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APTORCHIS MEGACETABULUS N. SP. (PLATYHELMINTHES: DIGENEA) FROM THE NORTHERN LONG-NECKED TURTLE, *CHELODINA RUGOSA* (PLEURODIRA: CHELIDAE), IN AUSTRALIA

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ABSTRACT: *Aptorchis megacetabulus* n. sp. is described from the intestine of the northern long-necked turtle, *Chelodina rugosa* (Pleurodira: Chelidae), in Northern Territory, Australia. This is the first helminth species reported from *C. rugosa*. This plagiorchioidean digenean differs from the 3 previously known species of *Aptorchis* in the relative size of the ventral sucker, overall body proportions, nature of the cirrus sac, and egg size. Comparison of approximately 2,700 bases of ribosomal DNA obtained from all known *Aptorchis* species strongly supports the status of *Aptorchis megacetabulus* n. sp. as a new species.

Aptorchis is currently represented by 3 species, all recovered from Australian freshwater turtles. Nicoll (1914) described *Aptorchis aequalis* from the saw-shelled turtle, *Elseya latisternum*, in northern Queensland. Jue Sue and Platt (1999) proposed a new genus *Dingularis* with 3 species, *Dingularis anfracticirrus*, *Dingularis pearsoni*, and *Dingularis megapharynx*. *Dingularis anfracticirrus* was later synonymized with *Aptorchis aequalis*, and *D. pearsoni* and *D. megapharynx* were transferred into *Aptorchis* by Platt and Jensen (2002). In addition to *E. latisternum*, *A. aequalis* has been reported from Krefft's river turtle, *Emydura krefftii*, and the Murray turtle, *Emydura macquarii*. These reports have come from northern and southern Queensland, with a single report from northern New South Wales (Jue Sue and Platt, 1999; Platt and Jensen, 2002). *Aptorchis pearsoni* and *A. megapharynx* are both reported from the broad-shelled river turtle, *Chelodina expansa*, in southern Queensland (Jue Sue and Platt, 1999). The current report describes a new species of *Aptorchis* from the northern snake-necked turtle, *Chelodina rugosa*, in Northern Territory, Australia.

Chelodina rugosa Ogilby, 1890, is distributed across tropical northern Australia and lives in variety of aquatic habitats including swamps, rivers, and billabongs (Cogger, 2000). These turtles are carnivorous, primarily feeding on aquatic arthropods and fish (Kennett and Tory, 1996), food items that should bring these turtles into contact with the infective stages of a number of helminth parasites. To date, however, no published reports exist of helminths from *C. rugosa*. In May of 2004 and May and June of 2005, we undertook a preliminary survey of parasites of *C. rugosa* in Northern Territory, Australia. A number of nematodes, cestodes, and digeneans were recovered, most of which appear to be new to science. Herein we describe a new species of *Aptorchis* from this collection, the first helminth species reported from *C. rugosa*.

MATERIALS AND METHODS

In 2004, 2 *C. rugosa* were collected from the Mary River on the Opium Creek Station in a baited crab trap. In 2005, 4 *C. rugosa* were collected by hand from the Daly River, near Oolloo Crossing, and 1 *C. rugosa* was collected by hand from the Daly River near the town of Daly River. All localities are in Northern Territory, Australia; collection proceeded under permits from the Northern Territory Parks and Wildlife Commission. Seven adult and 3 juvenile specimens of a new digenean species belonging to *Aptorchis* were recovered from the intestine of 3 *C. rugosa*. Live 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, dehydrated in a graded ethanol series, cleared in clove oil, and mounted permanently in Damar balsam.

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

Specimens of all 3 previously known *Aptorchis* species were collected for comparative morphological and molecular analysis from New South Wales and Queensland under permits from appropriate state agencies, and voucher specimens deposited (Table I) in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln. 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* QM G215065–G215066; *Aptorchis pearsoni* QM G215057–G215060.

