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APTORCHIS GLANDULARIS N. SP. (DIGENEA: PLAGIORCHIOIDEA) FROM THE NORTHWESTERN RED-FACED TURTLE, *EMYDURA AUSTRALIS*, (PLEURODIRA: CHELIDAE) IN THE KIMBERLEY, WESTERN AUSTRALIA

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ABSTRACT: *Aptorchis glandularis* n. sp. is described from the intestine of the northwestern red-faced turtle, *Emydura australis* (Pleurodira: Chelidae), in the Kimberley, Western Australia. This digenean is morphologically most similar to *Aptorchis aequalis* but can be differentiated readily from the latter species by the presence of ventral glands arranged in 3 rows. This feature is unique among plagiorhioidean digeneans and resembles the glands observed in some monostome digeneans in Notocotylidae and Microscaphidiidae. Comparison of approximately 2,600 bases of ribosomal DNA (partial 18S, complete ITS1+5.8S+ITS2, partial 28S), obtained from all 5 known *Aptorchis* species, strongly supports the status of *Aptorchis glandularis* n. sp. as a new species. Phylogenetic analysis of these sequence data demonstrates 2 strongly supported clades (*A. pearsoni* + *A. megacetabulus*) and (*A. aequalis* + *A. glandularis* n. sp.), with *A. megapharynx* representing a separate lineage. This is the first report of an endoparasite from *Emydura australis*.

The Kimberley Plateau is an isolated region in the wet–dry tropics of northwestern Western Australia. This region is home to 2 endemic species of freshwater turtles (Cogger, 2000), the sandstone snake-necked turtle *Chelodina burrungandjii* and the northwestern red-faced turtle *Emydura australis*, and to the more broadly distributed northern snapping turtle, *Elseya dentata*. A report of leeches from the 2 former species (Tucker et al., 2005) represents the extent of published information on the parasites of Kimberley turtles. As part of an examination of parasite biodiversity in Australian freshwater turtles, we examined the endoparasites of *E. australis* and discovered a number of helminths, some of them new to science. Among the digeneans is a new species of *Aptorchis*, a genus currently comprised of 4 species (see Tkach and Snyder, 2007a, for a brief review). The fifth species, described herein, is the first helminth described from *E. australis* and the first reported from turtles of the Kimberley.

MATERIALS AND METHODS

In July 2006, 20 *E. australis* were taken, by hand and by baited traps, from rivers and streams throughout the Kimberley region of northwestern Western Australia under a permit from the Western Australia Department of Conservation and Land Management. Numerous specimens of a new digenean species belonging to *Aptorchis* were recovered from the rectums of 11 *E. australis* collected in 4 different localities. 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 Mayer's hematoxylin, dehydrated in a graded ethanol series, cleared in methyl salicylate or clove oil, and mounted permanently in Damar balsam.

Measurements were taken from a compound microscope using digital imaging and Rincon measurement software (v. 7.1.2, Imaging Planet, Goleta, California) as well as with 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 a lower CV have values that are more stable around the mean than those with higher CVs.

Type and voucher specimens were examined from the collection of the Queensland Museum (QM), Brisbane, Queensland, Australia: *Aptorchis aequalis* (GL11844, QM G218820–G218822, G215048–G215050), *Aptorchis megapharynx* (G215065, G215066), and *Aptorchis pearsoni* (G215057, G215058). Sequences of DNA of 4 recognized *Aptorchis* species were taken from GenBank for comparison to the new species: *Aptorchis megacetabulus* (EF014730), *Aptorchis pearsoni* (EF014728), *Aptorchis megapharynx* (EF014727), and *Aptorchis aequalis* (EF014729). The sequence of *Choanocotyle hobbsi* (EU196356) was used as an outgroup in the phylogenetic analysis.

Genomic DNA for molecular analysis was isolated from 2 specimens of the new species collected in 2 different distant localities (Lake Argyle Spillway and Bell Creek, see taxonomic summary for details), according to Tkach and Pawlowski (1999). A single adult worm was used for each DNA extraction upon preliminary morphological identification. DNA fragments of approximately 2,700 base pairs, and spanning the 3′ end of 18S nuclear rDNA gene internal transcribed spacer region (ITS1 + 5.8S + ITS2) and 5′ end of the 28S gene (including variable domains D1–D3), were amplified by PCR on an Eppendorf Master Gradient thermal cycler (Eppendorf AG, Hamburg, Germany) using forward primer ITSf (5′-CGCCCGTCGCTACTACCGATTG-3′) and reverse primer 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′). Additionally, a matching sequence was obtained from a specimen of *Aptorchis aequalis* collected by Dr. Thomas Platt in 1993 from *Emydura kreffti* captured in Ross River Dam near Townsville, Queensland. PCR primers and several internal primers were used in sequencing reactions. Internal forward primers: digl2 (5′-AAGCATATCACTAAGCGG-3′), 300F (5′-CAAGTACCGTGAGGGAAAGTTG-3′), 900F (5′-CCGTCTTG AAACACGGACCAAG-3′); internal reverse primes: 300R (5′-CA ACTTTCCCTCACGGTACTTG-3′), digl2r (5′-CCGCTTAGTGATAT GCTT-3′), ECD2 (5′-CTTGGTCCGTGTTTCAAGACGGG-3′). PCR reactions were performed according to protocols described by Tkach et al. (2003).

