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TWO NEW SPECIES OF *CHOANOCOTYLE* JUE SUE AND PLATT, 1998 (DIGENEA: CHOANOCOTYLIDAE) FROM AN AUSTRALIAN FRESHWATER TURTLE (TESTUDINES: PLEURODIRA: CHELIDAE)

Thomas R. Platt and Vasyl V. Tkach*
Department of Biology, Saint Mary's College, Notre Dame, Indiana 46556. *e-mail: tplatt@saintmarys.edu*

ABSTRACT: *Choanocotyle hobbsi* n. sp. and *Choanocotyle juesuei* n. sp. are described from the small intestine of the oblong turtle *Chelodina oblonga* from the vicinity of Perth, Western Australia. These are the third and fourth species referred to *Choanocotyle*. *Choanocotyle hobbsi* is most similar to *Choanocotyle nematoides* but differs in the size and shape of the oral sucker and the absence of a median loop in the cirrus sac. *Choanocotyle juesuei* is most similar to *Choanocotyle elegans* but differs in the size of the oral sucker and other morphometric criteria. Comparative analysis of the sequences of different nuclear ribosomal deoxyribonucleic acid regions of *C. nematoides* and *C. hobbsi* has confirmed that they are closely related but distinct species.

There are no previous reports in the literature of digenetic trematodes from the oblong turtle *Chelodina oblonga* (Gray, 1841), which is endemic to southwestern Australia (Pichelin et al., 1999). Specimens clearly referable to *Choanocotyle* Jue Sue and Platt, 1998 were collected from this host species from a pond on the campus of the Veterinary School of Murdoch University in December 1993. The larger form is similar in size to *Choanocotyle nematoides* Jue Sue and Platt, 1998, whereas the smaller form is similar in size to *Choanocotyle elegans* Jue Sue and Platt, 1998. Detailed morphological comparison, statistical analysis, and comparison of ribosomal deoxyribonucleic acid (rDNA) sequences strongly indicate that the 2 forms are distinct species and new to science. These 2 forms are described herein as *Choanocotyle hobbsi* n. sp. and *Choanocotyle juesuei* n. sp., respectively.

MATERIALS AND METHODS

The hosts examined were captured during 2–5 December 1993 from Melaleuca Swamp on the campus of Murdoch University, using hoop nets baited with meat. Turtles were maintained in large holding tanks and necropsied within 48 hr of capture. They were injected with a lethal dose of sodium pentabarbitone and were considered dead when they no longer responded with an eye blink to the touch of a fine camel-hair paint brush on the cornea. Necropsies were conducted using standard procedures. Worms were killed and fixed with steaming 5% formalin. Worms were placed in a screw-cap vial with a small amount of 0.7% saline. Steaming formalin was added to the vial, and the vial was capped quickly and shaken vigorously for a few seconds. Worms were stained in Van Cleave's hematoxylin (Pritchard and Kruse, 1982), dehydrated, cleared in methyl salicylate, and mounted as whole mounts in Canada balsam. All measurements are in micrometers unless otherwise noted and are given as the mean followed by the range in parentheses. Analysis of covariance (ANCOVA) was conducted using VassarStats (Lowry, 2000). Figures were made with the aid of a camera lucida. Representative specimens have been deposited in the Queensland Museum (QM), Brisbane, Queensland, Australia.

The following specimens were examined for comparative purposes: *C. elegans* paratypes, QM nos. G213984 and G213985; and *C. nematoides* paratypes, QM nos. G213992 and G213993. Additional specimens of these species, from the collection of the first author, were examined.

A subsample of the digeneans collected was fixed in 80% ethanol in 1993 and stored in a refrigerator (~4 C) for 8 yr. Deoxyribonucleic acid (DNA) was extracted from specimens of *C. nematoides* (collected by T.R.P. from *Emydura macquarii* (Gray, 1831), MacCleay River, New South Wales) and a form, described below as *C. hobbsi* n. sp. (collected by T.R.P. from hosts that yielded the type series), which is morphologically similar to *C. nematoides*, was identified. Specimens of the second species described in the present article, *C. juesuei* n. sp., were not preserved for molecular study.

