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# *KREFFTASCARIS* (NEMATODA, ASCARIDOIDEA) FROM AUSTRALIAN SIDE-NECKED TURTLES WITH DESCRIPTION OF *KREFFTASCARIS SHARPILOI* SP. N. FROM *CHELODINA RUGOSA*

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***Krefftascaris*** **(Nematoda, Ascaridoidea) from Australian Side-Necked Turtles with Description of** ***Krefftascaris sharpiloi*** **sp. n. from** ***Chelodina rugosa*****. Tkach V. V., Kuzmin Yu. I., Snyder S. D.** — Previously known records of ascaridoid nematodes *Krefftascaris* Sprent, 1980 are summarized and new records of the genus reported. *Krefftascaris sharpiloi* Tkach, Kuzmin et Snyder, sp. n. is described from specimens found in the stomach of the northern snake-necked turtle *Chelodina rugosa* collected from two localities in Northern Territory, Australia. The new species differs from the only previously known *Krefftascaris* species, *K. parmenteri* Sprent, 1980, by the presence of thickened and bifurcated anterior edges of the lateral cuticular alae and a difference in the relative distance from the anterior end to the nerve ring which is 1.5 to 2 times greater in *K. parmenteri*. Comparison of approximately 2.100 bases of ribosomal DNA sequences This study contains first reports of *Krefftascaris* in *Chelodina rugosa*, *Chelodina burrungandjii*, *Chelodina canni* and *Emydura tanybaraga* and the first records of this genus in the Northern Territory, Queensland and Western Australia. Molecular phylogenetic analysis based on sequences of nuclear small ribosomal subunit gene has demonstrated close affinities between *Krefftascaris* and *Heterocheilus*, the type genus of the Heterocheilidae and Heterocheilinae. Parasitism of several species and genera of Heterocheilidae in crocodiles allows us to hypothesize that *Krefftascaris* may have been acquired by turtles from crocodilians.

Key words: Nematoda, Ascaridiidae, *Krefftascaris*, sp. n., Australia, turtles, *Chelodina*, *Elseya*, ribosomal DNA sequences, molecular phylogeny.

***Krefftascaris*** **(Nematoda, Ascaridoidea) из бокошейных черепах Австралии, с описанием** ***Krefftascaris sharpiloi*** **sp. n. из** ***Chelodina rugosa*****. Ткач В. В., Кузьмин Ю. И., Снайдер С. Д.** — Обобщены известные ранее и приведены новые данные о нематодах рода *Krefftascaris* Sprent 1980. Новый вид рода, *Krefftascaris sharpiloi* Tkach, Kuzmin et Snyder, sp. n., описан из желудка бокошейной черепахи *Chelodina rugosa* из Северной Территории (Австралия). Он отличается от ранее известного *K. parmenteri* Sprent, 1980 наличием разветвленных утолщений на переднем краю латеральных кутикулярных крыльев, а также положением нервного кольца относительно переднего конца тела. Это расстояние, отнесенное к длине тела, у нового вида в 1,5–2 раза меньше, чем у *K. parmenteri*. Сравнение последовательностей около 2100 оснований рибосомальной ДНК Виды рода *Krefftascaris* впервые обнаружены у хозяев *Chelodina rugosa*, *C. burrungandjii*, *C. canni* и *Emydura tanybaraga* и на территории австралийских шатов Северная Территория, Квинсленд и Западная Австралия. Молекулярно-филогенетический анализ, основанный на последовательностях нуклеотидов ядерного гена малой рибосомальной субъединицы, показал близость *Krefftascaris* к роду *Heterocheilus*, типовому в семействе Heterocheilidae и подсемействе Heterocheilinae. Паразитирование некоторых видов и родов Heterocheilidae у крокодилов позволяет нам предположить, что предки *Krefftascaris* перешли к паразитированию у черепах от этой группы хозяев.

Ключевые слова: Nematoda, Ascaridiidae, *Krefftascaris*, новый вид, Австралия, черепахи, *Chelodina*, *Elseya*, рДНК, последовательность нуклеотидов, молекулярная филогения.