PCR products were purified directly using Qiagen Qiaquick™ (Valencia, California) columns, cycle-sequenced using ABI BigDye™ chemistry, alcohol-precipitated, and run on an ABI Prism 3100™ automated capillary sequencer (Applied Biosystems, Foster City, California). Contiguous sequences were assembled and edited using Sequencher™ ver. 4.1.4 (GeneCodes Corp., Ann Arbor, Michigan) and submitted to GenBank: *Aptorchis glandularis* n. sp. (EU334367, EU334368), *Aptorchis aequalis* (EU334369).

Sequences of all 5 *Aptorchis* species and sequence of *C. hobbsi* were initially aligned using Clustal W as implemented in the BioEdit program, version 7.0.1 (Hall, 1999) and manually refined using BioEdit. The alignment was deposited with European Bioinformatics Institute and is available by anonymous FTP from FTP.EBI.AC.UK in directory/pub/databases/embl/align and from the EMBLALIGN database through SRS at http://srs.ebi.ac.uk (instructions on retrieval can be found at http://www3.ebi.ac.uk/Services/webin/help/webin-align/alignSRShelp.html) under accession ALIGN_001233; exclusion sets

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were added as comments. A maximum likelihood (ML) analysis was performed using PAUP* ver. 4.0b10 (Swofford, 2002). Nodal support was calculated based on 1,000 bootstrap replicates with 100 replicates at each step.

All measurements are in micrometers (μm) unless otherwise stated.

DESCRIPTION

Aptorchis glandularis n. sp.

(Figs. 1–3)

Measurements are based on 13 adult specimens, 7 of them from type locality and 6 from 3 other localities; measurements of holotype appear in text; measurements of entire series used in description appear in Table I. Body elongate, widest near level of testes; body length 3,913, body width at level of ventral sucker 1,000. Body width 25.6% of body length. Tegument thick, spination heaviest anterior of ventral sucker; spines decrease in size and density as they extend to posterior end and disappear a short distance posterior to level of testes. Ventral surface with 3 rows of glandular protrusions in hindbody (Figs. 1B, C, 3B, F). Each row contains 6 glands in the holotype and 6 or 7 glands in other specimens of the type series; total number of glands 18 to 21. Oral sucker rounded, subterminal, 235 long × 360 wide, slightly smaller than rounded ventral sucker, 284 × 297. Ventral sucker situated in anterior half of body at 36.3% of body length; body surface aspinose.

Prepharynx 151 long, widening posteriorly. Muscular pharynx, 185 long × 170 wide. Esophagus 240, surrounded by group of glandular cells. Intestinal bifurcation 776 from anterior end of body. Ceca nearly reach posterior end of body, terminating 128 from end.

Testes 2, opposite, spherical to subspherical, postovarian, ventral to ceca in posterior third of body, closer to posterior body end than to ventral sucker. Left testis 204 long × 194 wide, right testis 204 × 177. Testes intracecal with lateral margins occasionally overlapping ceca. Cirrus sac very large, 1,013 long, tortuous, passing intracecally, dorsal to and within margins of ventral sucker. In some specimens, middle part of cirrus sac forms a loop (Fig. 2). Base of cirrus sac well posterior of posterior margin of ventral sucker. Cirrus sac with bipartite internal seminal vesicle, very large pars prostatica. Everted cirrus not observed. Genital pore ventral, submedian, dextral or sinistral, anterior to ventral sucker, 1,136 from anterior end of body.

Ovary spherical, 153 long × 157 wide, situated midway between posterior portion of cirrus sac and anterior margin of testes. Seminal receptacle thick-walled, 119 long × 120 wide, immediately posterior to and slightly overlapping ovary. Vitellarium begins 215 posterior of ventral sucker, consists of small, irregularly shaped follicles, arranged in mostly extracecal lateral fields that merge at posterior end of body forming ''U.'' Ootype and Mehlis' gland just posterior to ovary and ventral to seminal receptacle. Laurer's canal not visible in total mounts. Uterus ventral to other organs, mostly intra- and post-cecal, with occasional extracecal loops. Metraterm approximately as long as cirrus sac. Metraterm passes dorsal to cirrus sac before opening into genital atrium. Eggs operculate, 28 × 16. Excretory pore dorsal, subterminal; excretory vesicle Y-shaped, reaches the level of ootype.

Taxonomic summary

Type host: Northwestern red-faced turtle, *Emydura australis* Gray, 1841 (**Chelonia**: Pleurodira: Chelidae).

Type locality: Lake Argyle Spillway, Ord River, Western Australia, 16°07.371′S, 128°44.271′E.