Genomic DNA for molecular analysis was isolated from specimens of *Aptorchis megacetabulus* n. sp. and 3 other *Aptorchis* species according to Tkach and Pawlowski (1999) or using the Qiagen DNAeasy tissue kit (Qiagen, Inc., Valencia, California) following the manufacturer's instructions. 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 using forward primer ITSf (5′-CGCCCGTCGCTACTACCGATTG-3′) and reverse primer 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′). PCR primers and several internal primers were used in sequencing reactions. Internal forward primers: digl2 (5′-AAGCATATCACTAAGCGG-3′), 300F (5′-CAAGTACCGTGAGGGAAAGTTG-3′), 900F (5′-CCGTCTTGAAACACGGACCAAG-3′); internal reverse primers: 300R (5′-CAACTTTCCCTCACGGTACTTG-3′), digl2r (5′-CCGCTTAGTGATATGCTT-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™ columns, 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 (see Table I). Sequences were manually aligned and compared using the BioEdit program, version 7.0.1 (Hall, 1999).

DESCRIPTION

Aptorchis megacetabulus n. sp.

(Figs. 1–2)

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

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TABLE I. Species of *Aptorchis*, host, geographical origin, GenBank accession numbers, and HWML accession numbers.

Digenean species	Host species	Geographic origin	GenBank no.	HWML no.
Aptorchis megacetabulus n. sp.	*Chelodina rugosa*	Mary River, Northern Territory	EF014730	HWML48423–48425
Aptorchis pearsoni	*Chelodina expansa*	Murray River, Albury, New South Wales	EF014728	HWML48426
Aptorchis megapharynx	*Chelodina longicollis*	Yass River, Yass, New South Wales	EF014727	HWML48427
Aptorchis aequalis	*Emydura krefftii*	Ross River, Townsville, Queensland	EF014729	HWML48428

elongate, widest at its midpoint; body length 860, body width at level of ventral sucker 230. Body width 26.7% of body length. Tegument thick, heavily armed at anterior end; spines decrease in size and density as they extend to posterior end. Oral sucker rounded, subterminal, 85 long × 95 wide, much smaller than rounded ventral sucker, 180 × 185. Ventral sucker situated in middle of body, surface covered with minute spines with 2 rings of larger spines at the periphery. Oral sucker/ventral sucker width 51.3%.

Prepharynx 60 long, widening posteriorly. Muscular pharynx, 75 long × 77.5 wide. Esophagus very short, sometimes almost indistinct, 10. Esophagus is surrounded by a group of single-cell glands. Intestinal bifurcation 250 from anterior end of body. Ceca nearly reach the posterior end of body terminating 65 from end.

Testes 2, rounded, oblique, postovarian, ventral to ceca in posterior third of body, midway between ventral sucker and posterior body end. Left testis usually anterior to right testis. Left testis 65 long × 50 wide, right testis 70 × 57. Cirrus sac very large, 248 long, ventral to ceca, arching extracecally along one lateral margin (usually right) of ventral sucker. Base of cirrus sac just posterior or at level of posterior margin of ventral sucker. Cirrus sac with bipartite internal seminal vesicle, very large pars prostatica and cirrus. Evaginated cirrus not observed. Genital pore submarginal, 315 from anterior end of body.

Ovary small, 60 long × 45 wide, median or slightly submedian, usually spherical, dorsal to ceca, situated just posterior to proximal part of cirrus sac, near posterior margin of ventral sucker. Vitellarium consists of small, irregularly shaped follicles, arranged in lateral fields partly overlapping ceca or extracecal. Vitelline fields extend posteriorly, 105 from posterior body end. Anteriorly, left vitelline field reaches 15 from posterior margin of ventral sucker, right vitelline field reaches 30 from posterior margin of ventral sucker. Vitelline fields not confluent. Seminal receptacle thick-walled, relatively large, 70 long × 60 wide, adjacent to ovary. Mehlis's gland ventral to seminal receptacle. Laurer's canal not visible in total mounts. Uterus inter- and postcecal, postacetabular; metraterm approximately as long as cirrus sac. Metraterm passes dorsal to cirrus sac before opening into genital atrium. Eggs operculate, 25 × 12.5. Excretory pore terminal; excretory vesicle reaches level of ovary.

