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Aptorchis kuchlingi n. sp. (Digenea: Plagiorchioidea) from the Oblong Turtle, *Chelodina oblonga* (Pleurodira: Chelidae), in Western Australia

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ABSTRACT: *Aptorchis kuchlingi* n. sp. is described from the rectum of the oblong turtle, *Chelodina oblonga* (Pleurodira: Chelidae), in southwest Western Australia. This digenean is morphologically most similar to *Aptorchis pearsoni* but can be differentiated from the latter species by: a relatively longer cirrus sac in relation to overall body length, a cirrus sac that reaches the ovary, testes that are situated much closer to the cirrus sac and separated from it only by the seminal receptacle, vitellarium that extends anteriorly beyond the ventral sucker, a relatively wider ventral sucker in relation to oral sucker width, and a pharynx equal in size or larger than the oral sucker. Comparison of approximately 2,400 bases of ribosomal DNA (complete internal transcribed spacer [ITS]1 + 5.8S + ITS2, partial 28S) obtained from all 5 known *Aptorchis* species also suggests a close relationship of the new species with *A. pearsoni* and strongly supports the status of *A. kuchlingi* n. sp. as a new species. This is the fourth species of *Aptorchis* reported from long-necked turtles (*Chelodina*) and the first species of the genus reported in *C. oblonga*.

KEY WORDS: *Aptorchis*, Digenea, Platyhelminthes, *Chelodina oblonga*, oblong turtle, Pleurodira, Chelidae, Western Australia.

The oblong turtle, *Chelodina oblonga*, is distributed over a small corner of southwest Western Australia and shares its range with only 1 other turtle (Cogger, 2000), the critically endangered *Pseudemydura umbrina*. *Chelodina oblonga* previously has been reported to host only 3 species of parasites (Platt and Tkach, 2003; Zelmer and Platt, 2008), digeneans belonging to *Choanocotyle* and *Aptorchis pearsoni*. As part of an ongoing project to examine comprehensively the parasite biodiversity of Australia's freshwater turtles, we collected oblong turtles from across the range and uncovered a number of parasites. The description of 1 novel species from this collection follows.

MATERIALS AND METHODS

In December 2007, 14 *C. oblonga* were taken in baited traps from bodies of water near Perth, Manjimup, and Albany, 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 8 *C. oblonga* collected in 5 different localities. Living worms were rinsed in saline, briefly examined before 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 with the use of 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 percent 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.

The *Aptorchis* collection of Lindsey Jue Sue was provided to us by Tom Cribb, University of Queensland. Paratypes and voucher specimens were examined, and specimens were deposited in the Harold W. Manter Laboratory (*Aptorchis aequalis* HWML 49238, *Aptorchis megapharynx* HWML 49239, and *A. pearsoni* HWML 49240). Sequences of DNA of 5 recognized *Aptorchis* species were taken from GenBank for comparison with the new species: *Aptorchis glandularis* (EU334367), *Aptorchis megacetabulus* (EF014730), *A. pearsoni* (EF014728); *A. megapharynx* (EF014727); *A. aequalis* (EF014729). Specimens identified by Zelmer and Platt (2008) as *A. pearsoni* were obtained from Tom Platt, St. Mary's College, Notre Dame, Indiana. Five voucher specimens were examined and deposited: HWML P-2010-027.

For DNA sequence comparison of the new specimens to other *Aptorchis* species and to assess whether differences in worm size were indicative of phenotypic variation in the new species, genomic DNA was isolated according to Tkach and Pawlowski (1999) from 2 small gravid worms from Lake Joondalup near Perth (31°45.694′S, 115°47.259′E), 1 small and 1 large gravid worm from Perup Pond near Manjimup (34°10.511′S, 116°35.503′E), and 1 large worm from the Tone River, Tone River Bridge, Boyup Brook Road (34°13.657′S, 116°42.318′E). Fragments of approximately 2,400 DNA base pairs spanning ITS1, 5.8S, ITS2, and 28S rDNA were amplified by PCR, sequenced, and assembled as described in Tkach and Snyder (2008).

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Sequences were submitted to GenBank under accession numbers HQ680841–HQ680845.