## Introduction

*Krefftascaris* Sprent, 1980 is a monotypic genus known only from Australian freshwater turtles, and has not been reported since original description. *K. parmenteri* Sprent, 1980 was described from fresh-water turtles *Chelodina longicollis* (Shaw, 1794) and *Elseya dentata* (Gray, 1863) from New South Wales (Sprent, 1980). Sprent did not clearly specify the systematic position of the new genus and only mentioned its probable close relationships with the parasites of crocodilians (*Ortleppascaris* Sprent, 1978 and *Gedoelstacaris* Sprent, 1978) and terrestrial reptiles (*Angusticaecum* Baylis, 1920, *Hexametra* Travassos, 1919 and *Polydelphis* Dujardin, 1845).

As part of a survey of the parasite fauna of Australian freshwater turtles (Snyder, Tkach, 2006, 2007, 2009; Tkach, Snyder, 2006, 2007 a, b, 2008; Kuzmin et al., 2009) we found ascaridiid nematodes in several species of freshwater turtles from multiple localities in Queensland, Western Australia, and Northern Territory, Australia. The majority of these specimens were identified as *Krefftascaris parmenteri*. However, specimens from *Chelodina rugosa* Ogilby, 1890 collected from two localities in Northern Territory appeared to be morphologically different from *K. parmenteri*. This material is described herein as a new species based on combined morphological and molecular evidence. A molecular phylogenetic analysis is attempted in order to clarify systematic position of *Krefftascaris*.

## Material and methods

Between June 2004 and December 2007, specimens belonging to 12 species of freshwater turtles, were collected by baited traps or by hand from numerous localities in New South Wales, Queensland, Northern Territory and Western Australia. All collections proceeded under permits from authorities of corresponding states.

Most turtles harboured one to several species of parasitic worms including digeneans, aspidogastreans, cestodes and nematodes; however, for the purpose of this work we refer only to nematodes. 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-glycerine (1 : 3 ratio). Measurements were taken from a compound microscope using digital imaging and Rincon measurement software (v. 7.1.2, Imaging Planet, Goleta, California). Photographs and drawings were made with DFC480 digital camera and drawing tube mounted on Leica DM5000 compound microscope equipped with DIC optics. Nematodes belonging to the new species were found in material from *C. rugosa* from two localities in Northern Territories (Hayes Creek Billabong and a small lagoon on Mango Farm in the Daly River floodplain). Specimens of *K. parmenteri* used for morphological differentiation with the new species originated from *C. rugosa* and *C. burrunganjii* from Western Australia, Queensland and Northern Territory.

All measurements are in micrometers unless otherwise stated.

Genomic DNA for molecular analysis was extracted from: six specimens of *Krefftascaris parmenteri* obtained from *Chelodina rugosa* collected from the Mary and Daly Rivers in Northern Territory) and Leichardt Lagoon in Northern Queensland; *Chelodina canni* collected from Armraynald Station and Ross River Dam in Northern Queensland; *Chelodina burrungandjii* collected from Parry Creek Farm Billabong in Western Australia and *Emydura tanybaraga* collected from a small lagoon on Mango Farm in Northern Territory. DNA was also extracted from two specimens of the new species obtained from *Chelodina rugosa* collected from Hayes Creek Billabong and a small lagoon on Mango Farm, both in Northern Territory (tabl. 1). Tissue for DNA extraction was taken from the middle part of the body while taxonomically important anterior and posterior parts were preserved as vouchers for morphological identification. DNA was extracted according to Tkach and Pawlowski (1999).

For species differentiation, DNA fragments spanning the 3' end of 18S nuclear rDNA gene, internal transcribed spacer region (ITS1 + 5.8S + ITS2) and 5' end of the 28S gene were amplified by PCR on an Eppendorf Master Gradient thermal cycler using the newly designed forward primer c1860f (5'– TGAAAA TCCTCCGTGCTCGG–3') and the reverse primer n900r (5'–GGTTCGATTAGTCTTTCGCC–3'). PCR primers as well as additional forward internal primer 28S 2 (5'–CCGCTGAATTTAAGCATAT–3') and reverse internal primers 28S 2R (5'–ATATGCTTAAATTCAGCGG–3'), 300R (5'–CAACTTTCCC TCACGGTACTTG–3') and ECD2 (5'–CTTGGTCCGTGTTTCAAGACGGG–3') were used for sequencing. PCR products were purified directly using Qiagen Qiaquick™ (Valencia, CA) 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: *Krefftascaris parmenteri* (GU245685– GU245690), *Krefftascaris sharpiloi* sp. n. (GU245691– GU245692). Sequences were aligned for pairwise comparison in the BioEdit program, version 7.0.1 (Hall, 1999).