Taxonomic summary

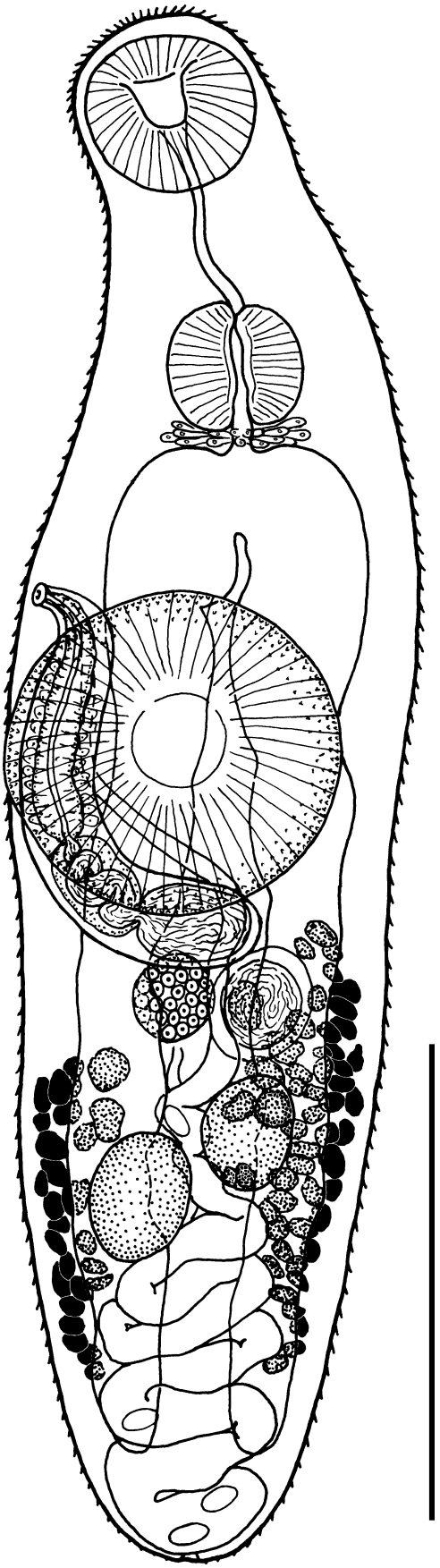
Type host: Northern long-necked turtle, *Chelodina rugosa* Ogilby, 1890 (Chelonia: Pleurodira: Chelidae).

Type locality: Daly River, near town of Daly River, Northern Territory, Australia, 13°44.33′S, 130°41.12′E.

TABLE II. Metric data for *Aptorchis megacetabulus* n. sp.

Character	n	Min-max	Mean	StD	CV*
Body length	4	860–1,070	938.8	141.3	15.1
Body width	6	205–330	268.3	46.8	17.4
Oral sucker length	4	65–95	83.8	13.1	15.7
Oral sucker width	4	75–105	95.0	14.1	14.9
Prepharynx length	4	45–100	71.3	23.9	33.6
Pharynx length	6	65–90	80.8	9.7	12.0
Pharynx width	6	60–90	79.6	12.3	15.4
Esophagus	6	10–30	16.7	7.5	45.2
Ventral sucker length	6	150–210	184.2	19.6	10.6
Ventral sucker width	6	160–220	195.0	21.4	11.0
End of cecum to posterior end	6	35–65	50.8	10.7	21.1
Cecal bifurcation to anterior end	4	220–320	275.0	48.0	17.4
Right testis length	6	70–100	80.8	10.7	13.2
Right testis width	6	55–95	66.1	15.0	22.7
Left testis length	6	65–105	81.7	14.7	18.0
Left testis width	6	50–92.5	67.9	15.5	22.9
Cirrus sac length	6	248–450	314.6	72.8	23.2
Cirrus sac width	6	50–67.5	55.8	6.5	11.6
Genital pore from anterior end	4	300–403	348.1	48.6	14.0
Ovary length	6	60–75	68.3	6.1	8.9
Ovary width	6	45–75	55.8	12.0	21.5
Seminal receptacle length	6	55–92.5	75.4	12.5	16.6
Seminal receptacle width	6	45–90	69.5	15.5	22.3
Vitelline field-posterior body end	5	70–130	106.0	10.7	13.3
Left vitelline field-ventral sucker	6	0–75	21.7	27.9	128.6
Right vitelline field-ventral sucker	6	0–50	29.2	18.8	64.5
Egg length	12	25–27	26.0	0.8	3.0
Egg width	12	12–14	12.9	0.9	6.6

* Coefficient of variation.



