

NEOSYCHNOCOTYLE MAGGIAE, N. GEN., N. SP. (PLATYHELMINTHES: ASPIDOGASTREA) FROM FRESHWATER TURTLES IN NORTHERN AUSTRALIA

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ABSTRACT: *Neosychnocotyle maggiae*, n. gen., n. sp., (Aspidogastrea) is described from the pig-nosed turtle, *Carettochelys insculpta*, and reported from the Victoria River red-faced turtle, *Emydura victoriae*, all from the Daly River, Northern Territory, Australia. *Neosychnocotyle* n. gen. is differentiated from all aspidogastrea genera but one by the absence of a cirrus sac. The similar *Sychnocotyle* also lacks a cirrus sac, but *Neosychnocotyle* n. gen. differs from the former genus by possessing a narrow, tapered anterior end, a ventral disk that covers the posterior end of the body, a genital pore that is displaced anteriorly, and a vas deferens that is less convoluted and less robust. Analysis of ribosomal DNA sequences demonstrates substantial sequence variability between representatives of *Sychnocotyle* and *Neosychnocotyle maggiae*, n. gen., n. sp. It is possible that this new parasite may reach sexual maturity only in *C. insculpta*. This is only the second aspidogastrea species reported from Australian freshwater turtles.

Aspidogastrea are globally distributed trematodes found as adults in molluscs, fish, and turtles in both marine and fresh waters. Only 1 species of Aspidogastrea, *Sychnocotyle kholo* Ferguson, Cribb and Smales, 1999, has been reported from Australian freshwater turtles. Representatives of this species were found as adults in the intestine of the Macquarie turtle, *Emydura macquarii*, and as juveniles in both corbiculid clams (Corbiculidae) and thiarid snails (Thiaridae) (Ferguson et al., 1999). As part of an ongoing examination of parasite biodiversity in Australian freshwater turtles, we report on a novel species of Aspidogastrea that does not conform to *Sychnocotyle* or any other known aspidogastrea genus.

MATERIALS AND METHODS

Carettochelys insculpta and *Emydura victoriae* 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 specimens of a new aspidogastrea species belonging to Aspidogastridae were collected from these turtles. Live worms were rinsed in saline, killed with hot water, and fixed in 70% ethanol. Specimens were stained with aqueous alum carmine, dehydrated in a graded ethanol series, cleared in clove oil, and mounted permanently in Damar balsam.

Measurements were taken using 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. All measurements in micrometers unless otherwise stated.

Specimens of *S. kholo* were obtained from Krefft's turtle, *Emydura krefftii*, collected from the Ross River in northeastern Australia, near Townsville, Queensland, and an undescribed species of *Sychnocotyle* was collected from *Emydura victoriae* in the Daly River, Northern Territory, Australia. *Aspidogaster conchicola* Baer, 1826, was collected from *Unio* spp. from the Dnieper River in Kiev, Ukraine. These specimens were compared to specimens of the new species using morphological and molecular techniques.

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.

Genomic DNA for molecular analysis was extracted from single eth-

anol-fixed specimens of the new species described in this work and 2 species of *Sychnocotyle*, following the protocol of Tkach and Pawlowski (1999). A fragment of nuclear ribosomal DNA at the 3' end of the 18S and including the entire ITS1, 5.8S, and ITS2 regions and a fragment of the 5' end of the 28S (including variable domains D1–D3) was amplified by PCR on an Eppendorf Master Gradient thermal cycler using forward primer ITSF (5'-CGCCCGTCGCTACTACCGAT TG-3') and reverse primer 1500R (5'-GCTATCCTGAGGGAAACTT CG-3'). PCR primers, the internal forward primer digl2 (5'-AAGCAT ATCACTAAGCGG-3'), and the internal reverse primers ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3'), d58r (5'-GTC GAT GTT CAA AGC AGT ATG C-3'), and 300R (5'-CAA CTT TCC CTC ACG GTA CTT G-3') were used in sequencing reactions.

PCR reactions were performed according to protocols described by Tkach et al. (2003). PCR products were purified directly using Qiagen Qiaquick™ columns (Valencia, California), cycle-sequenced using ABI BigDye™ chemistry, alcohol-precipitated, and run on an ABI Prism 3100™ automated capillary sequencer. Contiguous sequences were assembled and edited using Sequencher™ (GeneCodes Corp., ver. 4.1.4) and submitted to GenBank under accession numbers EF015578 (*Neosychnocotyle maggiae* n. sp.), EF015579 (*S. kholo*), and EF015580 (*Sychnocotyle* sp.). Sequences were manually aligned and compared using the BioEdit program, version 7.0.1 (Hall, 1999).