RESULTS

Aptorchis kuchlingi n. sp. (Figures 1, 2)

Description

All measurements are in micrometers (μm). Measurements based on 15 adult specimens, 5 from type locality and 10 from 4 other localities; measurements of holotype in text; measurements of entire series used in description in Table 1. Body elongate, widest near ventral sucker; body length 2,026, body width at level of ventral sucker 400. Body width 19.7% of body length. Tegument thick, spined, spination heaviest anterior of ventral sucker; spines decrease in size and density as they extend to posterior end. Oral sucker rounded, subterminal, 100 long × 103 wide, slightly smaller than rounded ventral sucker, 250 × 241. Ventral sucker situated in anterior half of body at 34.4% of body length, periphery of sucker surrounded with several rows of densely packed, minute spines; larger, less densely packed spines occupy remainder of sucker.

Prepharynx 191 long, widening posteriorly. Muscular pharynx 118 long × 100 wide. Esophagus 96, surrounded by group of glandular cells. Intestinal bifurcation 470 from anterior end of body. Ceca nearly reach posterior end of body, terminating 113 from end.

Testes 2, oblique, spherical to subspherical, postovarian, ventral to and slightly overlapping ceca in posterior third of body. Anterior testis 145 long × 123 wide, posterior testis 144 × 125. Cirrus sac very large, 727 long × 80 wide, ventral to ceca, arching extracecally along 1 lateral margin of ventral sucker. Base of cirrus sac well posterior of posterior margin of ventral sucker, extending to level of ovary. Cirrus sac dextral or sinistral, with bipartite internal seminal vesicle, very large pars prostatica. Everted cirrus not observed. Genital pore ventral, submedian, dextral or sinistral, anterior to ventral sucker, 590 from anterior end of body.

Ovary spherical to subspherical, 147 long × 117 wide, median to submedian, dorsal to ceca situated just anterior to anterior testis at level of proximal portion of cirrus sac. Seminal receptacle thick-walled, 85 long × 68 wide, posterio-oblique to ovary. Vitellarium consists of small, irregularly shaped follicles, arranged in lateral fields, occasionally confluent, partly overlapping ceca, extending between ventral sucker and posterior end of ceca. Ootype and Mehlis' gland ventral to seminal receptacle, overlapping or slightly posterior to seminal vesicle. Laurer's canal not observed. Uterus ventral to other organs, mostly intra- and postcecal, with occasional extracecal loops. Metraterm approximately as long as cirrus sac, originating ventral of proximal portion of cirrus sac and passing dorsal to cirrus sac before opening into genital atrium. Eggs operculate, 31 × 16. Excretory pore terminal; excretory vesicle Y-shaped with short branches, reaches level of ovary.

Taxonomic Summary

Type host: Oblong turtle, *Chelodina oblonga* Gray, 1841 (Chelonia: Pleurodira: Chelidae).

Type locality/collection date: Tone River, Tone River Bridge, Boyup Brook Road, Western Australia, 34°13.657′S, 116°42.318′E; December 2007, by Scott Snyder.

Other localities/collection dates: Perup Pond, Perup Nature Reserve, Western Australia, 34°10.511′S, 116°35.503′E; Lake Joondalup, Joondalup, Perth, Western Australia 31°45.694′S, 115°47.259′E; Lake Seppings, Albany, Western Australia, 35°00.752′S, 117°54.981′E; Marsea Lake, Manjimup, Western Australia, 34°06.643′S, 116°14.321′E; December 2007, by Scott Snyder.

Site of infection: Rectum.