For phylogenetic analysis, nearly complete nuclear small ribosomal subunit (18S) gene was amplified from the new species using forward primer G18S4 (5'–GCTTGTCTCAAAGATTAAGCC–3') and reverse primer c1960r (5'–CGACTTTTGCCCGGTTCAAGCCAC–3'). PCR primers and additional internal reverse primers n635r (5'–CGCCTGCTGCCTTCCTTGG–3') and n649r (5'–TAAGAACGGCCATG CACCAC–3') were used for sequencing. Sequence of *K. sharpiloi* nov. sp. was aligned with previously published sequences (Nadler, Hudspeth, 1998, 2000; Nadler et al., 2007) of 16 other species belonging to 7

**Table 1. Hosts and localities of *Krefftascaris* spp. in Australia**
**Таблица 1. Хозяева и места обнаружения *Krefftascaris* spp. в Австралии**

| Species | Hosts | Localities | Source of information |
|---|---|---|---|
| *K. parmenteri* | *C. longicollis* | Gara River, NSW | Sprent (1980) |
| | *E. dentata* | Gara River, NSW | Sprent (1980) |
| | *C. burrungandjii* | Parry Creek Farm Billabong, WA (15°35.817' S; 128°16.724' E) | present study |
| | *C. rugosa* | Leichardt Lagoon, QLD (17°51.040' S; 141° 07.707' E) | present study |
| | | Mary River, NT (12°34.624' S; 131°43.476' E) | present study |
| | | Daly River, NT (14°00.31' S; 131°14.46' E) | present study |
| | *C. canni* | Armraynald Station, QLD (17°57.432' S; 139°45.393' E) | present study |
| | | Ross River Dam, near Thuringowa, QLD (19°24.582' S, 146°44.302' E) | present study |
| | *E. tanyabaraga* | Daly River Mango Farm, NT (13°44.30' S; 130°40.84' E) | present study |
| *K. sharpiloi* sp. n. | *C. rugosa* | Hayes Ck Billabong, NT (13°39.589' S; 131°23.990' E) | present study |
| | | Daly River Mango Farm, NT (13°44.30' S; 130°40.84' E) | present study |

ascaridoid families and 3 outgroup taxa available in GenBank: *Brumptaemilius justini* (AF036589), *Raillietnema* sp. (DQ503461), *Nemhelix bakeri* (DQ118537), *Dujardinascaris waltoni* (EF180081), *Cruzia americana* (U94371), *Heterocheilus tunicatus* (U94373), *Contracaecum eudyptulae* (EF180072), *Porrocaecum depressum* (U94379), *Toxocara canis* (U94382), *Pseudoterranova decipiens* (U94380), *Anisakis* sp. (U94365), *Terranova caballeroi* (U94381), *Hysterothylacium reliquens* (U94376), *Iheringascaris inquies* (U94377), *Raphidascaris acus* (DQ503460), *Ascaris lumbricoides* (U94366), *Baylisascaris procyonis* (U94368), *Parascaris equorum* (U94378), *Toxascaris leonina* (U94383). Choice of ingroup and outgroup taxa was based on recent molecular phylogenetic studies of the Ascaridoidea and related nematode lineages (Nadler, Hudspeth, 1998, 2000; Nadler et al., 2007). Choice of the gene was defined by the selection of sequences currently available in the GenBank. Sequences were aligned using Clustal W module implemented in the BioEdit 7.01 (Hall, 1999) with subsequent refinement by eye using BioEdit. A Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses were performed using PAUP* ver. 4.0b10 (Swofford, 2002). Nodal support was calculated based on 1,000 bootstrap replicates with 100 replicates at each step for MP analysis and 1,000 bootstrap replicates for ML analysis.

***Krefftascaris sharpiloi*** Tkach, Kuzmin et Snyder, **sp. n.** (fig 1; 2, *a*, *c*, *d*)

Description

General. Body slender, irregularly coiled. Body cuticle transversely striated. Lateral alae present along the whole body from base of pseudolabia to the caudal region. At the head end, lateral alae possess characteristic bifurcate thickening of their edges (fig. 1, *a*). Pseudolabia trapezoid, rounded. Two large cephalic papillae present at base of each pseudolabium. Labial pulp with two rounded anterior processes. Buccal cavity small, elongated. Oesophagus with indistinct border between muscular and glandular parts, cylindrical, with somewhat dilated anterior end. Ventriculus three-lobed. Caecum long, its anterior end somewhat posterior to level of nerve ring (fig. 1, *b*). Excretory pore just posterior to the nerve ring. Deirids were not observed.