RESULTS

Description

Platyhelminthes; Aspidogastrea; Aspidogastridae; Aspidogastrinae.

Neosychnocotyle n. gen.

Diagnosis: Body elongate, nearly uniform in width, more narrow at anterior end than at posterior end. Tegument aspinose. Ventral disk wider than body; extends from just posterior to level of pharynx to slightly beyond posterior end of body. Ventral disk with 12 transverse rows of alveoli separated by transverse septa, each row divided by longitudinal septa to form up to 4 alveoli per row. Marginal bodies with small, thin-walled ampullae at both sides of each transverse septum. Oral sucker absent, prepharynx short, pharynx relatively large and oval. Esophagus distinct, leads to simple cecum, terminating near posterior body end. Testis single, median, postovarian, near posterior body end. Cirrus and cirrus sac absent. Slightly convoluted vas deferens terminates in thick-walled pars prostatica. Genital atrium at level of posterior half of pharynx. Ovary slightly submedian, somewhat lobed. Ootype between testis and ovary. Uterus coiled. Metraterm poorly developed. Vitellarium consists of 2 lateral vitelline fields merging immediately pos-

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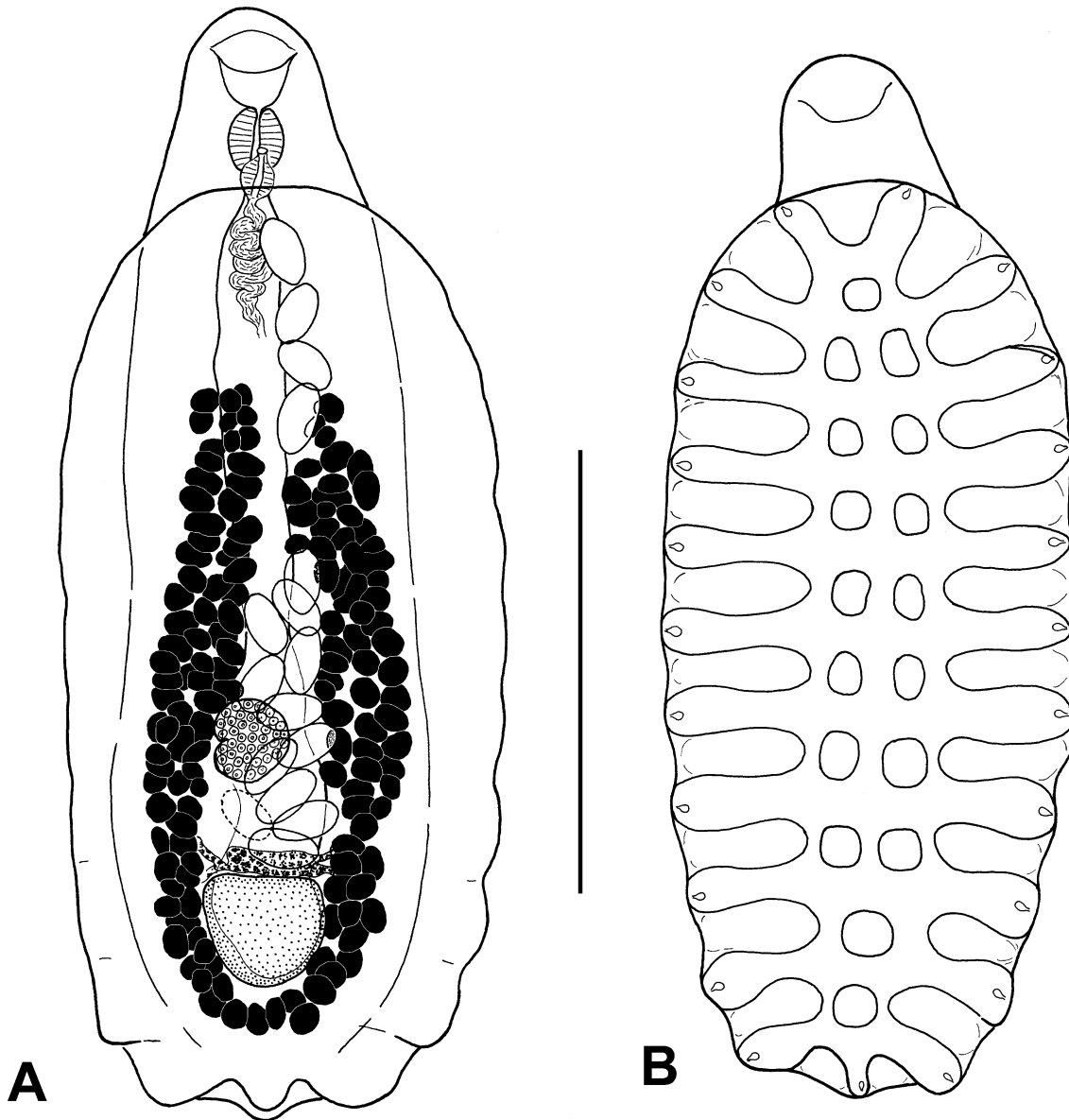


