

## *Camallanus tuckeri* n. sp. (Nematoda, Camallanidae) from Freshwater Turtles (Pleurodira: Chelidae), in the Kimberley, Western Australia

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**ABSTRACT:** *Camallanus tuckeri* n. sp. is described from specimens found in the intestine of the side-necked turtles *Emydura australis* and *Chelodina burrungandjii* collected from several localities in the Kimberley region of Western Australia. The new species differs from *Camallanus chelonius*, a parasite of African turtles, in the shape of the buccal capsule ridges, the number of precloacal papillae in males (7 pairs in the new species vs. 6 pairs in *C. chelonius*) and spicule shape. *Camallanus tuckeri* n. sp. differs from *Camallanus waelhrew* in the shape of buccal capsule ridges and the inflation of vulva lips in females. The new species differs from *Camallanus nithoggi* by possessing a smaller number of incomplete buccal capsule ridges (up to 2 in *C. tuckeri* n. sp. vs. 4 or 5 in *C. nithoggi*), larger spicules, and the inflation of a posterior vulva lip that is lacking in *C. nithoggi*. *Camallanus tuckeri* n. sp. is the first nematode reported from *Em. australis* and the first endoparasite reported from *Ch. burrungandjii*.

**KEY WORDS:** Nematoda, Camallanidae, *Camallanus tuckeri* n. sp., freshwater turtle, *Emydura australis*, *Chelodina burrungandjii*, Chelidae, Australia, 28S lsrDNA sequences.

Nematodes circumscribed within *Camallanus* Raillet et Henry, 1915, are common parasites of fishes worldwide and are also found in amphibians and reptiles (Petter, 1979). Yeh (1960) transferred all *Camallanus* species parasitic in turtles (mainly from suborder Cryptodira) from *Camallanus* to *Serpinema* Yeh, 1960, on the basis of the presence of a gap between dorsal and ventral groups of ridges on the lateral valves of the buccal capsule. Baker (1983) described *Camallanus chelonius* Baker, 1983, a parasite of the pleurodiran turtle *Pelosius sinatus* from South Africa. He believed that the buccal capsule morphology of *C. chelonius* was intermediate between that of *Camallanus* and *Serpinema*; however, the species possessed a median ridge in the buccal capsule and thus was assigned to the genus *Camallanus*. All other camallanid parasites of turtles, both pleurodiran and cryptodiran, were distributed in Eurasia and the Americas and remained in *Serpinema* (Petter, 1979).

The first *Camallanus* reported from Australia was *C. chelonius* from *Elseya latisternum* and *Emydura macquarii* collected in Queensland (Ferguson and Smales, 1998, 2006). Recently, Rigby et al. (2008) described 2 *Camallanus* species from freshwater turtles in New South Wales: *Camallanus nithoggi*

from *El. latisternum* and *Camallanus waelhrew* from *Emydura* spp. In this publication, we demonstrate that the *C. chelonius* of Ferguson and Smales (1998) was, in fact, *C. nithoggi*.

As part of a survey and inventory of the parasite fauna of Australian freshwater turtles, we identified *C. waelhrew* in *Em. macquarii* and *Chelodina expansa* from Queensland and New South Wales, and *C. nithoggi* in *El. latisternum* from Queensland. Intestinal nematodes found in *Emydura australis* and *Chelodina burrungandjii* from Western Australia appeared to be different from both *C. nithoggi* and *C. waelhrew*, as well as from other camallanids reported from turtles. This material is described herein.

### MATERIAL AND METHODS

In July 2006, 22 *Em. australis* and 16 *Ch. burrungandjii* were collected by baited traps or by hand in Lake Argyle Spillway, Donkey Hole, Minah Creek, Bell Creek, Ord River, and Geike Gorge, Western Australia. All collections proceeded under a permit from the Western Australia Department of Conservation and Land Management

Ten of 38 examined turtles harbored from 1 to 18 *Camallanus*. After recovery from the host intestine, the nematodes were rinsed in saline, killed with hot 70% ethanol, and stored in 70% ethanol. For light microscopy, they were cleared in phenol-glycerin (1:3 ratio). Measurements were taken from a compound microscope with the use of digital imaging and Rincon measurement software (v.