DNA was extracted from single digeneans according to the protocol of Tkach and Pawlowski (1999) and Tkach, Pawlowski, and Mariaux (2000). Three different fragments of the nuclear ribosomal ribonucleic acid (rRNA) were amplified and sequenced: the complete 18S gene, the fragment containing ITS1, 5.8S gene, and ITS2, and a fragment about 1,300 bp long at the 5′ end of the 28S gene. Polymerase chain reaction (PCR) reactions were performed in a total volume of 50.25 μl when conventional *Taq* polymerase was used (42 μl H_2O, 5 μl *Taq* buffer, 1 μl deoxynucleoside triphosphate (dNTP), 1 μl of each primer, 0.25 μl of Roche *Taq* polymerase at concentration 5 units/μl, 1 μl of template DNA solution), or in a total volume of 25 μl when Ready-To-Go (Amersham Pharmacia Biotech, Buckinghamshire, U.K.) PCR beads were used (22 μl H_2O, 1 μl of each primer, 1 μl of template DNA and a PCR bead containing 1.5 units of *Taq* polymerase, components of buffer and dNTPs). The thermocycling program consisted of 3 min denaturation held at 96 C, 40–41 cycles (30 sec at 94 C, 30 sec at 50–52 C, and 2 min at 72 C), and 7 min at 72 C for final elongation.

For amplification of 18S gene, we used primers 18S Worm-A (5′ GCG AAT GGC TCA TTA AAT CAG 3′) and 18S Worm-B (5′ CTT GTT ACG ACT TTT ACT TCC 3′) according to the protocol described by Littlewood and Olson (2001). The ITS region was amplified using the universal primer Br (5′-GTAGGTGAACCTGCAGG-3′) localized at the 3′ end of the 18S gene and digenean-specific reverse primer digl1 (5′-GTGATATGCTTAAGTTCAGC-3′), localized in the conserved region at the 5′ end of the 28S gene. The digenean-specific forward primer digl2 (5′-AAG CAT ATC ACT AAG CGG-3′) and the universal reverse primer L0 (5′-GCT ATC CTG AG(AG) GAA ACT TCG-3′) were used for amplification of the fragment of 28S gene.

PCR products were purified using High Pure PCR Purification kits (Roche Molecular Biochemicals, East Sussex, U.K.) or QIAquick PCR Purification Kit (Qiagen, West Sussex, U.K.) according to the manufacturer's instructions. Purified PCR products were sequenced directly on an ABI PRISM 377 automated DNA sequencer, using Big Dye (Perkin Elmer, Cambridge, U.K.) reactions according to manufacturer's instructions. Several internal sequencing primers (Tkach, Pawlowski and Mariaux, 2000; Tkach, Pawlowski and Sharpilo, 2000; Littlewood and Olson, 2001) were used for sequencing in addition to the amplification primers. The sequences obtained were assembled using Sequencher version 3.1.1 (GeneCodes Corp., Ann Arbor, Michigan) software and submitted to GenBank/EMBL. For comparison, sequences were aligned using BioEdit software (Hall, 1999). The nucleotide sequences used in this study are available in GenBank under the accession numbers AY116861–AY116868.

RESULTS

Complete sequences of 18S gene from single specimens of each species (*C. nematoides*, GenBank AY11687; *C. hobbsi*, GenBank AY116868) revealed a 10-base difference in the 1,869 bp long sequence. The variable sites were scattered throughout

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* Institute of Parasitology, Polish Academy of Sciences, 51/55 Twarda Street, 00-818 Warsaw, Poland.

the gene. Sequences of the fragment situated at the 5′ end of the 28S gene were obtained from 3 specimens of *C. nematoides* (GenBank AY116862–AY116864) and 2 specimens of *C. hobbsi* (GenBank AY116865, AY116866). The 28S fragment consisted of 1,251 bases in *C. nematoides* and 1,252 bases in *C. hobbsi*. A 10-base difference was found in this region between the 2 species and no intraspecific variability was observed. The variable sites, as in the 18S gene, were localized in different parts of the fragment. No intraspecific variability has been detected in this fragment of 28S rDNA, among plagiorchiate digeneans (about 20 species belonging to different families), when more than 1 specimen of the same species was examined (Tkach, pers. obs.).

The ITS1+5.8S+ITS2 region was successfully amplified and sequenced in *C. hobbsi* (GenBank AY116861); however, we failed to amplify the corresponding region from *C. nematoides* using DNA extractions that were successful in amplifying the other regions. Despite numerous attempts and using several different pairs of primers (all of which worked well with the DNA of *C. hobbsi*), amplification of *C. nematoides* resulted in numerous products of different lengths none of which was similar in length with that of *C. hobbsi*. We have no explanation for this failure; however, there might be several different variants of ITS1 present in the genome of *C. nematoides*, and thus its sequence would require cloning.

