiguous sequences were assembled using Sequencher™ (GeneCodes Corp., ver. 4.1.4) and submitted to GenBank under accession numbers: EU196355 (*C. platti* sp. nov.), EU196356 (*C. hobbsi*), EU196357 and EU196358 (*C. nematoides* from *Emydura krefftii*, Northern QLD), EU196359 and EU196360 (*C. nematoides* from *Emydura macquarii*, NSW). Sequences were manually aligned and compared using the BioEdit program, version 7.0.1 (Hall 1999).

Results

***Choanocotyle platti* sp. nov.** (Figs 1 and 2)

Description: Based on 11 adult specimens. Measurements of the holotype are given in the text; measurements of the entire type series are given in Table I.

Body elongate, 6510; body width at level of ventral sucker 410. Tegument heavily spinose anteriorly, decreasing posteriorly, with smallest spines sparsely distributed at posterior end. Oral sucker very wide, 1020, incised ventrally, much wider than forebody when fully expanded; shape variable, occasionally funnel-shaped when retracted. Oral sucker surface aspinose. Prepharynx 280 long. Posterior part of oesophagus folded in all specimens, looks like a pair of lateral diverticula (Fig. 1). Pharynx oval, 190 × 180. Oesophagus short, 70, surrounded by unicellular glands. Caecal bufurcation 750 from anterior end of body. Thin-walled caeca extend to posterior end. Ventral sucker round, 220 × 210; distance from center of ventral sucker to anterior end 2200; ventral sucker from anterior end as percent of total body length 31.5. Oral:ventral sucker width ratio 4.86:1. Area anterior of ventral sucker aspinose. Under scanning electron microscope 2 rings of sensory papillae observed on ventral sucker, the outer ring consists of 6 papillae and the inner ring consists of 9 papillae (Fig. 2).

Common genital pore located ventrally at posterior margin of ventral sucker. Dorsal protuberance large, raised; located medially on dorsal surface posterior to ovarian complex. Ovary spherical to oval 180 × 170, median, approximately 2/3 of body length from anterior end, 2290 from posterior end. Seminal receptacle oval, 65 × 80, postero-sinistral and dorsal to ovary, usually slightly overlapping it. Mehlis' gland large, posterior to ovary. Laurer's canal opens dorsally at anterior margin of dorsal protuberance. Uterus intercaecal, between ovary and ventral sucker. Distal portion of uterus passes dorsal to cirrus sac and ventral sucker, looping posteriorly along left margin of ventral sucker before entering genital atrium. Vitelline follicles small, scattered in posterior quarter of body; extending from posterior extremity anteriorly to posterior margin of ovary. Testes, oval, tandem, entire, in posterior quarter of body. Anterior testis 210 × 170; posterior testis 230 × 170; distance between testes 520 (separated by approximately 2 testis lengths); distance from posterior testis to posterior end of body 620. Cirrus sac length 730, maximum width 150, overlapping ventral sucker dorsally and curving posteriorly, entering genital atrium posterior to uterus. Bottom of cirrus sac at 420 from posterior margin of ventral sucker. Cirrus sac contains seminal vesicle, pars prostatica, cirrus. Bipartite seminal vesicle occupies approximately one-fourth of the cir-

Table I. Metric data for *Choanocotyle platti* sp. nov.

Characters	n	Min-Max	Mean	SD	CV*
Body length	9	6510–8080	7105.6	417.2	5.9
Body width†	11	300–640	477.7	97.7	20.4
% forebody*	9	24.2–34.1	31.0	2.8	9.2
Oral sucker width	11	740–1230	1004.5	132.2	13.2
Prepharynx length	10	180–280	212.0	30.9	14.6
Pharynx length	10	170–230	205.0	20.5	10.0
Pharynx width	10	150–220	191.0	25.3	13.2
Oesophagus	10	65–110	77.5	13.0	16.7
Anterior end to caecal bifurcation	10	750–1140	918.0	139.9	15.2
Ventral sucker length	11	200–280	242.7	25.4	10.5
Ventral sucker width	11	190–260	228.6	21.6	9.4
Ventral sucker to anterior end	10	1690–2450	2450.0	217.5	9.9
Seminal receptacle length	10	65–125	97.0	23.5	24.2
Cirrus sac length	11	670–810	754.5	51.6	6.8
Cirrus sac width	11	135–190	168.1	16.0	9.5
Posterior cirrus sac to posterior ventral sucker	11	290–480	388.6	49.6	12.8
Seminal receptacle width	10	75–170	106.0	29.7	28.0
Ovary length	10	150–190	172.0	14.2	8.2
Ovary width	10	170–230	195.5	21.8	11.1
Ovary to posterior end	10	2150–2800	2507.0	206.9	8.3
Anterior testis length	10	210–350	257.0	46.0	17.9
Anterior testis width	10	170–270	212.5	34.3	16.1
Posterior testis length	10	200–345	265.5	45.1	17.0
Posterior testis width	10	165–270	217.5	38.9	17.9
Distance between testes	10	320–520	437.0	67.6	15.5
Posterior testis to posterior body margin	10	620–1260	867.0	179.6	20.7
Egg length	11	30–40	34.0	2.8	8.2
Egg width	11	15–20	17.6	1.5	8.4