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.

Site of infection: Small intestine.

Prevalence and intensity of infection: Three of 7 *C. rugosa* were infected with 7, 1, and 1 worms, respectively.

Specimens deposited: The type series consists of 6 fully mature specimens, 2 without oral sucker. Holotype: Queensland Museum (QM) no. G227476; Paratypes QM no. G227477–G227478. Harold W. Manter Laboratory no. HWML48423–48425. All labeled identically: ex. *Chelodina rugosa*, Daly River, Northern Territory, Australia, June 2005.

Etymology: The specific epithet refers to the ventral sucker which is larger in size, compared to the total body length, than in congeneric species.

Remarks

Based on general morphology, the new species belongs to *Aptorchis* Nicoll, 1914, although it differs from the 3 previously described *Aptorchis* species in that it possesses a submarginal, rather than submedian, genital pore. In addition, *Aptorchis megacetabulus* n. sp. is smaller than all 3 previously described species, being nearly half the length of *A. pearsoni* and *A. megapharynx* and only 1/4 the length of *A. equalis*. The length of the ventral sucker in *A. megacetabulus* n. sp. is 19.6% (17.3–20.9%) of the total body length, a much greater proportion than in the other species of *Aptorchis* (9.5% in *A. aequalis*, 9.8% in *A. pearsoni*, 11.9% in *A. megapharynx*) (data from Jue Sue and Platt, 1999). The new species is also considerably more robust than those previously described. The body width of *Aptorchis megacetabulus* n. sp. is on average 28.6% (26.7–30.8%) of the body length, whereas this value is 18.9% in *A. aequalis*, 16.5% in *A. pearsoni*, and 19.6% in *A. megapharynx* (data from Jue Sue and Platt, 1999). The eggs of *Aptorchis megacetabulus* n. sp. range from 25 μm–27 μm long × 12 μm–15 μm wide, smaller than other known representatives of *Aptorchis*. *Aptorchis aequalis* eggs range from 32 μm–35 μm × 19 μm–21 μm, *A. pearsoni* eggs range from 29 μm–31 μm × 16 μm–18 μm, and *A. megapharynx* eggs range from 31 μm–32 μm × 18 μm–19 μm (Jue Sue and Platt, 1999).

In all 3 previously described *Aptorchis* species, the ovary is situated a substantial distance from the ventral sucker. In *A. pearsoni* and *A. megapharynx*, the ovary lies midway between the ventral sucker and the posterior end of the body, and in *A. aequalis* the ovary lies nearly equidistant between the ventral sucker and the testes. This contrasts with the close association of the ovary of *Aptorchis megacetabulus* n. sp. with the ventral sucker; it is separated from the ventral sucker by only the proximal portion of cirrus sac. The new species also clearly differs from *A. pearsoni* and *A. megapharynx* in the shape of the cirrus sac. In *A. megacetabulus* n. sp., the cirrus sac arches around the ventral sucker with the proximal part adjacent to the posterior margin of the ventral sucker. In both *A. pearsoni* and *A. megapharynx*, the proximal part of the cirrus sac is straight and extends well posterior of the posterior margin of ventral sucker. The above morphological differences allow for the reliable differentiation of *A. megacetabulus* n. sp. from previously described species of *Aptorchis*.

The fragments of DNA sequence from the 4 *Aptorchis* species ranged in length from 2,467 bp in *A. megapharynx* to 2,663 bp in *A. aequalis* and were 2,565 bp long in the new species. The substantial difference in length is largely due to presence of a 49-bp-long repeated 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. megacetabulus* n. sp. and the other 3 species has demonstrated substantial variability outside of the repeat zone (Table III). Interestingly, the number of variable sites in a rather short ITS2 region (9–14 variable sites) was comparable to the number of variable sites in a much longer ITS1 region (16–20) and partial 28S gene (12–22). Thus, sequence comparison (Tables III and IV) demonstrates substantial differences between *A. megacetabulus* n.