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**Table 1. Hosts, geographic origin, and GenBank accession numbers of sequenced *Camallanus* specimens.**

<i>Camallanus</i> species	Turtle species	Locality	Coordinates	GenBank No.
<i>C. tuckeri</i> n. sp.	<i>Emydura australis</i>	Lake Argyle Spillway, WA	16°07.371'S, 128°44.271'E	FJ969492
<i>C. tuckeri</i> n. sp.	<i>Emydura australis</i>	Ord River, Kununurra, WA	15°47.553'S, 128°42.310'E	FJ969493
<i>C. tuckeri</i> n. sp.	<i>Emydura australis</i>	Bell Creek, WA	17°10.159'S, 125°21.521'E	FJ969494
<i>C. tuckeri</i> n. sp.	<i>Chelodina burrungandjii</i>	Donkey Hole, WA	16°39.275'S, 125°29.338'E	FJ969495
<i>C. tuckeri</i> n. sp.	<i>Chelodina burrungandjii</i>	Minah Creek, WA	17°06.627'S, 125°21.012'E	FJ969496
<i>C. tuckeri</i> n. sp.	<i>Chelodina burrungandjii</i>	Ord River, Kununurra, WA	15°47.553'S, 128°42.310'E	FJ969497
<i>C. nithoggi</i>	<i>Eelseya latisternum</i>	Platypus Creek, QLD	17°21.815'S, 145°35.444'E	FJ969498
<i>C. waelhrew</i> (6 specimens, all from different turtle individuals)	<i>Emydura macquarii</i>	Mungabareena Reserve, Albury, NSW	36°05.579'S, 146°56.902'E	FJ969499–FJ969504
<i>C. waelhrew</i>	<i>Chelodina expansa</i>	Mungabareena Reserve, Albury, NSW	36°05.579'S, 146°56.902'E	FJ969505

\*NSW, New South Wales; QLD, Queensland; WA, Western Australia.

7.1.2, Imaging Planet, Goleta, California), as well as with an ocular micrometer. All measurements are in micrometres ( $\mu\text{m}$ ) unless otherwise stated.

Specimens used for scanning electron microscopy (SEM) were fixed in 70% ethanol, dehydrated in a graded series of ethanol, and dried with hexamethyldisilazane (Ted Pella Inc., Redding, California) as transition fluid. The specimens were mounted on stubs, coated with gold, and examined with the use of a Hitachi 4700 scanning electron microscope (Hitachi U.S.A., Mountain View, California) at an accelerating voltage of 10–15 kV.

Samples of *C. nithoggi* (13 specimens) from *El. latisternum* and *C. waelhrew* (25 specimens) from *Em. macquarii* and *Ch. expansa* collected in Queensland and New South Wales, respectively, were used for morphological comparison with the new species.

Genomic DNA for molecular analysis was extracted from 6 specimens of the new *Camallanus* species collected from 2 turtle species, *Em. australis* and *Ch. burrungandjii*, trapped in 5 different localities. For comparative purposes, DNA was also extracted from 1 specimen of *C. nithoggi* and 7 specimens of *C. waelhrew* collected in northern Queensland and New South Wales, respectively (Table 1). Tissue for DNA extraction was taken from the middle of the body whereas taxonomically important anterior and posterior parts were preserved as vouchers for morphological identification. DNA was extracted according to Tkach and Pawlowski (1999).

DNA fragments spanning the 3' end of the 18S nuclear rDNA gene, internal transcribed spacer region (ITS1 + 5.8S + ITS2), and 5' end of the 28S gene were amplified by polymerase chain reaction (PCR) on an Eppendorf Master Gradient thermal cycler with the use of the newly designed forward primer c1740f (5'-TGAAAATCCTCCGTGCTCGG-3') and reverse primer n900r (5'-GGTTCGATTAGTCTTTCGCC-3'). Polymerase chain reaction primers and additional internal primers 300R (5'-CAACTTTCCTCACGGTACTTG-3') and ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3') were used for sequencing. We were able to sequence complete amplicons of *C. waelhrew* without any problems; however, despite multiple attempts, we failed to sequence ITS1 in the new species and the entire ITS region in *C. nithoggi*. Therefore, only about a 510-base pair (bp)-long region at the 5' end of the 28S gene was used for