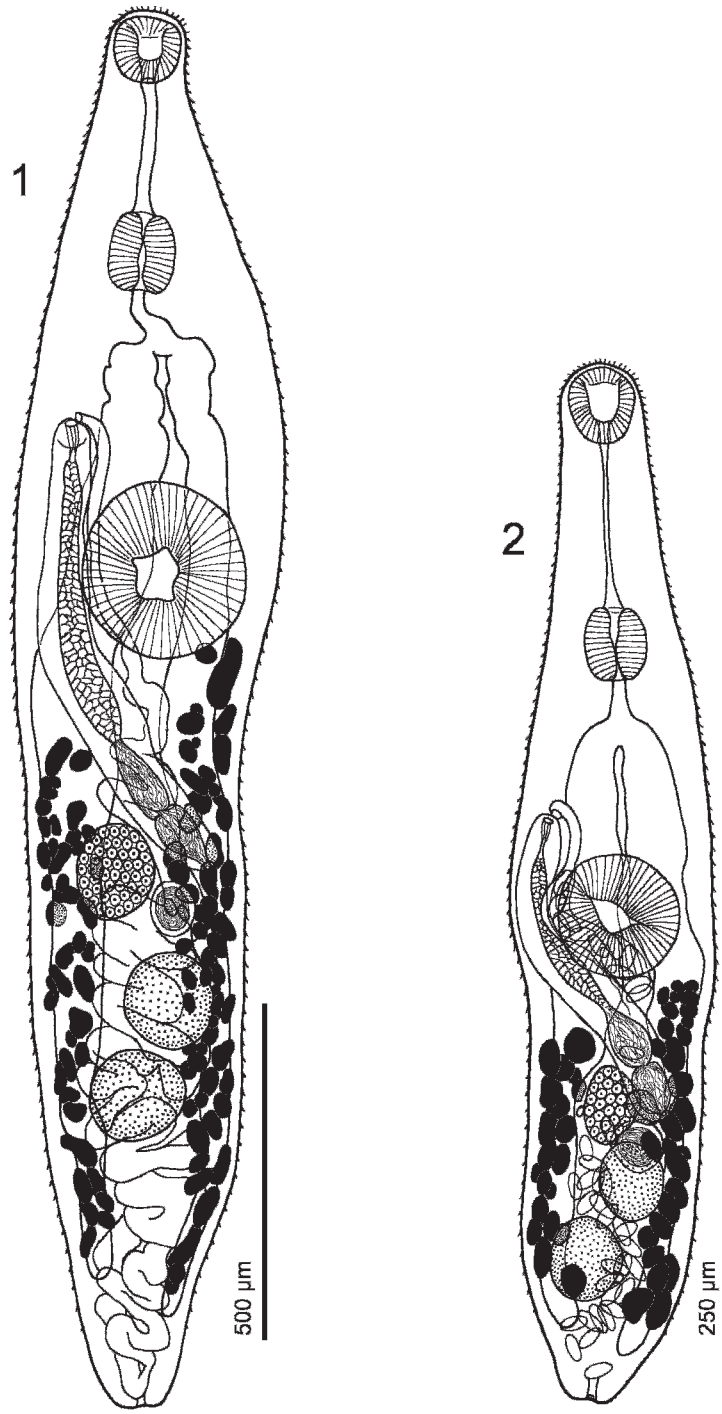
Prevalence and intensity of infection: One of 2 *Chelodina oblonga* from the type locality, 2 of 2 from Perup Pond, 2 of 3 from Lake Joondalup, 3 of 3 from Lake Seppings, and 1 of 1 from Marsea Lake were infected with the new species. Intensities ranged from 2 to more than 30.

Specimens deposited: The type series consists of 15 fully mature specimens. Holotype: Queensland Museum (QM) G230359. Paratypes: QM G230360; Harold W. Manter Laboratory 49241–49245.

Etymology: The species name honors Dr. Gerald Kuchling for his assistance in collection and his contributions to our knowledge and conservation of turtles, especially *P. umbrina*.

Remarks

Morphological data: On the basis of general morphology, *A. kuchlingi* n. sp. belongs to *Aptorchis* Nicoll, 1914 (Nicoll, 1914; Platt and Jensen, 2002; Tkach and Snyder, 2008), and a number of characters



Figures 1, 2. Line drawings of *Aptorchis kuchlingi* n. sp. **1.** Ventral view of holotype. **2.** Ventral view of a small-form specimen.