Other localities: Bell Creek, King Leopold Ranges Conservation Reserve, Kimberley Region, Western Australia, 17°10.159′S, 125°21.521′E; King River Pool, King River, Kimberley Region, Western Australia, 15°39.770′S, 128°05.237′E; Miner's Pool, Drysdale Station, Drysdale River, Kimberley Region, Western Australia, 15°40.810′S, 126°24.270′E.

Site of infection: Rectum.

Prevalence and intensity of infection: Three of 4 *E. australis* from Lake Argyle were infected with 3, 4, and 9 worms; 1 of 3 turtles from Bell Creek was infected with 10 worms; 4 of 4 turtles from King River Pool were infected with 1 to 4 worms each; and 3 of 4 turtles from Miner's Pool were infected with 2, 4, and 5 worms.

Specimens deposited: The type series consists of 7 fully mature specimens. Holotype: Queensland Museum (QM) no. G228979. Paratypes: QM no. G228980–228982; Harold W. Manter Laboratory no. HWML HWML48613 (a lot of three specimens). All specimens labeled ex. *Emydura australis*, Lake Argyle Spillway, Ord River, Western Australia, 29 May 2006, ATP-104A.

Etymology: The specific epithet refers to the unique glandular structures on the ventral surface of these parasites.

Remarks

Based on general morphology, the new species belongs to *Aptorchis* Nicoll, 1914 (Nicoll, 1914; Platt and Jensen, 2002), although it differs from the 4 previously described *Aptorchis* species in that it possesses large glandular structures on the ventral surface (Figs. 1, 3). In addition, *Aptorchis glandularis* n. sp. is considerably larger than 3 of the 4 described species (Jue Sue and Platt, 1999; Tkach and Snyder, 2007a), being as much as 4 times larger than *Aptorchis megacetabulus* and nearly twice the length of *A. pearsoni* and *A. megapharynx*. The new species also differs from these 3 *Aptorchis* in possessing a tortuous cirrus sac found in only 1 other species of *Aptorchis*, namely *A. aequalis*. In both size and overall morphology, the new species is strikingly similar to *A. aequalis* and would be difficult to confidently differentiate at first glance, were it not for the glandular ventral surface of *Aptorchis glandularis* n. sp. (ventral surfaces of both species can be compared on Fig. 3B, D). Examination of *A. aequalis* type and voucher specimens from the Queensland Museum, as well as numerous specimens collected by the authors, reveal that specimens of *Aptorchis glandularis* n. sp. have testes opposite to one another, whereas *A. aequalis* have a tendency towards oblique testes. In addition, the oral sucker of *Aptorchis glandularis* n. sp. is only slightly smaller than the ventral sucker, with an average oral sucker to ventral sucker width ratio of 0.9: 1. In contrast, 33 specimens of *A. aequalis* had a ratio of 0.72:1 (data from Platt and Jensen, 2002). The above morphological differences allow for the reliable differentiation of *A. megacetabulus* n. sp. from previously described species of *Aptorchis*.

Several new morphological features, especially the remarkable ventral glands, found in *Aptorchis glandularis* n. sp. but not in previously described species of the genus, necessitate an amendment of the generic diagnosis of *Aptorchis*. The generic diagnosis proposed by Platt and Jensen (2002) has been modified to incorporate additional features and is provided below. Some morphological details that we consider excessive in a generic diagnosis have been omitted. The excretory vesicle in adult *Aptorchis* is considered here a modification of the Y-shaped type.

Aptorchis Nicoll, 1914

Diagnosis: Plagiorchiida, Plagiorchioidea. Body elongate, slightly dorsoventrally flattened. Tegument armed; body-spines in quincunx, extend to testes or posterior end. Ventral surface posterior to ventral sucker may have large glands arranged in 3 longitudinal rows. Oral sucker approximately equal in size or smaller than ventral sucker; prepharynx of variable length. Pharynx larger or smaller than oral sucker. Esophagus usually short; anterior caecal diverticula present or absent; caeca extend almost to posterior end. Ventral sucker in anterior half of body, spined or not, with 9 papillae in mouth and 6 papillae around sucker. Entire reproductive system may demonstrate dextral or sinistral orientation. Testes 2, entire, inter-caecal, in posterior third of body, symmetrical, diagonal, or tandem. Cirrus sac large, sinuous or tortuous, may form complete loop. Cirrus sac extends well posterior to ventral sucker, contains unarmed cirrus, glandular pars prostatica and bipartite seminal vesicle occupying less than half its volume. Genital pore in forebody or at level of anterior margin of ventral sucker, submedian or sublateral, sinistral or dextral. Ovary median or submedian, in posterior half of body, between testes and ventral sucker. Seminal receptacle median or submedian, usually postero-dorsal, sometimes lateral to ovary. Mehlis' gland present, surrounds proximal end of uterus. Laurer's canal long, thick walled, opens onto dorsal surface anterior or posterior to testes. Uterus with single convoluted loop, comprising descending and ascending arms, inter- and post-caecal, extending to posterior extremity. Vitelline follicles large or small, extend in ventro-lateral extracaecal fields from posterior level of cirrus-sac almost to ends of caeca or to posterior extremity; in latter case vitelline fields may merge posteriorly. Excretory pore subterminal; excretory vesicle in adult worms Y-shaped with very long stem and short arms. In gut of Australian freshwater turtles.