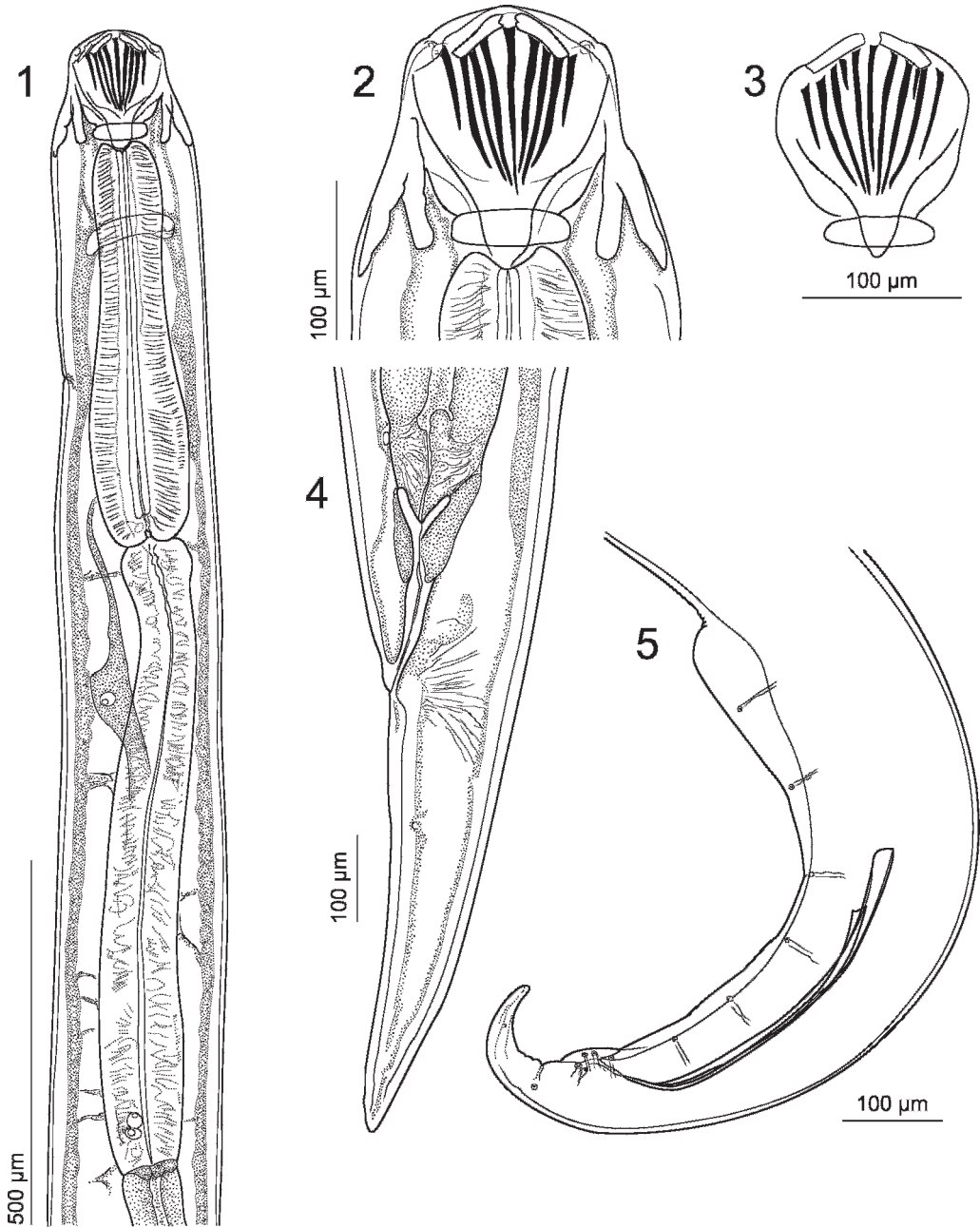
species differentiation. PCR products were purified directly with Qiagen Qiaquick<sup>TM</sup> (Valencia, California) columns, cycle-sequenced with ABI BigDye<sup>TM</sup> chemistry, alcohol-precipitated, and run on an ABI Prism 3100<sup>TM</sup> (Foster City, California) automated capillary sequencer. Contiguous sequences were assembled and edited with Sequencher<sup>TM</sup> (GeneCodes Corp., ver. 4.1.4) and submitted to GenBank: *Camallanus tuckeri* n. sp. (FJ969492–FJ969497), *C. nithoggi* (FJ969498), and *C. waelhrew* (FJ969499–FJ969505).