DESCRIPTION

Choanocotyle hobbsi **n. sp.**

(Figs. 1–3)

Measurements based on 8 stained whole mounts. Body elongate, 10.68 mm (7.75–16.23 mm); maximum width 419 (376–480); posterior end curved ventrally. Tegument heavily spined anteriorly, decreasing posteriorly, with no spines at posterior end. Oral sucker 482 (355–592) long, 672 (551–796) wide, incised ventrally, much wider than forebody when fully expanded; shape variable, funnel-shaped when retracted. Prepharynx 153 (25–271) long. Pharynx ovate 177 (138–213) long, 168 (140–188) wide. Esophagus 62 (42–100) long (n = 6). Ceca thin-walled, sinuous, extend almost to posterior end. Ventral sucker spherical 205 (183–240) long, 203 (185–240) wide, located 3,598 (2,250–5,625) from anterior end; ventral sucker from anterior end as percent of total body length 33.7 (29.0–40.4). Common genital pore located ventrally at posterior margin of ventral sucker. Dorsal genital protuberance large, raised; located medially on dorsal surface posterior to ovarian complex. Ovary ovate, 210 (168–251) long, 247 (228–277) wide, median to submedian; in posterior fifth of body. Seminal receptacle 119 (83–175) long, 141 (100–215) wide, located dorsally overlapping posterior margin of ovary. Mehlis' gland large, posterior to ovary. Laurer's canal, opening dorsally at anterior margin of dorsal genital protuberance. Uterus intercecal, anterior to ovary, narrows anteriorly near ventral sucker, loops dorsal to ventral sucker and cirrus sac, looping posteriorly along ventral surface of cirrus sac before entering ventral genital sinus, forming common genital pore. Vitelline follicles small, scattered in posterior fifth of body; extending from posterior extremity anteriorly to 131 (0–250) from posterior margin of ovary. Testes large, oval, tandem, entire, near posterior end. Anterior testis 451 (375–589) long, 262 (214–326) wide; posterior testis 458 (397–592) long, 252 (198–326) wide; testes separated by approximately 1 testis length. Cirrus sac 794 (668–997) long, 167 (150–188) wide, overlaps ventral sucker dorsally, reflexed ventrally, entering genital atrium posterior to uterus, containing seminal vesicle, pars prostatica, cirrus. Seminal vesicle bipartite; anterior compartment ovate, 138 (70–213) long, 119 (50–150) wide; posterior compartment 201 (88–271) long, 124 (50–150) wide. Pars prostatica elongate, glandular; cirrus unarmed. Excretory bladder elongate, intercaecal, extending to level of Mehlis' gland; excretory pore terminal. Eggs operculate, tanned; intrauterine eggs (n = 10) 35–40 long, 18–19 wide.

Taxonomic summary

Type host: Chelodina oblonga (Gray, 1841) (Testudines: Pleurodira: Chelidae).

Site of infection: Small intestine.

Type locality: Melaleuca Swamp, Veterinary School, Murdoch University, Murdoch, Western Australia, Australia (32°05′S, 115°50′E).

Prevalence and intensity: Prevalence 80% (4 turtles infected of 5 examined), mean intensity 10.75 (range 3–20).

Holotype: QM G219520.