FIGURE 1. *Neosynchnotyle maggiae* n. gen., n. sp. (A) Ventral view of holotype. (B) Arrangement of alveoli on ventral disc. Scale bar: 1 mm.

terior to testis. Vitelline fields do not merge anteriorly, vitelline reservoir present. Parasitic in the intestine of Australian freshwater turtles.

Type species: Neosynchnotyle maggiae n. sp.

DESCRIPTION

***Neosynchnotyle maggiae* n. sp.**
(Figs. 1, 2A, 2B)

Description based on 6 adult specimens: Measurements of holotype given in text; measurements of entire type series given in Table I. Body

2.57 mm, elongate, nearly uniform in width, with narrow anterior end and somewhat more rounded posterior end. Body margins nearly parallel (Fig. 1). Tegument aspinose. Ventral disk 2,120 × 1,050, wider than body; extends along most of body. Anterior margin of ventral disk 410 from anterior end, just posterior to level of pharynx (Figs. 1, 2A). Ventral disk extends beyond posterior end of body, in ventral view posterior end of body obscured by overhanging ventral disk. Ventral disk length:body length ratio 0.83:1. Ventral disk with 12 transverse rows of alveoli separated by transverse septa, alveoli within rows separated by longitudinal septa. First (anterior most) row consists of 1 alveolus, second row 3 alveoli, third through ninth rows each with 4 alveoli, rows 10 and 11 with 3 alveoli, and the twelfth and most pos-

FIGURE 2. (A, B) Anterior end of *Neosynchnotyle maggiae* n. gen., n. sp. Note numerous papillae and the genital pore at middistance between the oral opening and the ventral disc. (C) *Synchnotyle kholo*. (D, E) Anterior end of *Aspidogaster conchicola*. Scale bars: A = 500 μm; B = 100 μm; C = 500 μm; D = 200 μm; E = 100 μm.