Sequences were aligned for pairwise comparison in the BioEdit program, version 7.0.1 (Hall, 1999). All measurements are in micrometers ( $\mu\text{m}$ ) unless otherwise stated.

### *Camallanus tuckeri* n. sp. (Figs. 1–11)

#### Description

**General:** Slender worms. Body cuticle finely striated transversely, striation visible from posterior ends of tridents to tail end. Head end rounded, tail end tapering. Oral opening narrow, slit-like, with rounded corners. Eight cephalic papillae present in 2 circles: 4 minute flattened papillae closer to oral opening, and 4 large rounded papillae of outer circle (Fig. 6). Buccal capsule typical for the genus. Each valve of buccal capsule usually with 9 internal ridges: 1 median ridge, 4 ventral and 4 dorsal ridges (Figs. 2, 8). Ridges are angled medially from anterior to posterior. Anteriorly, distance between median ridge and nearest dorsal and ventral ridges somewhat larger than between other ridges. No incomplete or interrupted ridges were observed, however, 2 additional short ridges were present in one specimen (Fig. 3). Median ridge may be shorter than 2 proximate ridges. Thick, sclerotized ring present at base of buccal capsule. Buccal capsule valves supported by 2 prominent dorsoventral tridents

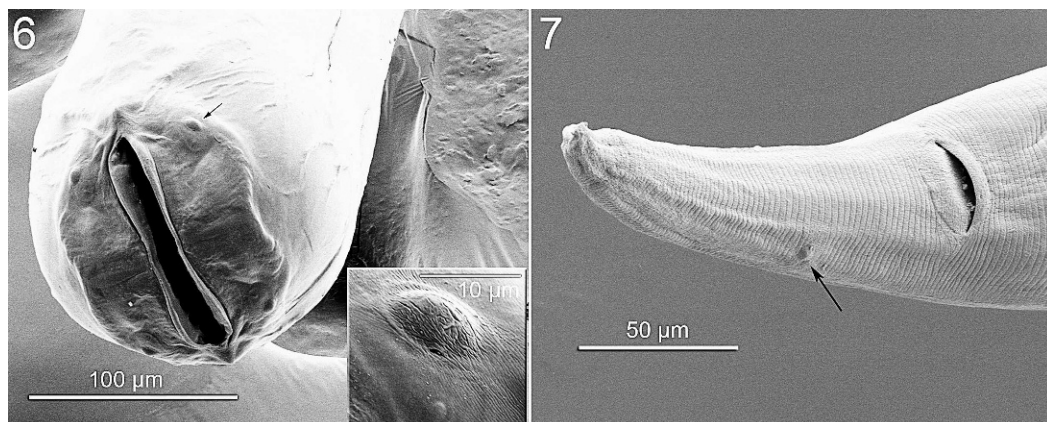


**Figures 1–5.** *Camallanus tuckeri* n. sp. 1. Anterior portion of holotype, lateral view. 2. Anterior end of holotype, lateral view. 3. Buccal capsule with 2 additional incomplete ridges, paratype. 4. Posterior end of female, lateral view of paratype. 5. Posterior body of male, lateral view of holotype.

having 3 posteriorly directed prongs (Fig. 9). Central prongs of tridents somewhat longer than sublateral ones. Tridents' posterior ends reach beyond level of basal ring. Deirids minute, papilla-shaped, situated

somewhat anterior to border between muscular and glandular esophagus (Fig. 1).

Muscular esophagus with elongated posterior bulb. Glandular esophagus about 1.6 times longer than



**Figures 6, 7.** Scanning electron micrographs of *Camallanus tuckeri* n. sp. **6.** Anterior end, subapical view. Inset shows an enlarged fragment of 1 of a pair of head papillae; arrow indicates region of inset. **7.** Posterior end of female showing phasmid (arrow), ventrolateral view.

muscular esophagus (Fig. 1). Three prominent large nuclei at posterior end of glandular esophagus. Intestine straight, narrow. Rectum straight, thin-walled.