Males (holotype and 1 paratype; measurements of paratype in parentheses). Smaller than females, body 37.3 (41.6) mm long, 405.0 (465.0) wide at midlength. Pseudolabia 35.0 (42.0) long. Oesophagus 3.9 (3.7) mm long [10.6 (9.0) % of body length], ventriculus 151.0 (152.0) long. Nerve ring at 343.0 (379.0), excretory pore at 430.0 (455.0) from anterior end. Posterior part of body bent ventrally. Tail short, conical, with pointed end (fig. 1, *d*). Distance from anus to tail tip 112.0 (98.0) [0.3 (0.2) % of body length].

Spicules strongly sclerotized (fig. 1, *c*). Right spicule 683.0 (678.0) long, with elongated funnel-shaped capitulum. Left spicule 583.0 (580.0) long, with shorter capitulum. Both spicules with smooth distal parts and sharply pointed ends. Gubernaculum 123.0 (130.0) long, with rounded proximal part and pointed distal end in lateral view. Four

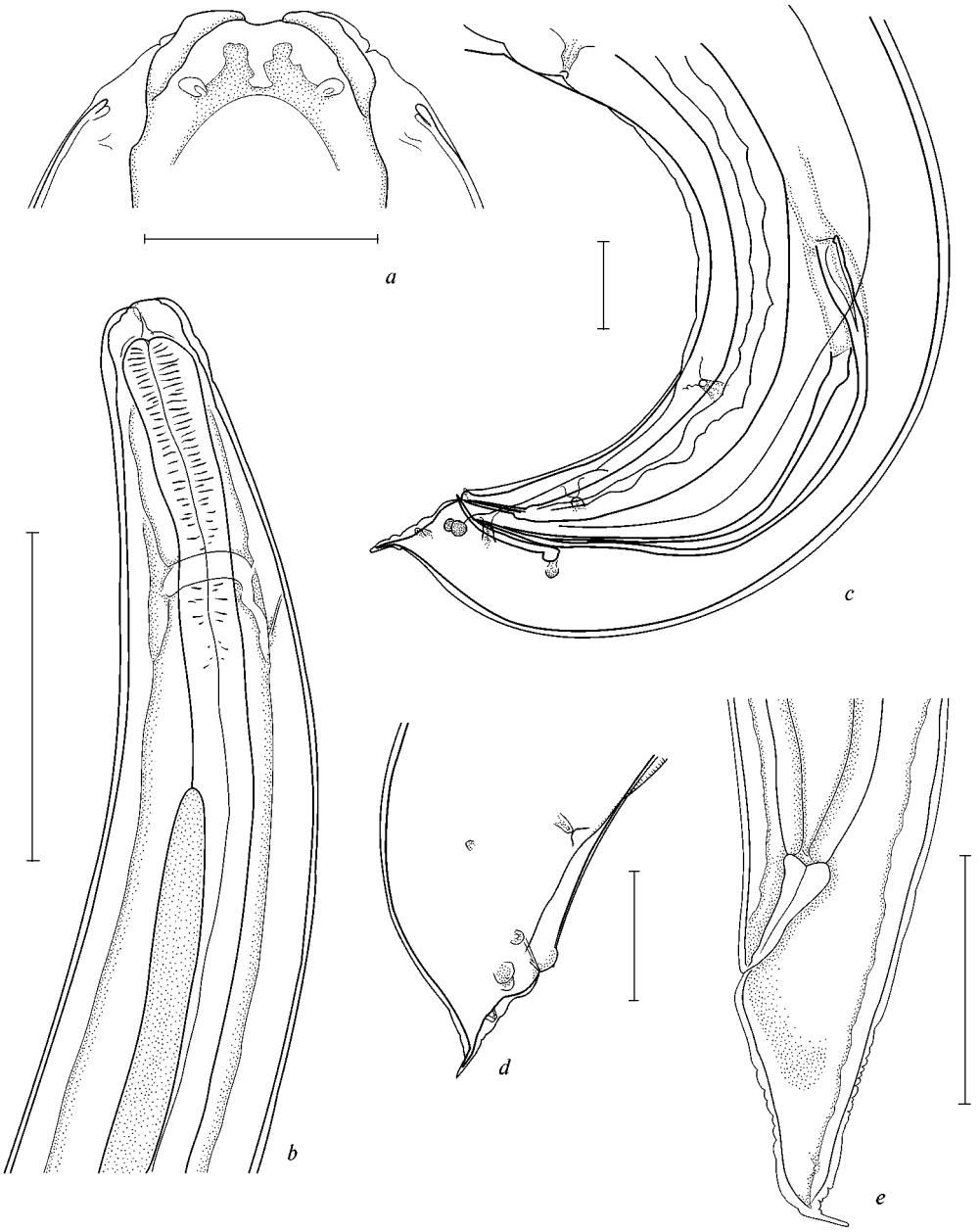


Fig. 1. *Krefftascaris sharpiloi* sp. n.: *a* — head end, dorsal view (female paratype); *b* — anterior end, lateral view (female paratype); *c* — male posterior end, lateral view (holotype); *d* — position of male caudal papillae, lateral view (first ventrolateral papilla not shown); *e* — female posterior end, lateral view (paratype). Scale bars: *a*, *c*, *d* — 0.1 mm; *b*, *e* — 0.5 mm.

Рис. 1. *Krefftascaris sharpiloi* sp. n.: *a* — головной конец, дорсальный вид (самка, паратип); *b* — передний конец тела, латеральный вид (самка, паратип); *c* — хвостовой конец самца, латеральный вид (паратип); *d* — расположение хвостовых сосочков самца, латерально (первый вентролатеральный сосочек не показан); *e* — задний конец тела самки (паратип). Масштабная линейка: *a*, *c*, *d* — 0,1 мм; *b*, *e* — 0,5 мм.