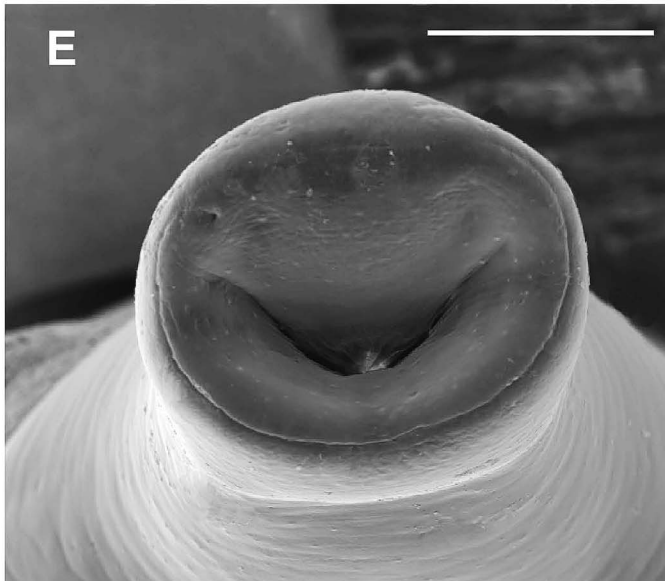
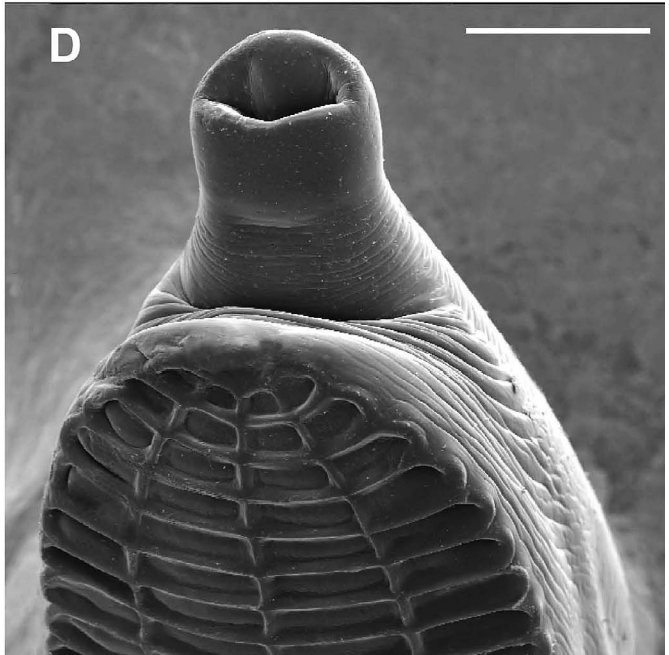
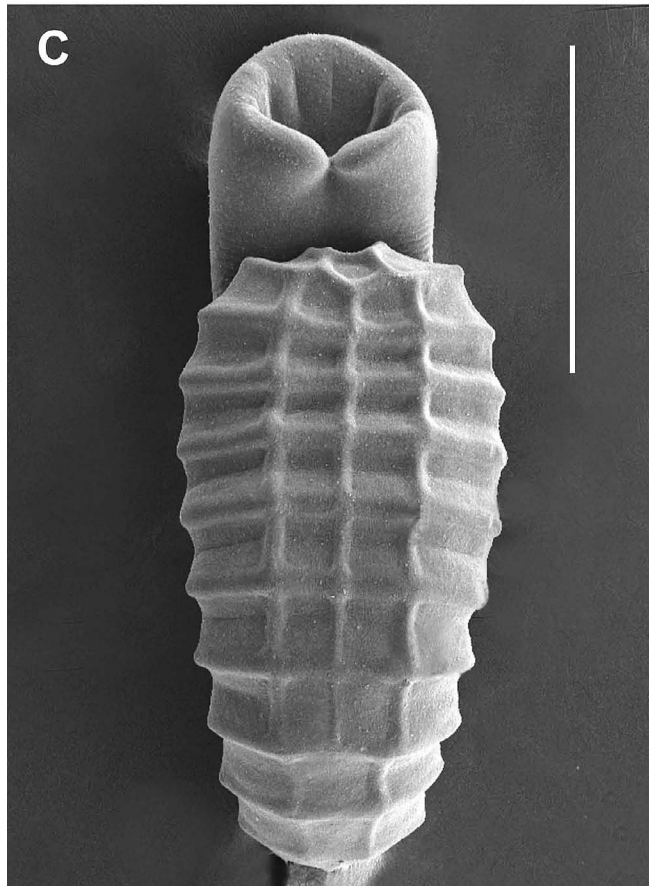
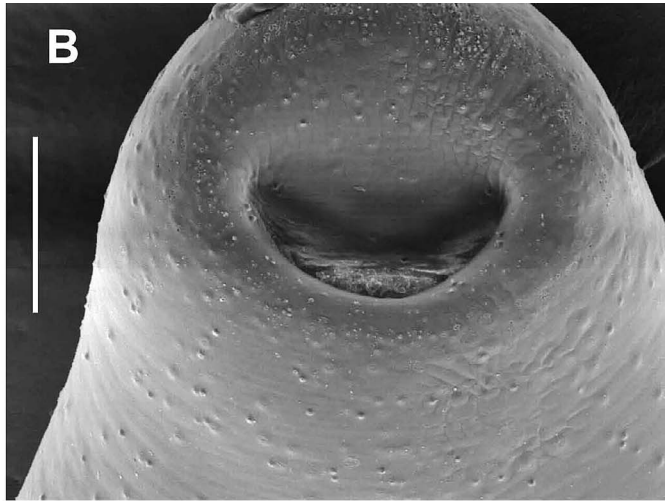
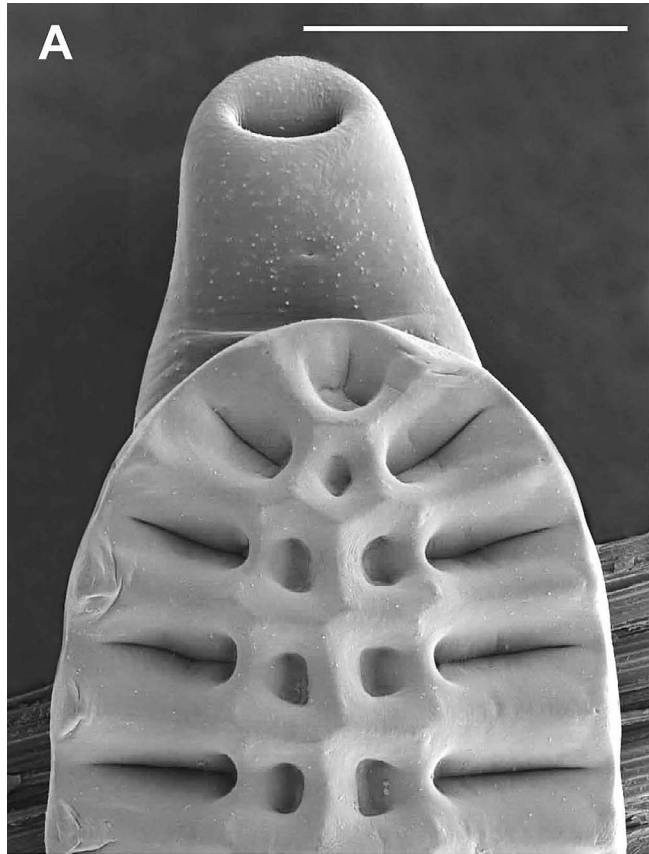