*Males* (measurements for holotype and 2 paratypes): Body 11.891 (10.205, 11.642) mm long, 195 (164, 189) wide. Posterior part of body coiled ventrally. Buccal capsule 152 (135, 146) long, valves 129 (113, 123) long and 125 (115, 117) wide in lateral view. Sclerotized ring 23 (22, 23) long and 76 (67, 78) wide. Muscular esophagus 560 (520, 547) long, glandular esophagus 873 (870, 939) long. Distance from anterior end of body to posterior end of esophagus 13.8% (14.2, 15.8) of body length. Nerve ring at 281 (248, 269) from anterior end. Deirids at 104 from border between muscular and glandular esophagus (measured in 1 male).

Anterior part of testis is slightly twisted. Caudal alae low, ventrolateral. Anterior part of alae somewhat elevated and joined on ventral surface forming pseudosucker. Posterior ends of alae behind level of tail midlength. In precloacal portion, alae supported by 7 pairs of pedunculate papilla. Anterior pair of papillae supporting posterior part of pseudosucker (Fig. 5). Five pairs of postcloacal papillae present. Posterior to cloacal opening, caudal alae supported by 2 pairs of ventrolateral papillae. Two pairs of subventral papillae situated closely to each of first ventrolateral papilla, thus forming a group of 3 papillae on each side. A pair of minute lateral papillae situated closer to tail tip (Fig. 5). A pair of papilla-shaped phasmids present at level of second pair of postcloacal ventrolateral papillae. Additionally, 2

pairs of sessile papilla, surrounding cloacal opening (Fig. 10). Spicules unequal (Fig. 5). Right spicule 394 (381, 390) long, more sclerotized, with elongated funnel-shaped anterior part and pointed end. Its distal part needle shaped, slightly curved ventrally. Left spicule 339 (312, 326), less visible, with sharply pointed end. Anterior ends of spicules posterior to level of pseudosucker. Tail conical, with rounded end, 186 (177, 194) long (1.6% [1.5, 1.9] of body length).

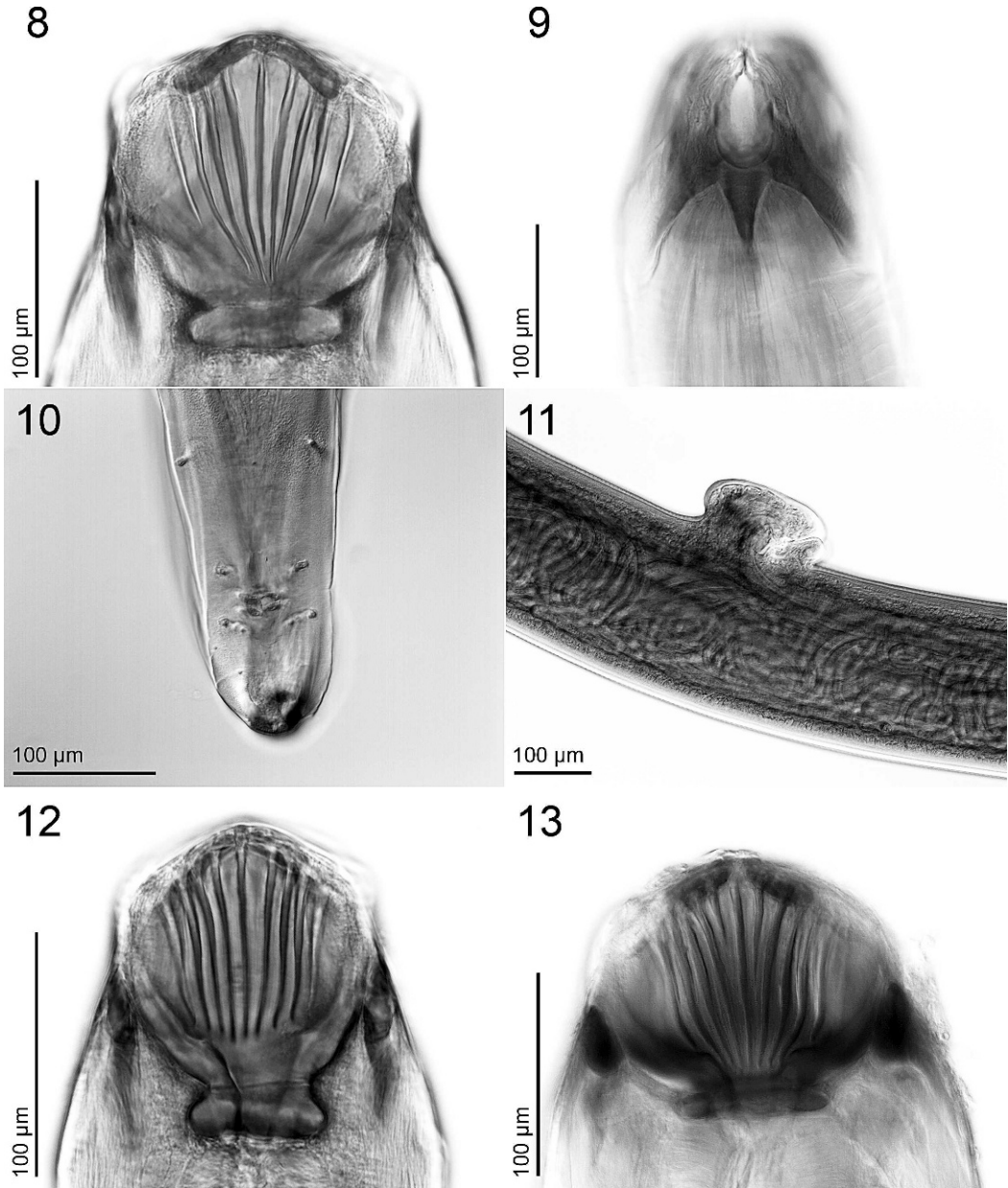
*Females* (measurements for 11 paratypes, mean value and limits): Body 16.368 (12.922–19.034) mm long, 232 (159–275) wide. Buccal capsule valves 143 (135–153) long and 149 (137–179) wide. Sclerotized ring at base of buccal capsule 88 (75–94) long, 23 (22–24) wide. Total length of buccal capsule 166 (157–176). Muscular esophagus 621 (555–658), glandular 991 (712–1062) long. Distance from anterior end of body to posterior end of esophagus 10.8% (9.8–11.9) of body length. Nerve ring at 310 (292–329) from anterior end.