†Body width at level of ventral sucker; *percent of body between ventral sucker and anterior end.

rus sac length. Pars prostatica glandular; cirrus unarmed, evaginated cirrus not observed. Excretory bladder elongate, intracaecal, extending almost to ovary; excretory pore terminal. Eggs operculate; fully formed eggs in distal part of uterus 40 × 20.

Taxonomic summary

Type host: *Chelodina rugosa* Ogilby, 1890 (Testudines, Pleurodira, Chelidae).

Site of infection: Small intestine.

Type locality: Small billabong at Daly River Mango Farm, near Nauiyu Community, Northern Territory, Australia, 13°44.302´S, 130°40.838´E.

Other localities: Daly River, near Oolloo Crossing, Northern Territory, Australia, 14°00.31´S, 131°14.46´E; Mary River, Opium Creek Station, Northern Territory, Australia, 12°34.62´S, 131°43.48´E.

Prevalence and intensity: 3 of 8 *C. rugosa* were infected with up to 10 worms each.

Specimens deposited: The type series consists of 11 fully mature specimens, 1 of which is incomplete. Holotype: Queensland Museum (QM) no. G228974 (labeled: ex. *Chelodina rugosa*, Daly River, Northern Territory, Australia, June 2005, SDS-05-53A). Paratypes: QM nos. G228975, G228976 (labeled: ex. *Chelodina rugosa*, Daly River, Northern Territory, Australia, June 2005, SDS-05-53A), and nos. G228977, G228978 (labeled: ex. *Chelodina rugosa*, Mary River, Opium Creek Station, Northern Territory, Australia, June 2005, SDS-04-59A). Paratypes: Harold W. Manter Laboratory (HWML) no. HWML48612 (6 slides labeled: ex. *Chelodina rugosa*, Daly River, Northern Territory, Australia, June 2005, SDS-05-32A).

Etymology: This species is named for Dr. Tom Platt, Saint Mary's College, Notre Dame, Indiana, for his contributions to helminthology in general and to our understanding of parasites of Australian turtles in particular.

Differential diagnosis

The presence of the large, highly specialized, ventrally incised oral sucker combined with other morphological features, such as the arrangement of gonads and the presence of a dorsal protuberance, clearly allows placement of these specimens within *Choanocotyle* Jue Sue et Platt, 1998.

Choanocotyle platti sp. nov. is morphologically most similar to *Choanocotyle hobbsi* Platt et Tkach, 2003 and quite similar to *Choanocotyle nematoides* Jue Sue et Platt, 1998. The new species differs from *C. nematoides* in body size, oral sucker size and proportions, cirrus sac shape and size, in addition to several other characters. The body of *C. platti* sp. nov. is half the length (6.51–8.08 mm, average 7.10 mm) of that of