Type-species: A. aequalis Nicoll, 1914. Additional species: *A. pear-*

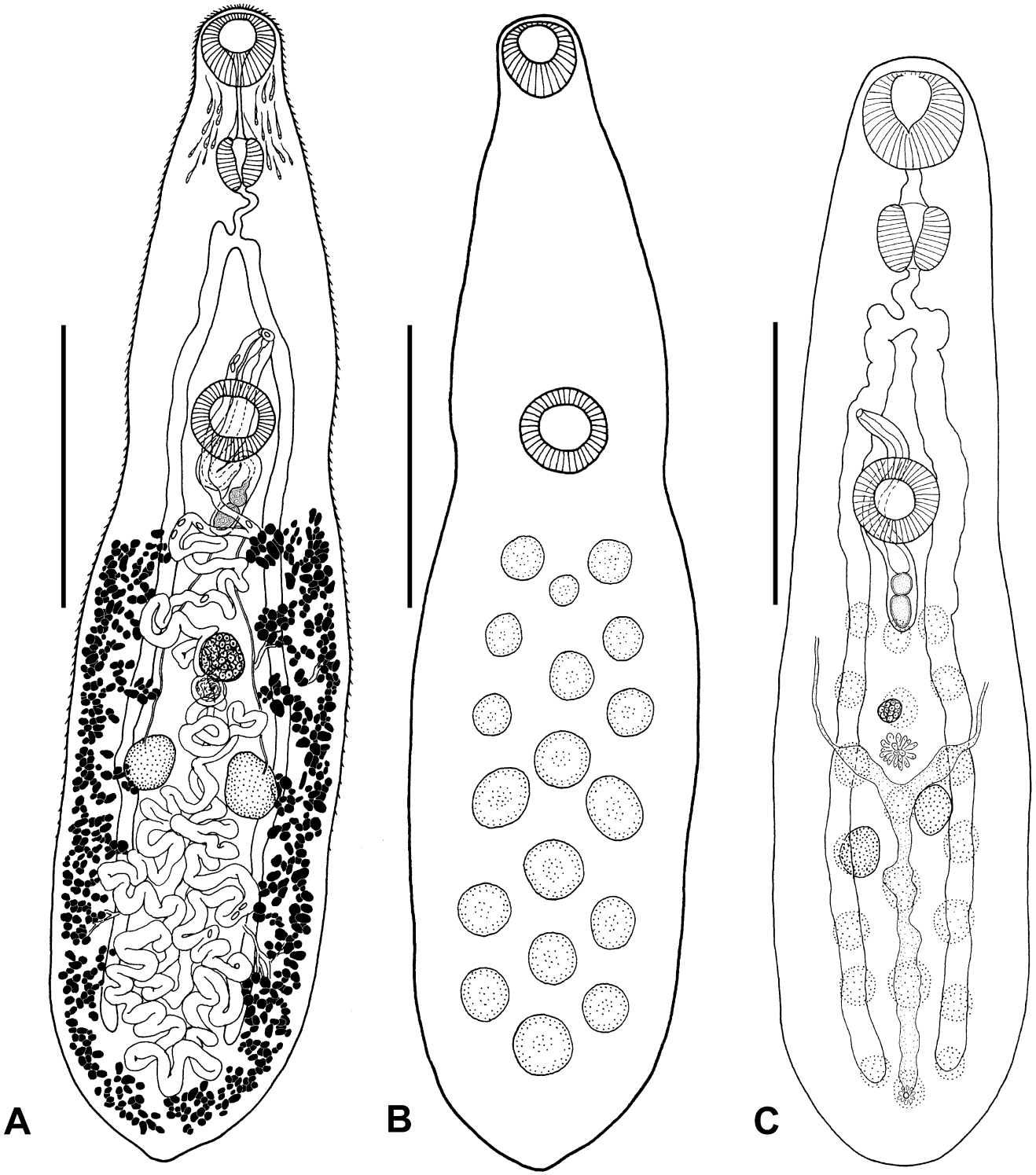

FIGURE 1. *Aptorchis glandularis* n. sp. (**A**) Ventral view of holotype not showing ventral glands. (**B**) Ventral view of holotype showing arrangement of ventral glands. (**C**) Immature specimen showing arrangement of ventral glands (note that there are 7 glands in lateral rows and 6 glands in middle row versus 6 glands in each row in holotype), position of excretory pore, and shape of excretory vesicle. Scale bars: A, B = 1,000 μm, C = 500 μm.