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FIGURE 1. Holotype of *Aptorchis megacetabulus* n. sp., ventral view. Scale bar = 250 μm.

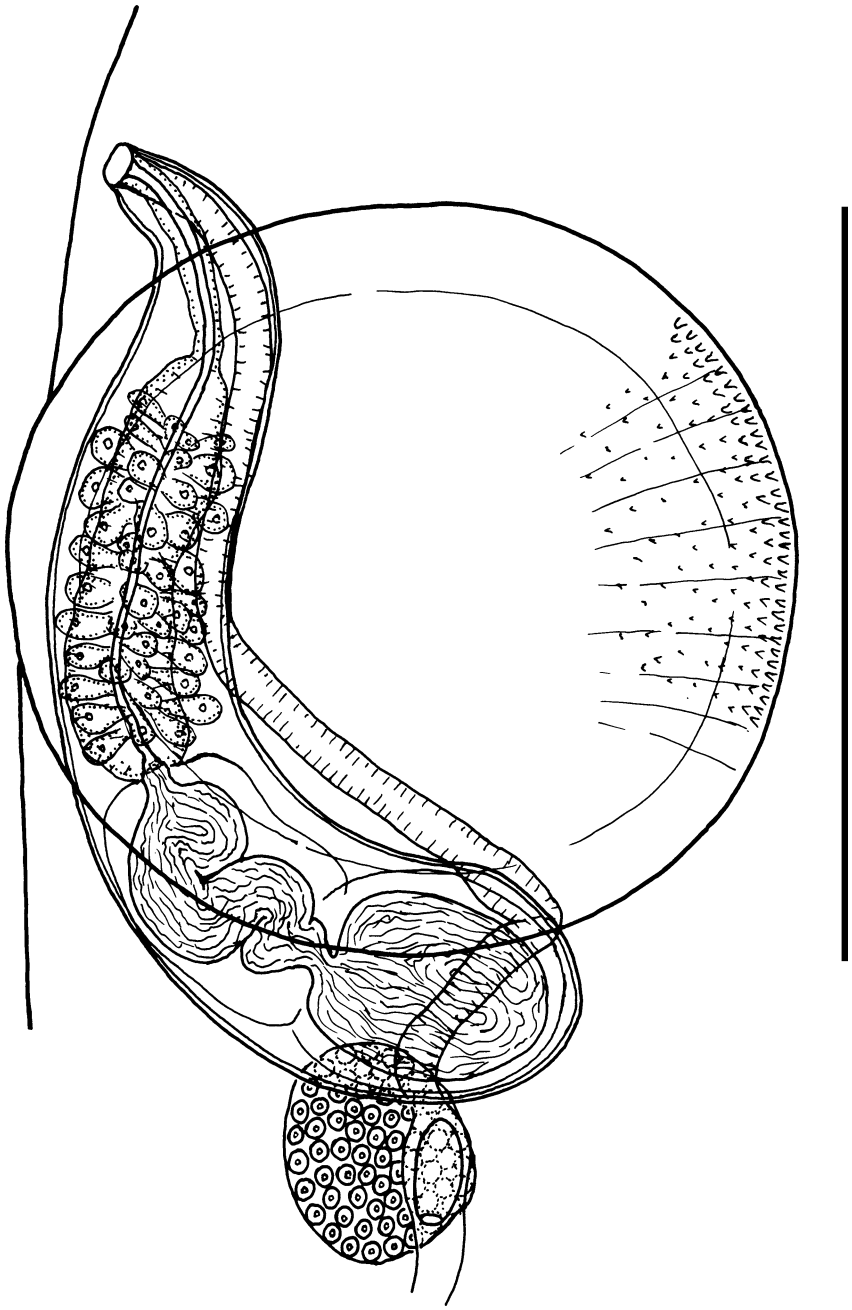

FIGURE 2. Cirrus sac, metraterm and ventral sucker of *Aptorchis megacetabulus* n. sp. Scale bar = 200 μm.

sp. and all previously described species of *Aptorchis*, confirming the status of *A. megacetabulus* n. sp. as a new species.