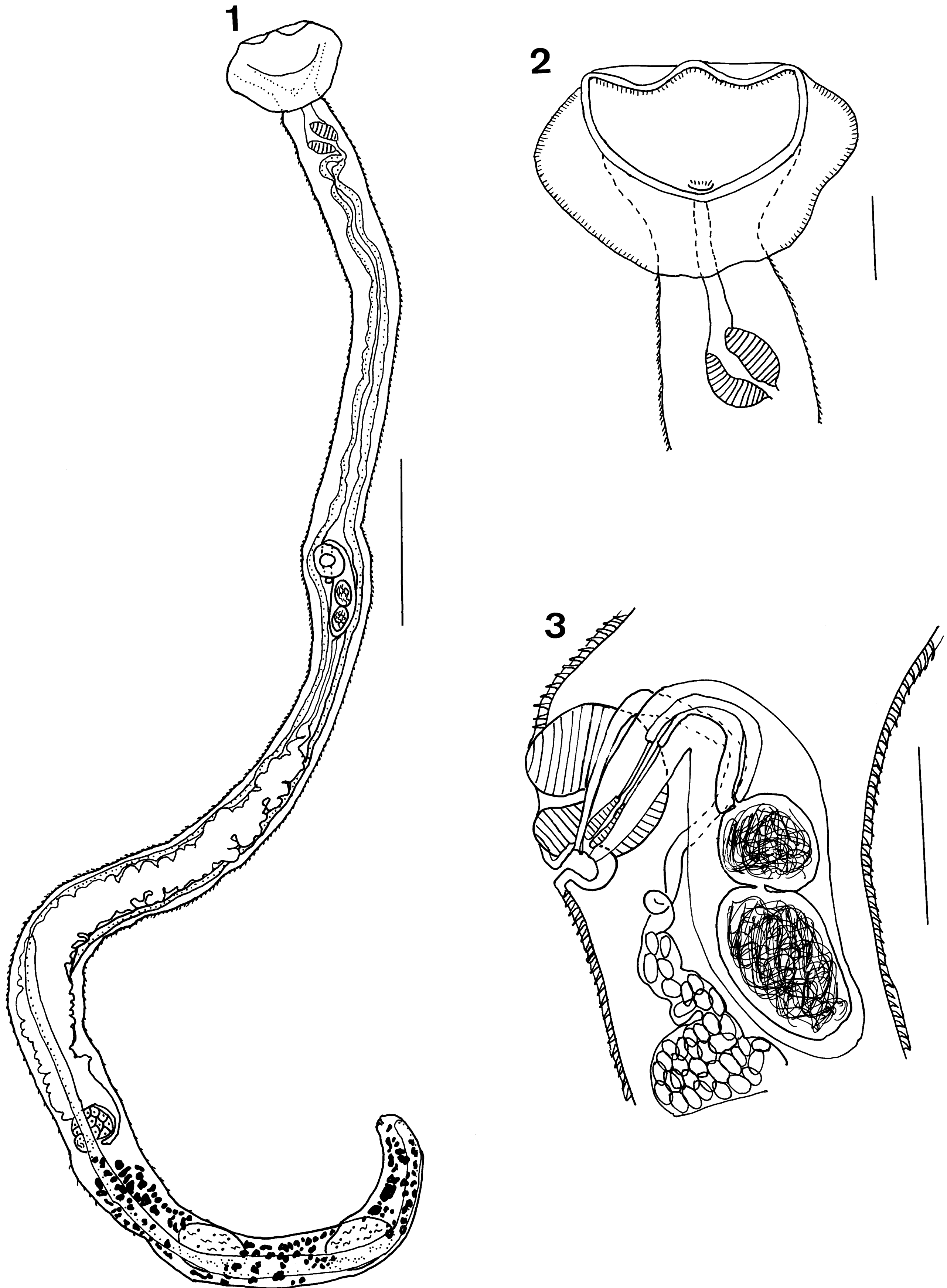
Paratypes: QM 219521–219527.

Etymology: This species is named after Mr. Russell P. Hobbs, Murdoch University, Australia, in recognition of his long-time friendship and outstanding contributions to parasitology.