TABLE I. Metric data for *Neosychnocotyle maggiae* n. gen., n. sp.

Characters	n	Min-max	Mean	StD	CV*
Body length	6	2,120–2,570	2,335.0	177.2	7.6
Body width	6	510–680	591.7	68.2	11.5
Ventral disk length	6	1,750–2,170	1,988.3	157.4	7.9
Ventral disk width	6	740–1,050	906.7	104.1	11.5
Anterior margin ventral disk from anterior end	6	270–430	366.7	55.7	15.2
Ventral disk length: body length	6	0.82–0.92	0.9	157.4	7.9
Anterior end width	6	260–290	282.5	11.7	4.2
Prepharynx length	6	0–10	1.7	4.1	244.9
Pharynx length	6	140–150	145.8	3.8	2.6
Pharynx width	6	115–130	123.3	6.1	4.9
Esophagus length	6	50–60	53.3	4.1	7.7
Testis length	6	220–260	245.0	13.8	5.6
Testis width	6	230–300	265.8	26.9	10.1
Testis to posterior end of ventral disk	6	235–380	301.7	52.0	17.2
Pars prostatica length	4	75–90	83.8	7.5	9.0
Pars prostatica width	4	60–70	65.0	4.1	6.3
Genital atrium to anterior end	6	280–320	301.7	14.7	4.9
Ovary length	4	125–135	130.0	4.1	3.1
Ovary width	4	130–140	132.5	5.0	3.8
Ovary to testis	5	190–225	203.0	14.8	7.3
Number of eggs in uterus	6	1–15	8.0	5.7	70.7
Anterior margin vitelline fields to anterior end	6	580–860	708.3	121.1	17.1
Egg length	15	165–180	172.7	5.0	2.9
Egg width	15	87–100	94.1	3.6	3.8

* Coefficient of variation

terior row with 2 alveoli. Marginal bodies at both sides of each transverse septum. Ampullae of marginal bodies small and thin walled.

Oral sucker absent, anterior end tapered, 290 wide at midpoint of oral opening. Mouth opens into very short, almost indistinct prepharynx. Pharynx 150 × 130, large, oval, elongated along anterior-posterior body axis. Esophagus distinct, 60 long, thick walled. Cecum simple, terminating blindly at posterior margin of testis, dorsal to reproductive organs.