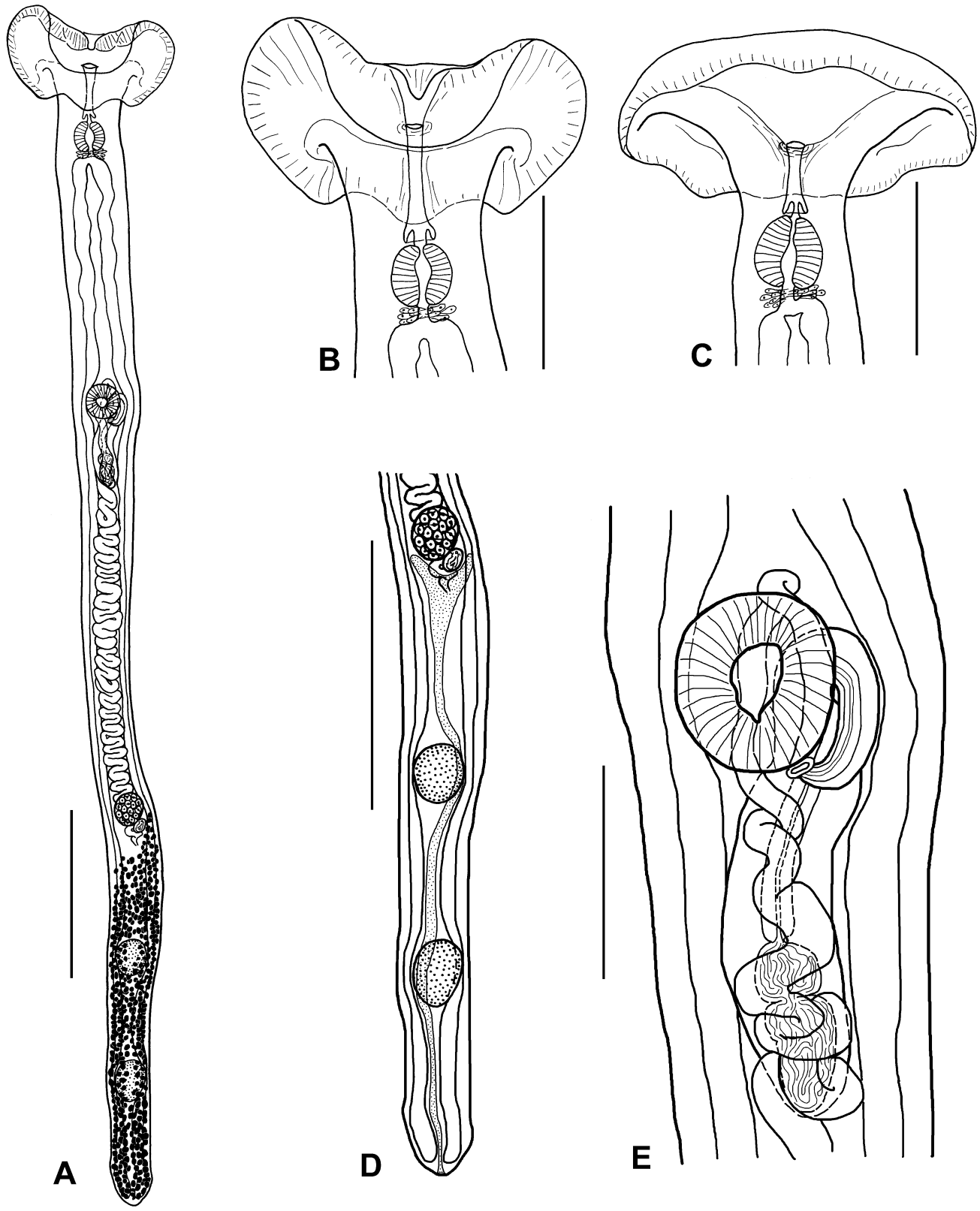

Fig. 1. *Choanocotyle platti* sp. nov.: Holotype **(**A, B, D, E) and paratype (C). **A** – overall view. **B** and **C** – fully everted oral suckers. **D** – posterior end of the body with vitellarium not shown. Note the shape of the excretory vesicle. **E** – distal parts of male and female reproductive system and ventral sucker. Scale-bars = 1 mm (A, D), 0.5 mm (B, C), 0.25 mm (E)

C. nematoides (16.3 mm). Despite a much smaller body, the oral sucker width in *C. platti* is 2–3 times larger (740–1230, average 1004) than that in *C. nematoides* (396). The cirrus sac in *C. platti* is much shorter (670–810, average 754) than in *C. nematoides* (1375) (data from Jue Sue and Platt 1998) and lacks the loop characteristic of *C. nematoides*.

Choanocotyle platti sp. nov. differs from *C. hobbsi* by possessing a smaller body length (6.51–8.08 mm, average 7.10 mm vs. 7.75–16.23 mm, average 10.68) and substantially larger oral sucker (740–1230, average 1004 vs. 551–796, average 672). Less prominent, but consistent features differentiating *C. platti* sp. nov. from both *C. nematoides* and *C. hobbsi*, is the greater relative distance between testes (about 2 testis lengths in the new species vs. only 1 testis length in the other 2 species) and the presence of a posterior infolding of the prepharynx in *C. platti*, but not in the other 2 species.