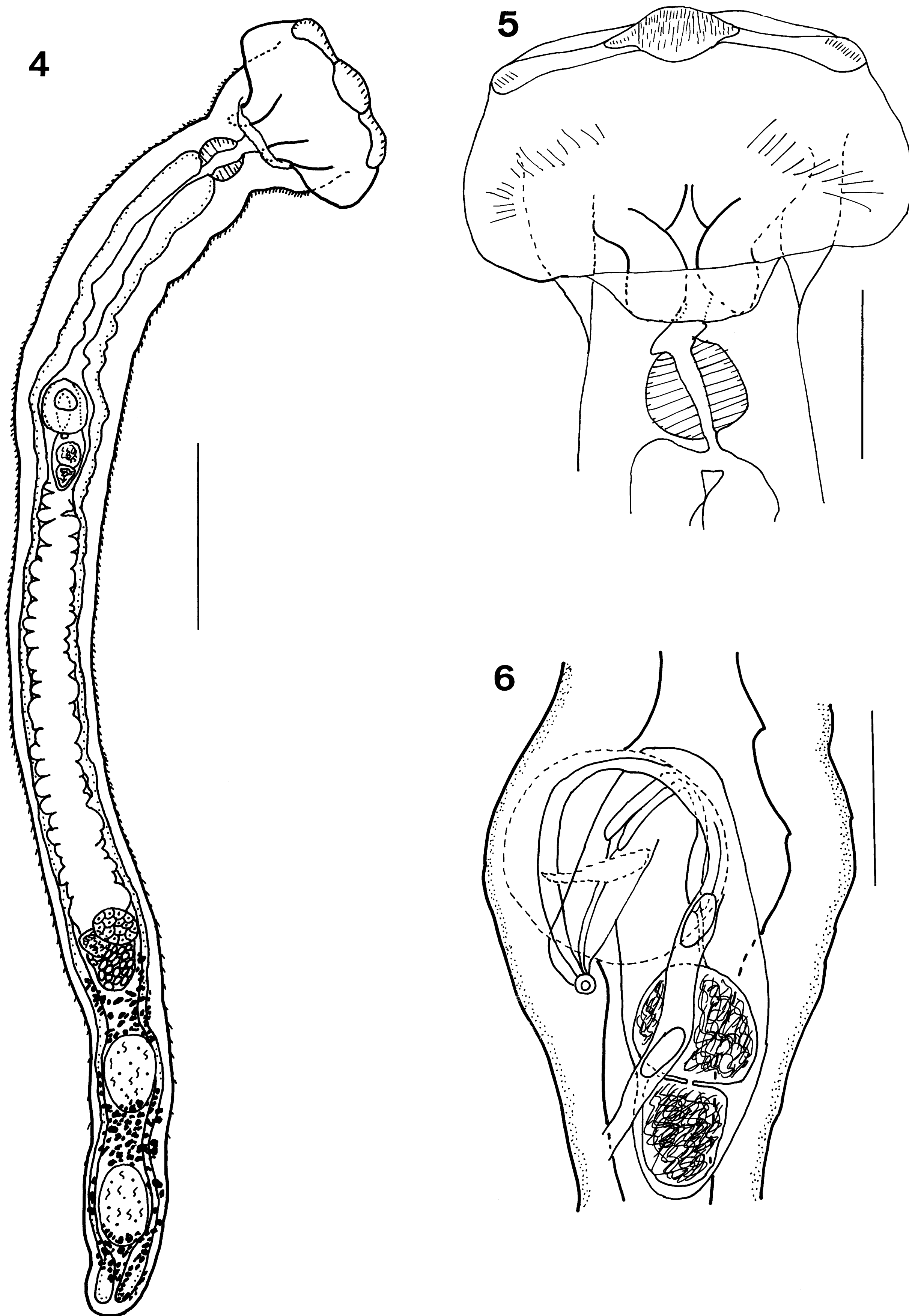
Choanocotyle juesuei **n. sp.**

(Figs. 4–6)

Measurements based on 9 stained whole mounts. Total body length 3,961 (3,050–4,765); maximum width 305 (256–386). Tegument heavily spined anteriorly, decreasing in number posteriorly with no spines at posterior end. Oral sucker 387 (345–438) long, 488 (392–551) wide, incised ventrally; much wider than forebody when fully expanded; shape variable, funnel-shaped when retracted. Prepharynx and esophagus short. Pharynx slightly ovate, 121 (103–138) long, 119 (113–138) wide. Ceca thin-walled, sinuous, extend almost to posterior end. Ventral sucker slightly ovate, 134 (120–155) long, 130 (115–143) wide; located 1,400 (1,150–1,650) from anterior end; ventral sucker from anterior end as percent of total body length 36.0 (29.5–39.2). Common genital pore located ventrally at posterior margin of ventral sucker. Dorsal genital protuberance large, raised, located medially on dorsal surface posterior to ovarian complex. Ovary ovate 122 (110–138) long, 122 (95–143) wide; median to submedian, in posterior quarter of body. Seminal receptacle 95 (75–113) long, 77 (63–93) wide, dorsally overlapping posterior margin of ovary. Mehlis' gland large, posterior to ovary. Laurer's canal opening dorsally at anterior margin of dorsal genital protuberance. Uterus intercecal, anterior to ovary, narrows anteriorly near ventral sucker, passes dorsal to ventral sucker and cirrus sac, looping posteriorly along ventral surface of cirrus sac before entering ventral genital sinus forming common genital pore. Vitelline follicles small, scattered in posterior fifth of body; extending from posterior extremity anteriorly to posterior margin of ovary. Testes oval, tandem, entire, near posterior end. Anterior testis 226 (193–261) long, 153 (135–180) wide; posterior testis 239 (213–271) long, 145 (128–165) wide; testes separated by less than 1 testis length. Cirrus



FIGURES 1–3. *Choanocotyle hobbsi* n. sp. adults from the small intestine of *Chelodina oblonga*. **1.** Adult (holotype), ventral. **2.** Oral sucker (holotype), ventral. **3.** Terminal genital complex (paratype), lateral. Scale bars: 1, 1 mm; 2 and 3, 200 μm.