pairs of ventrolateral genital papillae associated with small genital alae (fig. 1, *c*). First pair of genital papillae situated at some distance from other ones, second pair at midway between first pair and level of anus, fourth pair adanal. A pair of lateral papillae positioned between levels of the third and the fourth pairs of ventrolateral papillae

(fig. 1, *d*). Two pairs of short, robust ventrolateral adanal papillae not connected to genital alae. Two pairs of subventral papillae situated at tail midlength, all arranged in transversal row. One ventral preanal papilla present on anal anterior lip.

Females (4 paratypes). Somewhat larger than males, body 32.5–47.2 mm long, 492.0–692.0 thick at midlength. Pseudolabia 45.0–57.0 long. Oesophagus 4.0–4.6 mm long (9.6–12.4% of body length), ventriculus 89.0–190.0 long. Nerve ring at 321.0–416.0, excretory pore at 387.0–460.0 from anterior end. Tail short, conical, with cuticular needle on top (fig. 1, *e*). Tail length 502.0–604.0 (1.2–1.5% of body length). Vulva pre-equatorial, at 5.4–6.7 mm from anterior end (14.1–16.6% of body length). Lips of vulva indistinct. Vagina thick-walled, first directed anteriorly, then turned posteriorly (fig. 2, *c*). Uteri didelphic. Ovaries forming numerous loops in posterior part of body. Eggs rounded, with thick smooth transparent shell (fig. 2, *d*), 99.0–114.0 long and 62.0–78.0 wide (n = 10).

Taxonomic Summary

Type series: holotype (♂) and 5 paratypes (1 ♂ and 4 ♀).