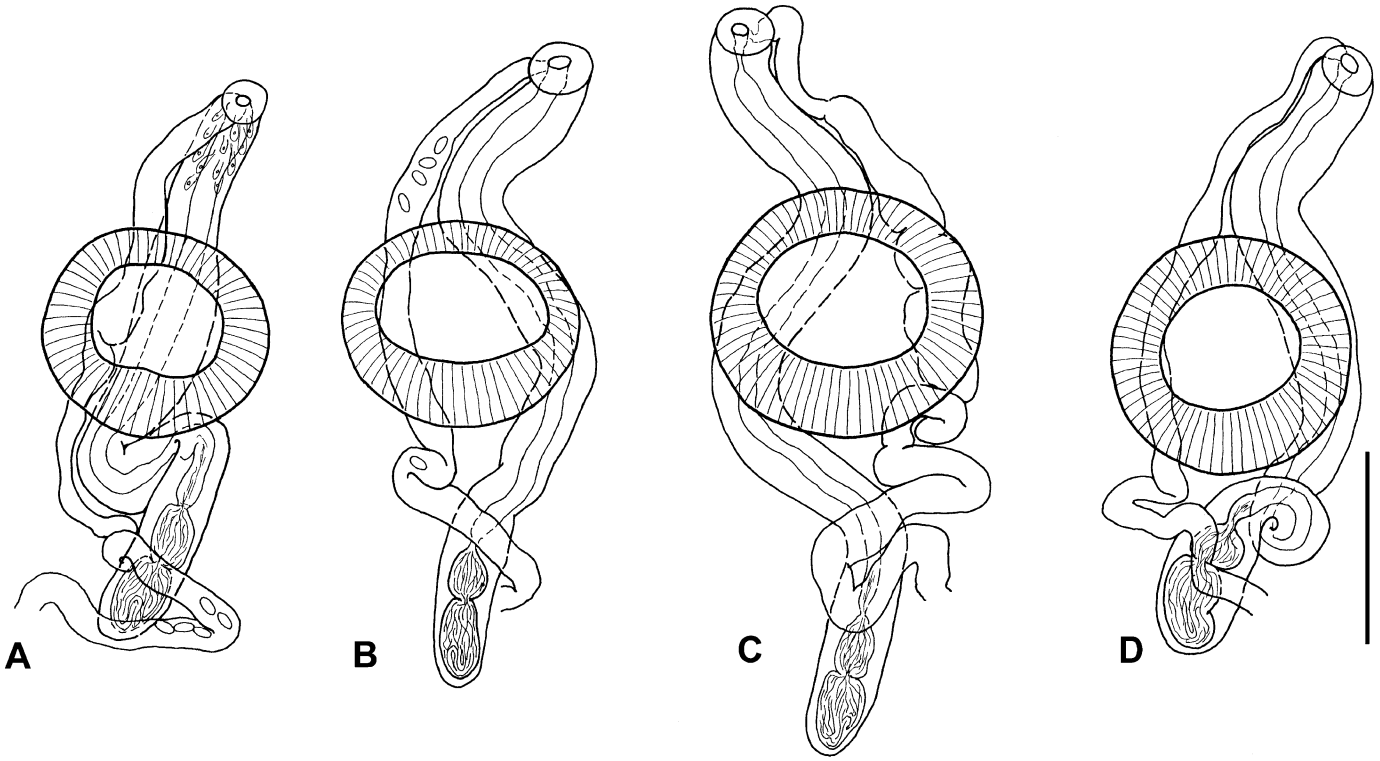

FIGURE 2. Variability of cirrus sac shape and position of genital pore in *Aptorchis glandularis* n. sp. (**A**) Holotype; (**B–D**) Paratypes. Scale Bar = 250 μm.

soni (Jue Sue & Platt, 1999); *A. megapharynx* (Jue Sue & Platt, 1999), *A. megacetabulus* Tkach and Snyder, 2007 and *A. glandularis* n. sp.

Molecular data: No intraspecific variability was observed among 2 specimens of *A. glandularis* n. sp. collected from different localities (Lake Argyle Spillway, Ord River, and Bell Creek, Kimberley Plateau) and situated at a distance of 380 km from each other. In addition to the distance, the 2 localities are separated in elevation and by several river drainages. Similarly, 2 individuals of *A. aequalis* collected from the same locality in 1993 and 2004 did not show any intraspecific sequence variability.

To compare interspecific sequences variability, we aligned seqeunces of *A. glandularis* n. sp. with sequences of all previously known *Aptorchis* species. The aligned fragments of DNA sequence from the 5 *Aptorchis* species ranged in length from 2,355 bp in *A. megapharynx* to 2,551 bp in *A. aequalis*, and was 2,358 bp long in the new species. The substantial difference in length is largely due to presence of a 49-bp-long repeat fragment in the ITS1 region. The number of repeats varies among species of *Aptorchis*, accounting for most, but not all, of the differences in sequence length. Pairwise sequence comparison among *A. glandularis* n. sp. and the other species in the genus demonstrates substantial variability outside of the repeat zone (Table II), with the new species most similar to *A. aequalis* across all ribosomal regions. Thus, the sequence data correspond with the morphological similarity of *A. glandularis* n. sp and *A. aequalis*, while demonstrating substantial differences between the new species and all previously described species of *Aptorchis*, confirming the status of *A. glandularis* n. sp. as a new species.