FIGURES 4–6. *Choanocotyle juesuei* n. sp. adults from the small intestine of *Chelodina oblonga*. **4.** Adult (holotype), ventral. **5.** Oral sucker (paratype), ventral. **6.** Terminal genital complex (paratype), ventral. Scale bars: 4, 500 μm; 5, 200 μm; 6, 100 μm.

sac 430 (363–530) long, 93 (78–110) wide, overlaps ventral sucker dorsally, reflexed ventrally, entering genital atrium posterior to uterus; containing seminal vesicle, pars prostatica, cirrus. Cirrus sac extends 208 (138–282) posterior to posterior margin of ventral sucker. Seminal vesicle bipartite; anterior compartment 77 (60–95) long, 72 (55–95) wide, posterior compartment 82 (53–118) long, 64 (50–95) wide. Pars prostatica elongate, glandular; cirrus unarmed. Excretory bladder elongate, intercaecal, extending to level of Mehlis' gland; excretory pore terminal. Eggs operculate, tanned; intrauterine eggs (n = 10) 35–38 long × 16–20 wide.

Taxonomic summary

Type host: Chelodina oblonga (Gray, 1841) (Testudines: Pleurodira: Chelidae).

Site of infection: Small intestine.

Type locality: Melaleuca Swamp, Veterinary School, Murdoch University, Murdoch, Western Australia, Australia (32°05′S, 115°50′E).

Prevalence and intensity: Prevalence 20% (1 turtle infected of 5 examined), intensity = 47.

Holotype: QM G219528.

Paratypes: QM G219529–G219536.

Etymology: This species is named after Dr. Lindsay Jue Sue, Atherton, Queensland, in recognition of his discovery of *Choanocotyle*.

Remarks

The presence of the large, ventrally incised oral sucker leaves no question that the specimens reported herein should be assigned to *Choanocotyle* as defined by Jue Sue and Platt (1998). The initial presumption was that the large and small specimens found represented different age classes of the same species, although all worms were mature and contained large numbers of uterine eggs. Hence, the only difference would be in size.

Most organs of *C. hobbsi* are larger than the corresponding structures in *C. juesuei*, as would be anticipated by the significant difference in total length ($\bar{x}$ = 10,675 and 3,961, respectively; t_{16} = 3.77, P = 0.0008). ANCOVA was employed to determine if the size differences of various structures were independent of total body length. The test for homogeneity of slopes was not significant for any of these comparisons, satisfying the primary condition for the analysis. ANCOVA showed that oral sucker length and pharynx diameter were dependent on total length, and there was no significant differences between these structures; there were, however, significant differences in ventral sucker diameter, cirrus sac length, and anterior and posterior testis length (Table I). It is possible that these structures might increase in a positive allometric relationship to body length; in the absence of any intermediates between the 2 sets of specimens, they are considered distinct and treated as separate species.

Both new species differ from *C. nematoides* in cirrus sac shape and position. The cirrus sac in *C. nematoides* possesses a median loop (Jue Sue and Platt, 1998) before continuing anteriorly and reflexing slightly, overlapping the posterior margin of the ventral sucker and terminating near the posterior edge of the ventral sucker. The cirrus sac of both *C. hobbsi* and *C. juesuei* course anteriorly, with no median loop, pass behind the ventral sucker, and reflex near the anterior margin of that structure before turning posteriad, terminating at the genital pore at the posterior edge of the ventral sucker. Both *C. hobbsi* and *C. juesuei* are similar to *C. elegans* in the shape of the cirrus sac.

TABLE I. Analysis of covariance of various structures of *Choanocotyle hobbsi* n. sp. and *Choanocotyle juesuei* n. sp. controlled for total length (df = 1,14).

Character	Observed means*	Adjusted means*	*F*-value	*P*-value
Oral sucker length	(387) (482)	(452) (408)	0.73	0.407
Ventral sucker diameter	(132) (204)	(145) (188)	12.33	0.0035†
Pharynx diameter	(120) (172)	(141) (151)	0.03	0.865
Cirrus sac length	(430) (794)	(517) (696)	5.04	0.0414†
Anterior testis length	(226) (451)	(253) (421)	7.2	0.0178†
Posterior testis length	(239) (458)	(256) (439)	11.59	0.0043†

* Mean size of structure in (*C. juesuei*) (*C. hobbsi*) rounded to the nearest micrometer.
† Significantly different.

