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 Microschaphidiidae. 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. megachelis) and (A. aequalis + A. glandularis n. sp.), with A. megachelis 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 burrengandjii 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 Dumar 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 megachelis (G215086, G215086), and Aptorchis pearsoni (G215057, G215058). Sequences of DNA of 4 recognized Aptorchis species were taken from GenBank for comparison to the new species: Aptorchis megachelis (EF014730), Aptorchis pearsoni (EF014728), Aptorchis megachelis (EF014727), and Aptorchis aequalis (EF014729). The sequence of Choanoctyle 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 TSR (5’-CGCCCGCTGCCTACTACGGATGTG-3’) and reverse primer 1500R (5’-GCTATCCTGGAGGAAAACCTCG-3’). Additionally, a matching sequence was obtained from a specimen of Aptorchis aequa-ulis 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’-AACTATATACATACTACGCGG-3’), 300F (5’-CAAGTACCGTGAGGGAAACTTCG-3’), 900F (5’-CCGCTTCTTAAACCCGACCAAG-3’); internal reverse primers: 300R (5’-CACTTCCCTCAGGGTACTCTT-3’), digl2r (5’-CGCCTTTGATATGCTT-3’), ECD2 (5’-TTTGGTCCGGTTTCAAGAAGGG-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://www.3.67.4.80/Services/webin/help/align.html) under accession ALIGN _001233; exclusion sets

Received 22 August 2007; revised 23 December 2007; accepted 28 December 2007.  
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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 1. 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. Tegment 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 × 210 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 sub-spherical, 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. Circus sac very large, 1,013 long, tortuous, passing intraceally, dorsal to and within margins of ventral sucker. In some specimens, middle part of circus sac forms a loop (Fig. 2). Base of circus sac well posterior of posterior margin of ventral sucker. Circus sac with bipartite internal seminal vesicle, very large pars prostatica. Everted circus 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. Vitellaria 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. Metatremar approximately as long as cirrus sac. Metatremar 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, 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.


**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 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 exces- sive 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:** Plagiorchiidae, Plagiochiroidea. Body elongate, slightly dorso-ventrally flattened. Tegment 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 extracecal 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; excre- tory 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.
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 sequences 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 burrungangdijji*. 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.

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* Coefficient of variation.

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-Régagnon 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.

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;
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 *Emydoura + 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.

**ACKNOWLEDGMENTS**

We thank Dr. Tony Tucker, Dr. Nancy FitzSimmons, and Maggie Snyder for collection assistance, and Gordon Graham of CALM Nunurra for making available laboratory space. This material is based upon work supported by the National Science Foundation under Grants NSF 0515460, 0132289, and 0515492. Support was also received from NSF 0515460, 0132289, and 0515492. Support was also received from the Research Committee of the Department of Biology, University of North Dakota (VVT) and the University Committee on Research of the University of Nebraska at Omaha (SDS).

**LITERATURE CITED**


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