DISCUSSION

Aptorchis megacetabulus n. sp. is the first species of helminth reported from *Chelodina rugosa* and was not found in 4 additional species of turtles (*Carettochelys insculpta* Ramsay, 1886, *Elseya dentata* Gray, 1863, *Emydura tanybaraga* Cann, 1997, and *Emydura victoriae* Gray, 1842) collected at or near the type locality. The new species is the third *Aptorchis* reported from long-necked turtles, *Chelodina*. *Aptorchis pearsoni* and *A. megapharynx* were described from *Chelodina expansa* taken from sites in southern Queensland (Jue Sue and Platt, 1999) and are reported from *Chelodina* spp. from southern New South Wales as part of the current study (Table I). *Aptorchis aequalis* is a parasite of species of *Emydura* and *Elseya*, but all 3 previously described species utilize the planorbid snails *Glyptophysa gibbosa* as first intermediate hosts and form metacercariae in tadpoles and snails (Jue Sue and Platt, 1999). *Glyptophysa gibbosa* occurs only in southeast Australia, but *Glyptophysa badia* is found in Northern Territory, as are species representing *Amerianna* and *Bayardella*, 2 other planorbid genera (Walker, 1988). The likely occurrence of planorbid snails in habitats utilized by *C. rugosa* and the presence of snails and tadpoles in the diet of

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

	DNA region			
Digenean species	ITS1 excluding repeat zone (644 bp)	5.8S (157 bp)	ITS2 (252 bp)	28S (1,309 bp)
A. pearsoni	16	0	14	12
A. aequalis	18	0	14	12
A. megapharynx	20	1	9	22

TABLE IV. Sequence identity (%) matrix based on 2,666-bp-long sequences of ribosomal DNA (partial 18S, complete ITS1, complete 5.8S, complete ITS2, partial 28S) of 4 *Aptorchis* species.

	Sequence identity			
Digenean species	*A. megacetabulus* n. sp.	*A. pearsoni*	*A. megapharynx*	*A. aequalis*
A. megacetabulus n. sp.	—			
A. pearsoni	95.0	—		
A. megapharynx	94.2	97.8	—	
A. aequalis	94.2	90.6	90.8	—

this turtle (Kennett and Tory, 1996) indicate potential intermediate hosts of *Aptorchis megacetabulus* n. sp.

Similar to other *Aptorchis* species (Jue Sue and Platt, 1999), *Aptorchis megacetabulus* n. sp. demonstrates both dextral and sinistral forms. The genital pore in all but 1 specimen was found on the right side of the body, with the cirrus sac arching around the right side of the ventral sucker and the left testis situated anteriorly to the right testis. In the single inverted specimen, the orientation of these organs was the mirror opposite, a relatively wide spread phenomenon among xiphidiatan digeneans (Tkach et al., 2000; Tkach and Bray, 2001).

Members of *Aptorchis* demonstrate high intrageneric sequence variability in the ITS1 ribosomal DNA, and even the more conserved 28S region is more variable than within most genera of plagiorchioidean digeneans studied thus far (Tkach et al., 2000; Snyder and Tkach, 2001; Platt and Tkach, 2003; Tkach et al., 2003). *Aptorchis pearsoni* and *A. megapharynx* have ribosomal sequences with the greatest degree of similarity, and the sequences of these species are most different from *A. aequalis* (Table IV). These 3 species occur sympatrically, but *A. pearsoni* and *A. megapharynx* are parasites of species of *Chelodina*, whereas *A. aequalis* is found in species of *Emydura* and *Elseya*. *Aptorchis megacetabulus* n. sp. is a parasite of *C. rugosa*, but has sequence identity of between 94% and 95% with all 3 previously described species (Table IV). Our results suggest that any of the ITS1, ITS2, or 28S fragments alone provide good resolution to differentiate among *Aptorchis* species, although ITS2 is the most practical target for quick differentiation as it is the shortest of these regions. Initial attempts to examine evolutionary relationships among the 4 species of *Aptorchis* did not provide a satisfactory degree of resolution. Putative new species of *Aptorchis* have been collected from additional *Chelodina* and *Emydura* in northern Australia, and a comprehensive phylogeny of the genus will be published separately.

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