Choanocotyle hobbsi does bear a striking resemblance to *C. nematoides* in overall size, with both species reaching lengths in excess of 10 mm. In addition to lacking the median loop characteristic of *C. nematoides*, the cirrus sac in *C. hobbsi* is smaller, never exceeding 1 mm in length. The new species also differs in having a much larger oral sucker ($\bar{x}$ = 482 long, 672 wide) than *C. nematoides* (holotype = 275 long, 396 wide), and larger testes than *C. nematoides* in specimens of comparable total body length.

Differences of 10 bp in the 18S gene (1,251 bp) and the fragment from the 5′ end of the 28S gene (1,869 bp) are considered biologically meaningful. The failure to amplify the ITS1+5.8S+ITS2 from *C. nematoides* also suggests significant differences in the sequence and structure of the ITS region in these forms, confirming that they represent distinct species.

Choanocotyle juesuei is similar to *C. elegans* in overall size and form. In addition having similar cirrus sacs (simple, no median loop, reflexed postero-ventrally behind the acetabulum and opening at a common genital pore near the posterior edge of the acetabulum), both species are similar in size and many of the characters overlap morphometrically. There are differences between the new species and the original description of *C. elegans* (see Jue Sue and Platt [1998]) that warrant recognition at the specific level. On comparing specimens that overlap in total length and maximum width, it was found that *C. juesuei* has a smaller oral sucker length (387 [345–438] vs. 599 [483–609]) and oral sucker width (488 [392–551] vs. 739 [672–819]), a smaller ventral sucker length (134 [120–155] vs. 205 [197–252]) and ventral sucker width (130 [115–143] vs. 199 [160–202]), as well as pharyngeal length (121 [103–138] vs. 210 [147–294]) and pharyngeal width (119 [113–138] vs. 202 [147–231]), respectively. These morphological differences, combined with their presence in different host species located on opposite extremes of the Australian continent, and temporally isolated for possibly tens of millions of years (see below), strongly argue for the recognition of distinct species status.

DISCUSSION

Despite the high sequence similarity in the 2 relatively conserved regions of rDNA (18S and partial 28S) demonstrated by

C. nematoides and *C. hobbsi*, the level of variability, at least in the 28S sequences, is comparable to that previously observed among members of other genera of plagiorchiate digeneans. Approximately equal or even lower variability has been observed between species of *Plagiorchis* Lühe, 1899 and *Parabascus* Looss, 1907 (see Tkach, Pawlowski, and Sharpilo, 2000; V. Tkach, unpubl. obs.) and even among closely related genera, such as *Leptophallus* Lühe, 1909; *Metaleptophallus* Yamaguti, 1958; *Paralepoderma* Dollfus, 1950; and *Macrodera* Looss, 1899 (see Tkach et al., 1999). For instance, the sequence variability between *Plagiorchis vespertilionis* (Müller, 1780) and *Plagiorchis muelleri* Tkach and Sharpilo, 1990, was only 6 nucleotides in the same 28S rDNA fragment (Tkach, Pawlowski, and Sharpilo, 2000; GenBank numbers AF184250, AF151931). A similar level of intrageneric variability has been reported recently by Snyder and Tkach (2001) among species of *Haematoloechus* Looss, 1899. A case of extreme sequence similarity in this DNA fragment has been reported by Tkach et al. (2001) for species belonging to 2 morphologically distinct genera of Omphalometridae; *Neoglyphe sobolevi* (Schaldybin, 1953) and *Rubenstrema exasperatum* (Rudolphi, 1819), showed only a 3-base difference. In most of the cases mentioned above, however, sequences of the more variable ITS regions, obtained from the same DNA extractions, demonstrated a much higher level of variability (Tkach et al., 1999; V. Tkach, unpubl. obs.) than partial 28S sequences. Our data on *Choanocotyle*, as well as the cases mentioned above, suggest that interspecific differences of rDNA sequences can be rather minor in certain digenean groups, especially those undergoing an active adaptive radiation.

The question remains as to what degree of difference between different rDNA markers is indicative of the separation of species. We agree that it is impossible to have a common benchmark (a minimum number of nucleotide substitutions) for different large taxa of digeneans (Anderson and Barker, 1998). In each particular group, this level is different and corresponds to its own rate of molecular evolution.