Table 1. Morphological measurements (μm) of *Aptorchis kuchlingi* n. sp.*

Character	*n*	Min–Max	Mean	SD	CV
Body length	15	863–2026	1,336.2	404.1	30.2
Body width	15	145–463	272.9	103.1	37.8
Oral sucker length	15	60–103	77.7	16.3	21.0
Oral sucker width	15	53–107	79.3	18.4	23.2
Prepharynx length	15	84–280	157.5	52.7	33.4
Pharynx length	15	58–118	86.1	19.5	22.6
Pharynx width	15	50–109	77.1	18.5	23.9
Esophagus	14	35–96	65.9	18.6	28.1
Cecal bifurcation to anterior end	15	275–584	373.9	89.9	24.0
Ventral sucker length	15	85–250	164.2	52.1	31.8
Ventral sucker width	15	87–241	158.8	48.3	30.4
Ventral sucker to anterior end	14	365–782	511.1	132.8	26.0
Cirrus sac length	12	316–790	505.2	194.8	38.6
Cirrus sac width	13	40–82	61.3	14.8	24.1
Seminal vesicle length	12	70–240	147.0	61.6	41.9
Genital pore from anterior end	14	340–683	456.4	105.6	23.1
Ovary length	15	55–147	95.5	28.9	30.3
Ovary width	15	47–117	77.0	22.3	29.0
Ovary to ventral sucker	15	79–250	147.1	52.7	35.8
Seminal receptacle length	13	42–95	69.5	17.4	25.0
Seminal receptacle width	13	39–80	57.8	12.8	22.1
Anterior testis length	14	65–145	103.4	28.2	27.3
Anterior testis width	14	58–144	90.0	30.1	33.4
Posterior testis length	14	71–150	107.7	28.5	26.5
Posterior testis width	14	56–135	92.6	31.4	34.0
Egg length	45	28–36	31.0	1.7	5.5
Egg width	45	14–18	16.0	1.0	5.9

* CV, coefficient of variation; Min, minimum; Max, maximum; SD, standard deviation.

allow for differentiation of the new species and the 5 previously described species of *Aptorchis*. Superficially, *A. kuchlingi* n. sp. is most similar in appearance to *A. pearsoni* (Jue Sue and Platt, 1999). The new species is similar in size to *A. pearsoni* and also shares a pronounced widening of the body at the level of the ventral sucker; however, the cirrus sac length of *A. pearsoni* is on average considerably smaller (24.6%; data from Jue Sue and Platt, 1999) as a percentage of body length than in the new species (37.8%), although the cirrus of *A. pearsoni* is more robust. The testes in *A. kuchlingi* n. sp. are separated from the proximal end of the cirrus sac only by the seminal receptacle, whereas in *A. pearsoni,* the testes are distant from the cirrus sac, separated by the ovary, seminal receptacle, and numerous uterine loops. In addition, the pharynx of *A. pearsoni* is smaller than the oral sucker, whereas the pharynx of the new species is equal in size or slightly larger than the oral sucker. In the largest specimens of *A. kuchlingi* n. sp., the ventral sucker reaches 241 μm in width and is about twice as large the oral sucker (oral sucker width:ventral sucker width is 0.49:1). The widest ventral sucker reported for *A. pearsoni* was 159 μm, and the oral sucker:ventral sucker ratio was 0.59:1 (data from Jue Sue and Platt, 1999). Finally, at least 1 vitelline field in *A. kuchlingi* n. sp. extends anteriorly to the posterior margin of the ventral sucker, whereas in *A. pearsoni,* the anterior border of vitellarium is situated much farther posteriorly, approximately at the level of the proximal end of cirrus sac.

The proximal end of the cirrus sac in the new species reaches the level of the ovary, but these

Table 2. Number of variable sites in different nuclear ribosomal DNA regions (complete ITS1, complete 5.8S, complete ITS2, partial LSU) among *Aptorchi kuchlingi* n. sp. and 5 known *Aptorchis* species. Alignment length (excluding repeat zones) for each fragment is shown in parentheses.

	No. of variable sites			
Aptorchis species	ITS1 (595 bp)	5.8S (157 bp)	ITS2 (252 bp)	LSU (1,306 bp)
A. pearsoni	7	0	6	7
A. megacetabulus	13	1	3	11
A. megapharynx	15	0	11	21
A. aequalis	23	1	17	23
A. glandularis	23	2	16	23

organs are separated by uterine coils in *A. pearsoni*, *A. megapharynx* (Jue Sue & Platt, 1999), *A. glandularis* Tkach and Snyder 2008, and *A. aequalis* Nicoll, 1914. The length of the cirrus sac as a percentage of body length is much smaller in *A. megapharynx* (24.5%) than in *A. kuchlingi* n. sp. (37.8%), and the internal morphology of the cirrus sac varies between the 2 species as well. In the new species, the proximal and distal portions of the bipartite seminal vesicle are approximately equal in size, whereas in *A. megapharynx,* the proximal portion is more than twice as large as the distal. The entire seminal vesicle of *A. megapharynx* takes up nearly one half of the cirrus sac, but the same structure accounts for 30% of the cirrus sac length in *A. kuchlingi* n. sp. Additionally, the vitellaria of the new species extend to the level of the ventral sucker, at the approximate midpoint of the body. The vitellaria of *A. megapharynx* are restricted to the posterior third of the body.