Our search for the most appropriate outgroup for phylogenetic analysis of *Aptorchis* identified *Choanocotyle* as the most closely related taxon of the numerous species of plagiorchiate digeneans available in the GenBank and in the authors' extensive unpublished sequence database. The tree resulting from a maximum likelihood analysis confirmed the close interrelationships (100% bootstrap support) among *A. glandularis* n. sp. and *A. aequalis* (Fig. 4). Another strongly supported clade includes *A. megacetabulus* and *A. pearsoni*, while *A. megapharynx* apparently represents a third lineage.

DISCUSSION

Aptorchis glandularis n. sp. is the first species of helminth reported from *Emydura australis* and was not found in syntopic *Chelodina burrungandjii*. The new species is the second *Aptorchis* reported from short-necked chelid turtles, along with *Aptorchis aequalis* found in species of *Emydura* and *Elseya*. The most remarkable morphological feature of *A. glandularis* n. sp. is the presence of ventral glandular structures similar to those known in monostome digeneans such as members of Notocotylidae and Microscaphidiidae. The genus *Aptorchis* is not closely related to these groups and belongs to a different and much more derived group of digeneans, namely the Plagiorchioidea. To the best of our knowledge, such structures have not been reported previously for any member of the Plagiorchioidea or any xiphidiatan digenean. At present, we do not have an adequate explanation for this unusual evolutionary acquisition in a single species. It is now obvious, however, that these structures evolved multiple times, independently, in the course of digenean evolution. Interestingly, these glandular structures are found only in digeneans living in the hind gut or rectum of vertebrates.

Aptorchis glandularis n. sp. is characterized by a highly variable cirrus sac shape, i.e., from moderately tortuous to forming a complete loop (Fig. 2). In some closely related plagiorchiatan taxa, i.e., *Choanocotyle* spp., the presence of a cirrus sac loop is considered a stable morphological feature suitable for differentiation among species (Jue Sue and Platt, 1998; Platt and Tkach, 2003). This is clearly not the case with *A. glandularis* n. sp. (Fig. 2). Similar to other *Aptorchis* species (Jue Sue and