Testis 260 × 290, single, median, postovarian, 290 from posterior end of ventral disk, with rounded posterior end and concave anterior surface. Cirrus and cirrus sac absent. Portion of vas deferens anterior to vitelline fields slightly convoluted. Vas deferens terminates in thick-walled, muscular pars prostatica, 90 × 70 pars prostatica empties into genital atrium. Genital atrium at 310 from anterior end of body, ventral to pharynx, ranging from midlevel to posterior margin of pharynx.

Ovary 135 × 145, slightly submedian, slightly lobed, 200 anterior of testis. Ootype between testis and ovary, slightly dextral. Laurer's canal not visible in whole mounts. Uterus coiled, with up to 15 eggs. Weak, thin-walled metraterm begins posterior to seminal vesicle. Vitellarium consists of 2 lateral vitelline fields merging immediately posterior to testes. Lateral vitelline fields do not merge anteriorly and are comprised of large globular follicles. Anterior margin of vitelline fields at 860 from anterior end of body. Left and right common vitelline ducts merge to form pronounced vitelline reservoir immediately anterior to testis. Eggs 175 × 95, operculate.

Taxonomic summary

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

Other host: Victoria River red-faced turtle, *Emydura victoriae* (Gray, 1842) (Chelonia: Pleurodira: Chelidae).

Type locality: Daly River, near Ooloo 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 1 to 3 worms and 1 of 5 *E. victoriae* was infected with 3 immature worms.

Specimens deposited: The type series consists of 6 fully mature spec-

imens. Holotype: Queensland Museum (QM) no. G227473. Paratypes: QM nos. 227474 and 227475, Harold W. Manter Laboratory (HWML) nos. 48419 and 48420. Three juvenile worms deposited HWML48421 and HWML48422. Slides labeled identically: ex. *Carettochelys insculpta*, Daly River, Northern Territory, Australia, June 2005.

Etymology: The generic name reflects the similarity of this genus to *Sychnocotyle*. The specific name is in honor of valuable field assistant Maggie Snyder, who helped collect these and many other Australian worms.

Remarks

Immature worms showed the general morphological characteristics of the adult parasites, including the position of developing gonads. Pharynx length, the size of the ovary, and egg size were the most stable among metric characters, as revealed by the coefficient of variation (Table I).

Based on overall morphology the new species is clearly circumscribed within Aspidogastrinae (*sensu* Rohde, 2002) and most similar to *S. kholo*, the only known species of *Sychnocotyle*. The morphology of the ventral disk (number of alveoli) resembles both *Sychnocotyle* and *Aspidogaster* (Figs. 2D, E). However, species of *Aspidogaster* possess a cirrus sac (Rohde, 2002), a character lacking in both *N. maggiae* n. sp. and *S. kholo*. Despite the similarities between these 2 species there exist numerous differences.

In *S. kholo*, the genital pore is at the anterior junction of the body and the ventral disk (Ferguson et al., 1999), whereas in the new species the genital pore is anterior of this junction, at the level of the pharynx. The anterior end of the body of *S. kholo* is funnel shaped and wider than the posterior end (Fig. 2C). The anterior end of *N. maggiae* n. sp. is tapered and considerably narrower than the posterior end (Fig. 1). The ampullae of the marginal bodies of *S. kholo* are thick walled and pronounced under microscopic examination, whereas these structures are much less prominent in the new species. The ventral disk of *N. maggiae* n. sp. extends further posterior than in *S. kholo* and covers the posterior end of the body. In the latter species, the body extends posterior of the ventral disk. The vas deferentia in the 2 species also differ substantially; in *S. kholo*, this structure is much more robust and convoluted than in the new species. The number of eggs in the uterus of

the new species (up to 15) is much greater than that observed for *S. kholo* (up to 5). Based on these and other differences we propose a new genus, *Neosychnocotyle*, with *N. maggiae* n. sp. as the type and only species.