Vulva pre-equatorial, 7.625 (6.076–9.273) from anterior end. In gravid females, both lips of vulva prominently elevated (Fig. 11). Tail conical, elongated, 232 (201–249) long (1.6% [1.4–1.8] of body length), with rounded tip. Phasmids situated at about midlength of tail (Fig. 7).

### Taxonomic summary

*Type host:* Northwestern red-faced turtle *Emydura australis* Gray, 1841. Prevalence 7/22 (31.8%), intensity 1–18 specimens.





**Figures 8–11.** *Camallanus tuckeri* n. sp. **8.** Anterior end, lateral view showing ridges. **9.** Anterior end, dorsal view showing trident. **10.** Male posterior end, ventral view. **11.** Female vulva region, lateral view. **12.** Anterior end of *Camallanus waelhreow*, lateral view, showing ridges. **13.** Anterior end of *Camallanus nithoggi*, lateral view, showing ridges.

*Other hosts:* Sandstone snake-necked turtle *Chelodina burrungandjii* Thomson, Kennett et Georges, 2000. Prevalence 3/16 (18.7%), intensity 2–7 specimens.

*Site of infection:* Intestine.

*Type locality:* Ord River, Kununurra, Western Australia (15°47.533'S; 128°42.310'E).

*Other localities:* Lake Argyle Spillway (16°07.371'S, 128°44.271'E), Donkey Hole (16°39.275'S, 125°29.338'E), Minah Creek (17°06.627'S,

125°21.012'E), Bell Creek (17°10.159'S, 125°21.521'E), Geike Gorge (18°06.259'S; 125°42.056'E), all in Western Australia.

The type series consists of 14 fully mature specimens. Holotype (male): Queensland Museum, Brisbane, Australia (QM) G231372. Paratypes (2 males and 11 females): QM G231373–G231377. All specimens labeled ex. *Emydura australis*, Kununurra, Western Australia, 19 June 2006. Coll. Snyder & Tkach.

*Etymology*: The species is named in honor of turtle biologist Dr. Tony Tucker in recognition of his knowledge of turtles of the Kimberley and his logistical assistance to our efforts in the Kimberley.