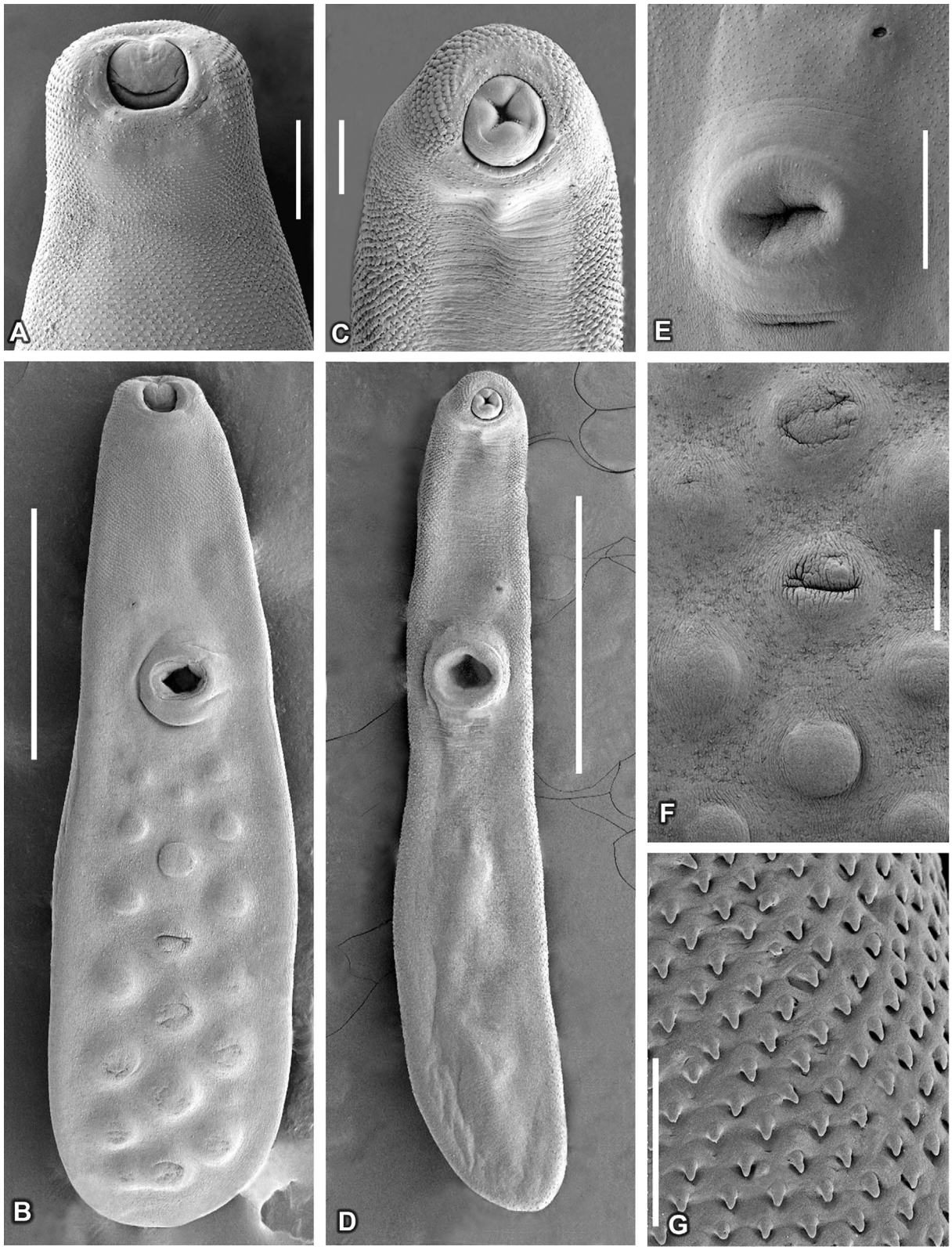

FIGURE 3. Details of the external morphology of *Aptorchis glandularis* n. sp. (**A, B, E, F, G**) and *Aptorchis aequalis* (**C, D**). (**A, C**) Anterior portions of body of both species showing oral sucker and spination. Note areas without spines just posterior to the sucker. (**B, D**) Total view of both species demonstrating presence of ventral glands in *A. glandularis* n. sp. and their absence in *A. aequalis*. (**E**) Area of ventral sucker and genital pore. Note lack of spines around both of these structures. (**F**) Ventral glands. (**G**) Tegumental spines at the ventral side of the body between oral sucker and genital pore. Scale bars: A, C, F = 100 μm; B = 500 μm; D = 1,000 μm; E = 200 μm; G = 30 μm.

TABLE I. Metric data for *Aptorchis glandularis* n. sp.

Characters	n	Min–Max	Mean	StD	CV*
Body length	13	2036–4017	3201.9	748.6	23.4
Body width	13	613–1108	861.2	185.5	17.4
Oral sucker length	13	164–279	223.0	39.9	17.9
Oral sucker width	13	185–305	242.2	40.8	16.8
Prepharynx length	13	21–151	93.9	38.6	41.1
Pharynx length	13	134–209	168.5	23.5	14.0
Pharynx width	13	123–179	157.5	22.0	14.0
Esophagus	13	131–262	182.5	50.1	27.4
Cecal bifurcation to anterior end	13	451–815	607.6	117.5	19.3
Ventral sucker length	13	158–383	259.6	68.0	26.2
Ventral sucker width	13	190–391	275.2	68.2	24.8
Cirrus sac length	12	509–1105	807.4	208.7	25.8
Cirrus sac width	12	49–108	79.4	15.8	19.9
Genital pore from anterior end	13	613–1196	869.0	186.4	21.5
Vitelline field-ventral sucker	13	0–380	193.7	101.7	52.5
Ovary length	13	99–241	144.2	38.5	26.7
Ovary width	13	99–248	146.4	39.2	26.8
Ovary to ventral sucker	13	747–1577	1122.4	250.0	22.4
Seminal receptacle length	11	55–134	100.5	27.6	27.4
Seminal receptacle width	11	56–167	107.9	32.2	29.9
Right testis length	13	108–269	176.8	48.4	27.4
Right testis width	13	103–207	161.5	41.5	25.7
Left testis length	13	111–243	179.5	46.8	26.1
Left testis width	13	92–245	164.2	49.2	30.0
End of cecum to posterior end	11	128–432	276.2	112.6	40.8
Egg length	39	26–37	29.2	3.2	11.1
Egg width	39	14–20	16.4	1.5	8.9

* Coefficient of variation.

Platt, 1999; Tkach and Snyder, 2007a), *Aptorchis glandularis* n. sp. possesses both dextral and sinistral forms. The genital pore can be situated to the left (Figs. 1, 2A, B, D, 3E), or to the right (Figs. 1C, 2C, 3B) of the median line of the body.

Examination of 4 rDNA regions, sequenced as part of this study, revealed that the greatest differences among *A. glandularis* n. sp. and previously described species occur in the ITS1 rDNA (Table II). The level of variability among *A. glandularis* n. sp. and *A. aequalis* was lower than that among any other pair of *Aptorchis* (see Table II and discussion in Tkach and Snyder, 2007a), but was similar to, or greater than, interspecific variability observed within most genera of plagiorchioideans and other digeneans studied thus far (Tkach et al., 2000; Snyder and Tkach, 2001; León-Règagnon and Paredes-Calderón, 2002; Platt and Tkach, 2003; Tkach et al., 2003; Nolan and Cribb, 2005; Olson and Tkach, 2005; Curran et al., 2006; Tkach and Snyder, 2007a, 2007b). In addition, the ITS1 sequence of *A. aequalis* is almost 200 bp longer than that of *A. glandularis* n. sp., due to the presence of several repeats in the ITS1 of the former species and their absence in the latter. Although we did not use this region in pairwise sequence comparisons because of the unclear mutagenic origin of repeats, it nevertheless provides additional evidence that the 2 species are distinct.