The cirrus sac of *A. megacetabulus* Tkach and Snyder, 2007, has a length accounting for 33.5% total body length (data from Tkach and Snyder, 2007), similar to the 37.8% seen in the new species. These 2 species are also similar in that they possess cirrus sacs that run to the level of the ovary. However, the species can be easily distinguished on the basis of a number of features. The ventral sucker length of *A megacetabulus* is 19.6% body length (Tkach and Snyder, 2007) but only 10.0% in *A. kuchlingi* n. sp., and the body of the former species does not noticeably widen at the level of the ventral sucker as it does in the latter. The proximal portion of the cirrus sac in the new species extends well posterior of the ventral sucker, but this structure extends only slightly posterior to the ventral sucker in *A. megacetabulus*. The esophagus of *A. megacetabulus* is indistinct but averages 16.7 μm compared with 69.9 μm in the new species, and the vitelline fields are more extensive in the new species than they are in *A. megacetabulus*.

The most dramatic differences lie between *A. kuchlingi* n. sp. and the large worms, *A. glandularis* and *A. aequalis*. In addition to the previously mentioned separation of cirrus sac and ovary in the latter 2 species, these worms are, on average, nearly twice as long as the new species (Jue Sue and Platt, 1999; Tkach and Snyder, 2008), do not have body margins that expand at the level of the ventral sucker, and have a tortuous cirrus sac unlike that of the new species. The new species does share a rectal habitat with the two large *Aptorchis* species, the other three species occurring in the small intestine.

Zelmer and Platt (2008) identified *A. pearsoni* from *C. oblonga* collected near Perth, Western Australia (32°05′S, 115°50′E). This parasite species was previously reported from *Chelodina expansa* in southern Queensland and southern New South Wales (Jue Sue and Platt, 1999; Tkach and Snyder, 2007), approximately 2,900 km and 3,600 km from Perth, respectively. Specimens from the Zelmer and Platt (2008) study were obtained and examined. These specimens conform to the diagnosis of *A. kuchlingi* n. sp.

Molecular data: Gravid specimens of *A. kuchlingi* n. sp. vary greatly in body size (Table 1), suggesting that sexually mature worms continue to grow well after egg formation commences. To ensure that differences in body size did not indicate the presence of 2 species that were otherwise morphologically similar, we examined ribosomal DNA sequences of five worms. Two individuals of the large form and 2 individuals of the small form had identical sequences across 2,391 bases of the entire ITS and partial LSU rDNA sequence. One smaller worm had a sequence length of 2,343 across the same region and, with the exception of these 48 contiguous missing bases, was identical in sequence composition to the other four worms. These 48 bp occur as a single repeat zone in the ITS1 region, a region of the *Aptorchis* rDNA that accounts for most of the sequence length variability across the genus (Tkach and Snyder, 2008). Sequence

similarity and the consistent morphological similarities among large and small forms support the presence of a single new species.

Aligned sequences of *A. kuchlingi* n. sp. were compared with sequences of all previously known *Aptorchis* species and demonstrate substantial variability outside of the repeat zone (Table 2), with the new species most similar to *A. pearsoni* across all ribosomal regions. The sequence data correspond to the morphological similarity of *A. kuchlingi* n. sp and *A. pearsoni*, while demonstrating substantial differences between the new species and all previously described species of *Aptorchis*, confirming the status of *A. kuchlingi* n. sp. as a new species.

Aptorchis kuchlingi n. sp. is the sixth species of *Aptorchis* to be described, the fourth species reported from long-necked turtles (*Chelodina*), and the first species of the genus reported in *C. oblonga*. The new species shares the greatest DNA sequence similarity with *A. pearsoni* and *A. megacetabulus* (Table 2), parasites of long-necked turtles and sister taxa in a recent phylogenetic analysis of the genus (Tkach and Snyder, 2008). Future phylogenetic analysis will reveal relationships among *A. kuchlingi* n. sp., a representative of a parasite lineage that potentially has been isolated in southwestern Australia since the Cretaceous (Burbridge et al., 1974; Platt and Tkach, 2003), and other members of *Aptorchis*.

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