Molecular data

Contiguous sequences spanning nearly 4300 bp of the nuclear ribosomal 18S gene, ITS region (ITS1, 5.8S, ITS2) and 28S gene were obtained from specimens of *C. platti* sp. nov. and *C. hobbsi*. Almost complete sequences of the 18S gene and partial sequences of the 28S gene were obtained from *C. nematoides*, however, despite numerous attempts, we failed to sequence most of the ITS region of specimens of *C. nematoides* collected from two different localities. Other regions were amplified from the same DNA extracts of *C. nematoides* without difficulty. For possible explanations of the situation see Platt and Tkach (2003) who were also unable to obtain an ITS sequence from this species.

The sequenced fragment of the 18S gene was 1906 bp long in all three studied species. The complete ITS1 region consisted of 595 bp in *C. platti* and 644 bp in *C. hobbsi*. The complete 5.8S gene was 157 bp long in both *C. platti* and *C. hobbsi* and the complete ITS2 region was 252 bp long in both *C. platti* and *C. hobbsi*. The sequenced fragment at the 5' end of the 28S gene consisted of 1340 bp in *C. platti*, 1339 bp in *C. hobbsi* and 1338 bp in *C. nematoides*. Sequences of *C. nematoides* and *C. hobbsi* were identical to 18S and 28S sequences of these species published by Platt and Tkach (2003).

Pairwise sequence comparison reveals differences among *C. platti*, *C. nematoides* and *C. hobbsi* in all analyzed DNA sequence regions (Table II). Additionally, a 38 bp long indel was found in the ITS1 sequence of *C. hobbsi*, but was not found in *C. platti*. Except for this indel the variable sites were localized in different parts of the alignment. Thus, the ob-