## Remarks

*Camallanus tuckeri* n. sp. is morphologically closest to 3 *Camallanus* species, all described from turtles: *C. chelonius*, *C. nithoggi*, and *C. waelhrew*. All 4 species show similarity in possessing a comparatively small number of buccal capsule ridges, an elongated tail in females, and the infection of pleurodirid turtles.

The new species differs from *C. chelonius* in the number of precloacal ventrolateral papillae in males: 6 in *C. chelonius* vs. 7 in *C. tuckeri* n. sp. The distal part of the right spicule in *C. tuckeri* n. sp. lacks a blunt projection and curved, sharply pointed distal end characteristic of *C. chelonius* (Baker, 1983). In females of *C. tuckeri* n. sp. lips of vulva are prominently elevated, whereas only an anterior lip swelling is present in *C. chelonius* (Baker, 1983; Fig. 11). In addition, these 2 species are separated geographically, with *C. chelonius* found in African turtles.

*Camallanus tuckeri* n. sp. resembles the Australian nematodes *C. nithoggi* and *C. waelhrew* in the shape of the spicules and the number and position of male genital papillae. The new species differs from *C. waelhrew* in the shape of buccal ridges (Figs. 2, 8, 12, 13). In the latter species, the proximal ridges run almost parallel to the longitudinal axis of the worm and each other (Fig. 12), with only the distal ridges slightly angled toward the longitudinal axis of the body (Rigby et al., 2008; personal observations), whereas in *C. tuckeri* n. sp., all dorsal and ventral ridges are angled toward the median ridge (Figs. 2, 8). Vulva lips in females of *C. tuckeri* n. sp. are swollen (Fig. 11). Such swelling is absent in gravid females of *C. waelhrew* (Rigby et al., 2008; personal observations). *Camallanus tuckeri* n. sp. is larger than

*C. waelhrew*, with the males of the new species 10.2–11.9 mm long and females 12.9–19.0 mm long. In *C. waelhrew*, males are 6.5–9.8 mm long and females are 10.9–15.9 mm long (Rigby et al., 2008).

*Camallanus tuckeri* n. sp. differs from *C. nithoggi* in the lower number of incomplete buccal capsule ridges. The new species normally has all ridges complete (Figs. 2, 8) or, rarely, 2 incomplete ridges (Fig. 3), whereas *C. nithoggi* has 2–6 (average 4) incomplete ridges in males and 2–12 (average 5) incomplete ridges in females (Rigby et al., 2008). Gravid females of *C. nithoggi* lack the swelling of the posterior lip of vulva (Rigby et al., 2008; personal observations), which is characteristic of *C. tuckeri* n. sp. Although male body length in both species is similar (10.2–11.9 in *C. tuckeri* n. sp. and 7.9–12.2 mm in *C. nithoggi*), spicules in *C. tuckeri* n. sp., especially the right one, are shorter than those in *C. nithoggi*. In the former species, the right spicule is 381–394 and the left spicule is 312–339, whereas in the latter, the right spicule is 500–550 and the left spicule is 300–420. These morphological characters and molecular evidence (below) strongly support the status of *C. tuckeri* n. sp. as new to science.

## Molecular differentiation

The ribosomal fragment sequenced was 509 bp long in *C. tuckeri* n. sp., 507 bp in *C. waelhrew*, and 502 bp in *C. nithoggi*. We sequenced multiple specimens of *C. tuckeri* n. sp. and *C. waelhrew*, and a single specimen of *C. nithoggi* (Table 1). No intraspecific variability was observed among 6 specimens of *C. tuckeri* n. sp. collected from 2 different turtle species and 5 different localities situated at a distance of up to 380 km from each other. These localities were separated by numerous river drainages and substantial differences in elevation. Similarly, there was no sequence variability among 7 specimens of *C. waelhrew* collected from 7 representatives of 2 turtle species at a single locality.

To examine interspecific sequence variability, we conducted pairwise sequence comparison among the 3 species using BioEdit software (Hall, 1999). The level of base differences was relatively high, with *C. waelhrew* and *C. nithoggi* most similar to one another (47 variable sites or 9.2% of alignment length). The species pair *C. tuckeri* n. sp.–*C. waelhrew* had 63 variable sites (12.2% of the alignment), and the pair *C. tuckeri* n. sp.–*C. nithoggi* had 57 variable sites (11.2% of the alignment). Thus, substantial sequence differences strongly support

observed morphological differences among all 3 species of *Camallanus* found thus far in Australian freshwater turtles and confirm the status of *C. tuckeri* n. sp. as a new species.