As part of this study, we sequenced a 2,454 bp fragment of the nuclear ribosomal DNA gene from *N. maggiae* n. sp. and 2 species of *Sychnocotyle*, *S. kholo* and *Sychnocotyle* sp. (an undescribed species). Alignment of the sequences required the inclusion of very few gaps, which suggests that these 3 species are closely related. In the 572 bp fragment of the ITS1, sequences of the 2 *Sychnocotyle* species varied at only 6 positions, whereas *N. maggiae* n. sp. differed at 78 positions from *Sychnocotyle* sp. and 76 positions from *S. kholo*. In 397 bases of the ITS2, *N. maggiae* n. sp. differed from *Sychnocotyle* sp. and *S. kholo* by 43 and 38 bases, respectively. The 2 species of *Sychnocotyle* differed from one another in the ITS2 at 11 of 397 bases. Across 1,328 bases of the 28S rDNA the 2 species of *Sychnocotyle* differed by 5 bases, whereas *N. maggiae* n. sp. differed from *Sychnocotyle* sp. by 51 bases and from *S. kholo* by 56 bases. The substantial level of sequence variability between *N. maggiae* n. sp. and the 2 species of *Sychnocotyle* strongly supports the status of *Neosychnocotyle* as a new genus.

Specimens of *N. maggiae* n. sp. are placed in the genus *Sychnocotyle* according to the most recent key to the Aspidogastrea (Rohde, 2002). To assist investigators using this key we suggest the following amendments:

- 4b Papillae on adhesive disc absent 5
 5a Genital pore at anterior junction of body and ventral disk; anterior end wider than posterior end
 *Sychnocotyle* Ferguson, Cribb & Smales, 1999
 5b Genital pore at level of pharynx, anterior of junction of body and ventral disk; anterior end much narrower than the posterior end *Neosychnocotyle*

Neosychnocotyle maggiae n. sp. is only the second freshwater aspidogastrea species reported from Australia and the first from Northern Territory. These parasites were found in both pig-nosed turtles, *C. insculpta*, and Victoria River red-faced turtles, *E. victoriae*. *Carettochelys insculpta* is the only representative of the Cryptodira (hidden-necked turtles) found in Australia and is considered to be a recent migrant to the continent, arriving less than 15,000 yr ago (Cogger and Heatwole, 1981). Like all other Australian turtles, *E. victoriae* represents Pleurodira (side-necked turtles). These 2 turtle lineages are thought to have diverged some 200 mya (Gaffney and Kitching, 1994), and the ability of *N. maggiae* n. sp. to parasitize such an evolutionarily diverse range of turtles seems remarkable at first glance. However, only 1 of 5 *E. victoriae* was infected, and the 3 specimens found in this turtle were not gravid. All 4 *C. insculpta* were infected, and 3 of 4 harbored gravid worms.

The relatively low prevalence and lack of mature worms in *E. victoriae* suggests that Victoria River turtles may be unsuitable hosts of *N. maggiae* n. sp. Indeed, the other 2 pleurodiran turtles, *Elseya dentata* and *Chelodina rugosa*, collected syntopically with *E. victoriae* and *C. insculpta* did not harbor *N. maggiae* n. sp. The absence of the new species from *El. dentata* may be another indication that these parasites cannot reach maturity in Pleurodira. In the Daly River, *C. insculpta* feed almost exclusively on ribbonweed, *Vallisneria spiralis*, a major component of the diet of *E. dentata* in this river (see Tkach and Snyder, 2006). As a result of eating *V. spiralis*, both turtle species ingest large numbers of snails (S. Snyder, unpubl. obs.), one of the hosts of *S. kholo*, the other Australian freshwater aspidogastrea (Ferguson et al., 1999). This information suggests that *C. insculpta*, *E. victoriae*, and *El. dentata* come into contact with the infective stages of *N. maggiae* n. sp., but that this aspidogastrea reaches sexual maturity only in *C. insculpta*.

As mentioned above, *S. kholo* utilizes thiarid snails and corbiculid clams as intermediate hosts and apparently requires ingestion by a freshwater turtle to reach sexual maturity (Ferguson et al., 1999). Other as-

pidogastrea species found in turtles also appear to use molluscs as intermediate hosts, or develop into adults in molluscs and use vertebrates as facultative hosts when both mollusc and parasite are ingested (Ward and Hopkins, 1931; Wharton, 1939; Hendrix and Short, 1972; Rohde, 1972). The putative molluscan host of *N. maggiae* n. sp. is unknown, although over 100 unidentified prosobranch snails were collected from *V. spiralis* in the Daly River and crushed. This examination revealed no parasites, but the morphological similarity between the new species and *S. kholo* suggests that further examinations of both snails and corbiculids provide the best opportunity for uncovering the life cycle of *N. maggiae* n. sp.

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