TABLE II. Number of variable sites in different nuclear ribosomal DNA regions (partial 18S, complete ITS1, complete 5.8S, complete ITS2, partial 28S) among *Aptorchis glandularis* n. sp. and 4 known *Aptorchis* species. Alignment length for each fragment is shown in parentheses; 3′ end of 18S gene is not included because it is extremely conserved and shows no variability, excluding repeat zone.

	DNA region			
Digenean species	ITS1 (642 bp)	5.8S (157 bp)	ITS2 (252 bp)	LSU (1309 bp)
A. aequalis	12	1	3	7
A. megacetabulus	21	1	14	20
A. pearsoni	26	2	13	23
A. megapharynx	21	2	14	18

Phylogenetic analysis of all 5 known *Aptorchis* species indicates the presence of 3 distinct evolutionary lineages (Fig. 4). The morphologically similar *A. aequalis* and *A. glandularis* n. sp. are parasites of the short-necked chelid turtles, *Emydura*, with *A. aequalis* also reported from another short-necked turtle, *Elseya latisternum*. *Aptorchis aequalis* has been recovered from Queensland and New South Wales (Nicoll, 1914; Jue Sue and Platt, 1999; Platt and Jensen, 2002), but was not found in our examinations of short-necked turtles from multiple sites in the Northern Territory or Western Australia. The second strongly supported lineage of *Aptorchis* spp. includes *A. pearsoni* and *A. megacetabulus*, both parasites of long-necked chelids; *Chelodina* spp. *Aptorchis pearsoni* is reported from eastern Australia, in both Queensland and New South Wales, whereas *A. megacetabulus* has been found in the Northern Territory and the Kimberley region of Western Australia (Platt and Jensen, 2002;

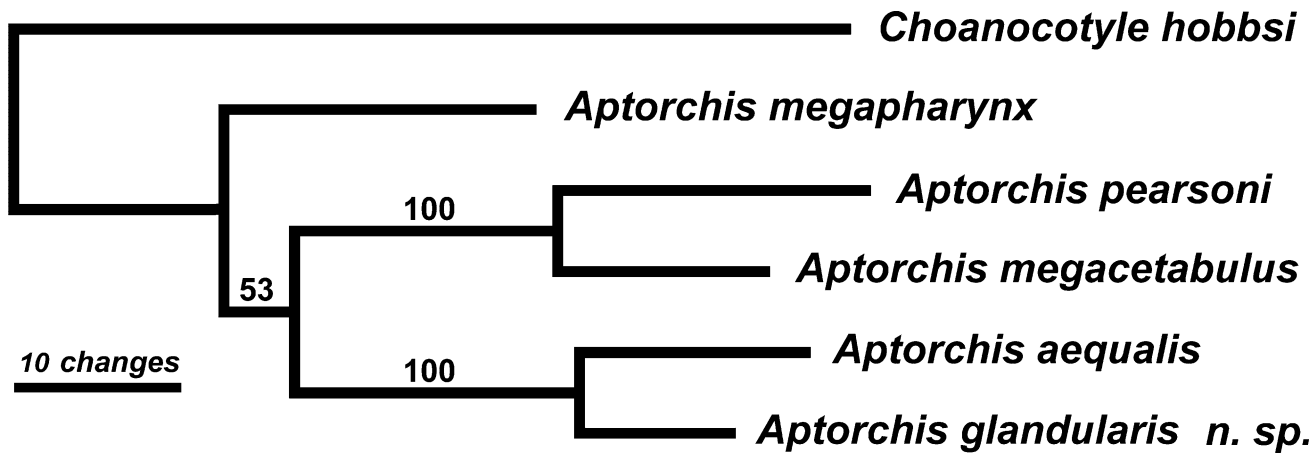

FIGURE 4. Phylogenetic relationships among species of *Aptorchis* spp. Numbers above nodes show bootstrap support from maximum likelihood analysis in PAUP (1,000 bootstraps with 100 replicates at each step).

data not shown). The third lineage is represented by *A. megapharynx*, a parasite of *Chelodina* from southern Queensland and New South Wales (Platt and Jensen, 2002; Tkach and Snyder, 2007a). Interestingly, *A. megapharynx* had the shortest sequence of all 5 *Aptorchis* species compared.

Chelodina spp. represent a distinct evolutionary lineage from the *Emydura* + *Elseya* lineage (Georges and Thomson, 2006), a pattern reflected in the apparent specificity of *Aptorchis* species to distinct turtle groups. Moreover, 2 of the 3 parasite lineages are comprised of species with discreet geographical distributions. We anticipate that additional species of *Aptorchis* await discovery in Australia, and that these parasites will reflect patterns of host specificity and geographical distribution uncovered thus far.

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