## DISCUSSION

Camallanidae are characterized by the presence of 8 cephalic papillae (Ivashkin et al., 1971), however 4 of these papillae are very difficult to discern by light microscopy and are frequently overlooked. For example, Sharma et al. (2002) report only 4 cephalic papillae, but close inspection of a scanning electron micrograph in their redescription of *Serpinema octorugatum* reveals the presence of 2 circles of 4 papillae each. Rigby et al. (2008) found only 4 cephalic papillae in *C. nithoggi* and *C. waelhrew*, but examination of our specimens of these species demonstrates the presence of 8 cephalic papillae. In this study, we observed 8 cephalic papillae in *C. tuckeri* n. sp. Four of them are large and prominent under the light microscope, whereas the other 4 are minute and indistinct, though easily observed by SEM (Fig. 6). Apparently, 8 cephalic papillae, 4 larger and 4 minute, are present in all camallanids from turtles. Thus, the number of cephalic papillae is not useful in distinguishing species or genera of Camallanidae.

Camallanids from turtles are circumscribed within the genera *Camallanus* and *Serpinema* and can be differentiated by the separation of buccal ridges into dorsal and ventral groups in *Serpinema*, whereas *Camallanus* lacks such a division (Yeh, 1960; Baker, 1983). *Camallanus tuckeri* n. sp. is the second species of the genus *Camallanus* possessing a median buccal capsule ridge that is only slightly separated from the other ridges of the buccal capsule, similar to the situation observed in *C. chelonius*. Baker (1983) considered this character as intermediate between the 2 genera and indicative of a close relationship among *Camallanus* spp. from pleurodiran turtles and *Serpinema* spp. described from pleurodirans in South America. The presence of such an intermediate, and possibly continuous, character suggested to Baker (1983) that the validity of *Serpinema* might be in question.

Conversely, the presence of a separation between dorsal and ventral ridges might not be the most important character for differentiation between *Camallanus* and *Serpinema*. *Camallanus tuckeri* n. sp. has a median buccal capsule ridge separated from other ridges, a character not found in *C. nithoggi* and *C. waelhrew*. However, the new species is similar to these species in a number of other characters (shape

of spicules, number and position of genital papillae, etc.). A molecular phylogenetic study might be necessary to provide an additional examination of the generic status of *Serpinema* and the utility of morphological characters for camallanid systematics above the species level.

Rigby et al. (2008) concluded that *Camallanus* found in *El. latisternum* in Queensland and reported as *C. chelonius* by Ferguson and Smales (1998) was, in fact, *C. nithoggi*, and that specimens from *Emydura krefftii* were morphologically consistent with *C. waelhrew*, although the authors did not make a definitive conclusion on the identity of these worms. More recently, Ferguson and Smales (2006) reported *C. chelonius* in *Em. macquarii* from northern and central Queensland. Although we did not examine these specimens, we have examined numerous turtles representing 3 genera from New South Wales and northern and southern Queensland. All *Camallanus* from *Emydura* and *Chelodina* from these regions belonged to *C. waelhrew*, whereas *C. nithoggi* was found only in *Elseya*, similar to the findings of Rigby et al. (2008). We therefore suggest that the specimens reported by Ferguson and Smales (1998, 2006) from *Emydura* as *C. chelonius* are *C. waelhrew*.

*Camallanus tuckeri* n. sp. is the first species of the genus reported from turtles in Western Australia. The 2 previously described species were found in New South Wales and Queensland (Rigby et al., 2008; our data). The new species is also the first nematode reported from *Em. australis* and the first endoparasite reported from *Ch. burrungandjii*. Specimens of *C. tuckeri* n. sp. found in both *Emydura* and *Chelodina* were adult and fully mature, which suggests it does not show obvious preference to turtles of either genus. The same is true for *C. waelhrew*, which is also found in both genera of turtles. Of 3 *Camallanus* species presently known from Australian turtles, only *C. nithoggi* seems to be specific to a single host species, *El. latisternum* (Rigby et al., 2008; personal observations).

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