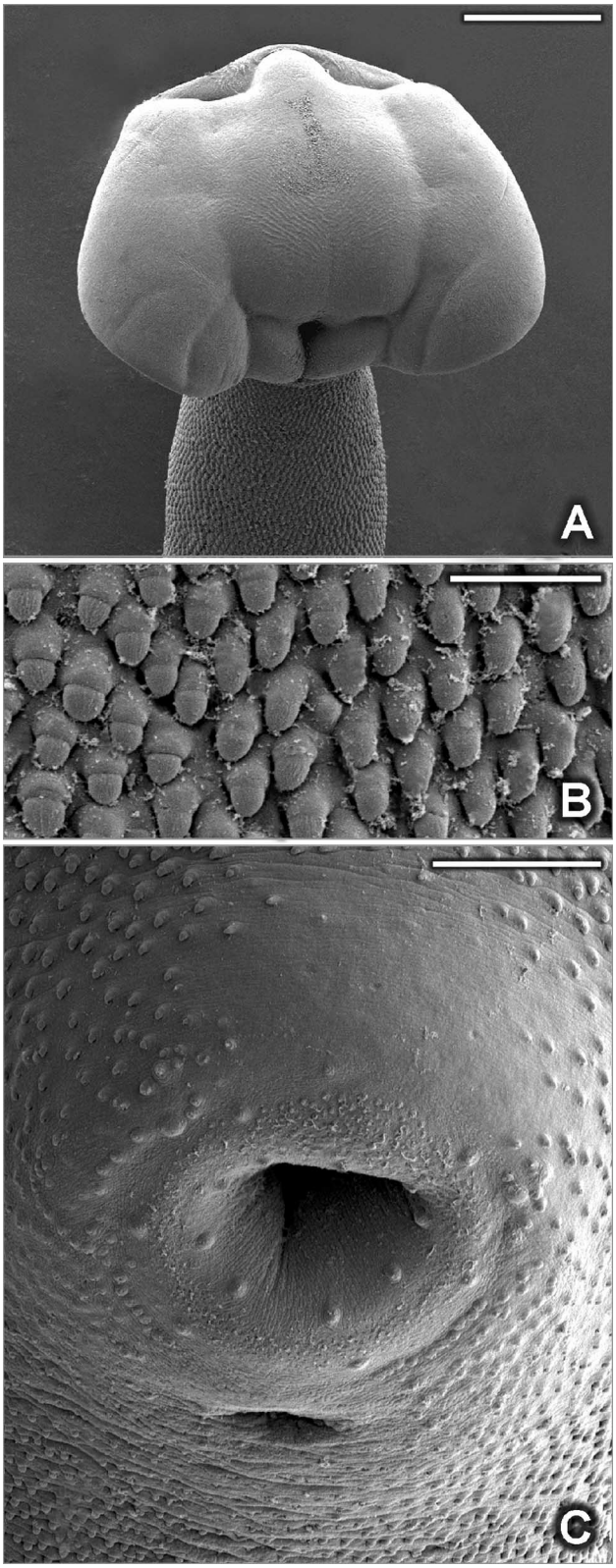

Fig. 2. *Choanocotyle platti* sp. nov.: SEM micrographs were taken from the same specimen that was used to obtain DNA sequence (GenBank # EU196355). **A** – anterior part of the body. Note the unique structure of the oral sucker. **B** – tegumental spines on the ventral side of the body posterior to the oral sucker. **C** – ventral sucker region. Note the genital pore posterior to the ventral sucker and two circles of sensory papillae around the sucker. Scale-bars = 250 μm (A), 25 μm (B), 50 μm (C)

Table II. Number of variable sites in different nuclear ribosomal DNA regions (partial 18S, complete ITS1, complete 5.8S, complete ITS2, partial 28S) among *Choanocotyle platti* sp. nov. and two morphologically similar *Choanocotyle* species. Total alignment length was 4299 bp. Alignment length for each fragment is shown in parentheses. 38 bp long indel region in the ITS1 sequence of *C. hobbsi* is not included in this table

Digenean species	DNA region				
	18S (1906 bp)	ITS1 (644 bp)	5.8S (157 bp)	ITS2 (252 bp)	28S (1340 bp)
C. hobbsi	4	18	0	3	13
C. nematoides	10	N/A	N/A	N/A	14

served morphological differences were strongly supported by molecular data (Table II) which supports the status of *C. platti* as a new species.

Discussion

This parasite represents the sixth trematode species described from the freshwater turtles of Northern Territory, after the recent descriptions of 4 species of digeneans and 1 species of aspidogastrean (Snyder and Tkach 2006, 2007; Tkach and Snyder 2006, 2007). The discovery of a relatively rich fauna of previously unknown digenean species in freshwater turtles from Northern Territory suggests that numerous additional helminth species await discovery in the unexplored turtles of Australia. *Choanocotyle platti* sp. nov. joins *C. juesui* and *C. hobbsi* as Choanocotylidae reported only from long-necked turtles of the genus *Chelodina*. The new species was not recovered from the short necked chelids (*Elseya dentata*, *Emydura tanybaraga*, *Emydura victoriae*), nor the cryptodiran turtle (*Carettochelys insculpta*) collected syntopically with *C. rugosa* from the Daly River and surrounding billabongs.

The level of sequence variability observed among *C. platti* sp. nov., *C. hobbsi* and *C. nematoides* (Table II) is consistent with the levels of variability observed among closely related congeneric species in other digenean groups (for references see Tkach *et al*. 2000; Jousson and Bartoli 2001, 2002; León-Règagnon and Paredes-Calderón 2002; Platt and Tkach 2003; Nolan and Cribb 2005; Olson and Tkach 2005; Curran *et al*. 2006; Tkach and Snyder 2007). As with many digeneans the ITS1 fragment of the *Choanocotyle* species examined in the current study was more variable than other regions of ribosomal DNA. Despite being three times shorter than the 18S gene, the ITS1 fragments of *C. platti* and *C. hobbsi* had 3.5 times as many variable sites as did the 18S gene (Table II). Similarly, ITS1 had substantially more variable sites than did the 28S fragment, despite being twice shorter. Based on examinations of sequence data and morphology the new species appears to be more closely related to *C. hobbsi* than to *C. nematoides*. The failure of our multiple attempts to amplify and sequence the ITS region of *C. nematoides* also suggests substantial differences in DNA sequence/structure of the ITS region among *C. nematoides* and 2 other species. However, a phylogenetic inference of interrelationships among just 3 species would not be particularly informative and we will await the addition of other species of Choanocotylidae to our sequence database before undertaking a more comprehensive molecular phylogenetic analysis.

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