Pichelin et al. (1999) provided a checklist of all helminths reported from Australian reptiles. The only helminth reported from *Chelodina oblonga* (Gray, 1841) was the stomach-dwelling nematode *Spiroxys chelodinae* Berry, 1985. *Choanocotyle hobbsi* n. sp. and *C. juesuei* n. sp. represent the first digenetic trematodes reported from *Chelodina oblonga* and the first reported from any freshwater turtle in Western Australia. Jue Sue and Platt (1998) erected *Choanocotyle* and Choanocotylidae to contain 2 highly distinct species of digenetic trematodes from freshwater turtles in southern Queensland: *C. elegans* from the long-necked turtle, *Chelodina expansa* Gray, 1857 and *C. nematoides* from a short-necked turtle *Emydura macquarii* (Gray, 1830).

Turtles of the family Chelidae (side-necked turtles) are Gondwanan in origin and found only in South America, Australia, and New Guinea (Heatwole, 1987). Manning and Kofron (1996), in a review of the literature on the evolution and biogeography of Australian freshwater turtles, suggested that southwestern Australia became isolated from the eastern portion of the continent by an inland sea during the early Cretaceous. The sea subsided during the late Cretaceous forming ''waterless desert equally impenetrable'' (Manning and Kofron, 1996). If this hypothesis is correct, *Chelodina oblonga* and its parasite fauna have been separated from their eastern counterparts for well over 100 million yr, resulting in speciation and providing ample time for the accumulation of genetic and morphological differences in both host and parasite lineages.

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LITERATURE CITED

ANDERSON, G. R., AND S. C. BARKER. 1998. Inference of phylogeny and taxonomy within Didymozoidae (Digenea) from the second internal transcribed spacer (ITS2) of ribosomal DNA. Systematic Parasitology **41:** 87–94.

HALL, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41:** 95–98.

HEATWOLE, H. 1987. Major components and distributions of terrestrial fauna. *In* Fauna of Australia: Vol. 1A, general articles, G. R. Dyne and D. W. Walton (eds.). Australian Government Printing Services, Canberra, Australia, p. 101–135.

JUE SUE, L., AND T. R. PLATT. 1998. Description and life-cycle of two new species of *Choanocotyle* n. g. (Trematoda: Plagiorchiida), parasites of Australian freshwater turtles, and the erection of the family Choanocotylidae. Systematic Parasitology **41:** 47–61.

LITTLEWOOD, D. T. J., AND P. D. OLSON. 2001. Small subunit rDNA and the Platyhelminthes: Signal, noise, conflict and compromise. *In* Interrelationships of the Platyhelminthes, D. T. J. Littlewood, and R. A. Bray (eds.). Taylor & Francis, London, U.K., p. 186–193.

LOWRY, R. 2000. VassarStats: web site for statistical computation. http://faculty.vassar.edu/~lowry/VassarStats.html.

MANNING, B., AND C. P. KOFRON. 1996. Evolution and zoogeography of Australian freshwater turtles. Memoirs of the Queensland Museum **39:** 319–331.

PICHELIN, S., P. M. THOMAS, AND M. N. HUTCHINSON. 1999. A checklist of helminth parasites of Australian reptiles. Records of the South Australian Museum, Monograph Series Number **5:** 1–61.

PRITCHARD, M. H., AND G. O. W. KRUSE. 1982. The collection and preservation of animal parasites. Technical Bulletin No. 1. University of Nebraska Press, Lincoln, Nebraska, 141 p.

SNYDER, S. D., AND V. V. TKACH. 2001. Phylogenetic and biogeographical relationships among some holarctic frog lung flukes (Digenea: Haematoloechidae). Journal of Parasitology **87:** 1433–1440.

TKACH, V., B. GRABDA-KAZUBSKA, J. PAWLOWSKI, AND Z. SWIDERSKI. 1999. Molecular and morphological evidences for close phylogenetic affinities of the genera *Macrodera*, *Leptophallus*, *Metaleptophallus* and *Paralepoderma* (Digenea, Plagiorchioidea). Acta Parasitologica, **44:** 170–179.

———, ———, AND Z. SWIDERSKI. 2001. Systematic position and phylogenetic relationships of the family Omphalometridae (Digenea, Plagiorchiida) inferred from partial lsrDNA sequences. International Journal of Parasitology **31:** 81–85.

———, AND J. PAWLOWSKI. 1999. A new method of DNA extraction from the ethanol-fixed parasitic worms. Acta Parasitologica **44:** 147–148.

———, ———, AND J. MARIAUX. 2000. Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. International Journal for Parasitology **30:** 83–93.

———, ———, AND P. SHARPILO. 2000. Molecular and morphological differentiation between species of the *Plagiorchis vespertilionis* group (Digenea: Plagiorchiidae), occurring in European bats, with a re-description of *P. vespertilionis* (Müller, 1780). Systematic Parasitology **47:** 9–22.