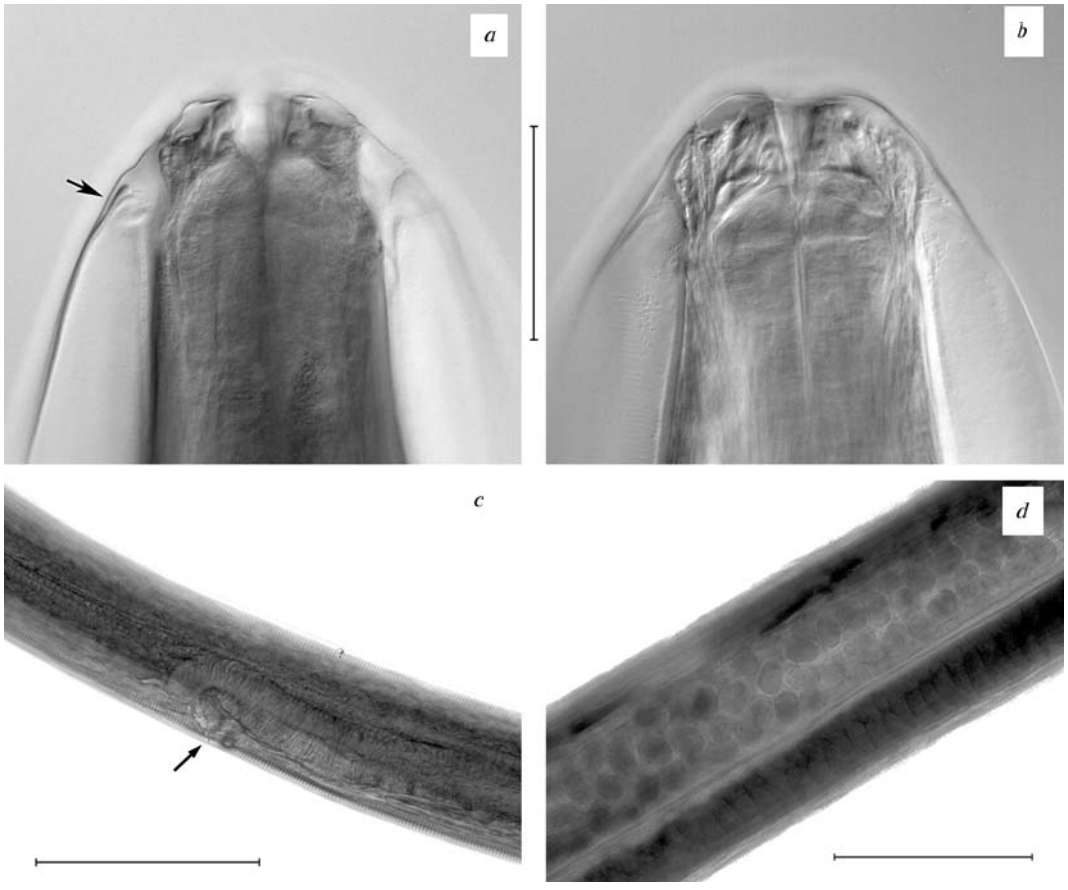


Fig. 2. *K. sharpiloi* sp. n. (*a*, *c*, *d*) and *K. parmenteri* (*b*): *a*, *b* — head end, dorsal view (arrow indicates thickening of cuticle on anterior edge of lateral alae in *K. sharpiloi* sp. n.); *c* — fragment of juvenile female showing vulva (arrow) and distal part of vagina; *d* — fragment of gravid female showing eggs in uterus. Scale bars: *a*, *b* — 0.1 mm; *c*, *d* — 0.5 mm.

Рис. 2. *K. sharpiloi* sp. n. (*a*, *c*, *d*) и *K. parmenteri* (*b*): *a*, *b* — головной конец, дорсально (утолщение кутикулы на переднем краю латеральных крыльев у *K. sharpiloi* sp. n. показано стрелкой); *c* — фрагмент тела ювенильной самки с вульвой (стрелка) и дистальной частью вагины; *d* — фрагмент тела взрослой самки со зрелыми яйцами в матке. Масштабная линейка: *a*, *b* — 0,1 мм; *c*, *d* — 0,5 мм.

Type specimens deposited in the Queensland Museum, Brisbane Australia. Holotype: G231391 (♂); 5 paratypes: G231392–231393 and G231689–231691 (1 ♂ and 4 ♀).

Type host: Northern snake-necked turtle *Chelodina rugosa* Ogilby, 1890. Prevalence: 10%; intensity: 3–6 specimens.

Type locality: Hayes Creek Billabong, Northern Territory, Australia; 13°39.589' S, 131°23.990' E.

Other localities: Daly River Mango Farm, Northern Territory, Australia; 13°44.30' S, 130° 40.84' E.

Etymology. The species is named in honour of prominent helminthologist Viktor Sharpilo in recognition of his contributions to helminthology and particularly to our knowledge of reptilian nematodes. He was a mentor of two co-authors of this publication, Vasyl Tkach and Yuriy Kuzmin.

Remarks

*Krefftascaris sharpiloi* sp. n. is morphologically most similar to *K. parmenteri* Sprent, 1980, the only species of *Krefftascaris* known prior to this study. The two species are similar in body size and shape, size and shape of the head structures, and the shape and position of male and female genital structures. *Kreftascaris sharpiloi* sp. n. differs from *K. parmenteri* in the presence of prominent bifurcating thickenings on the anterior edges of the lateral alae, which are less pronounced and lack bifurcation in *K. parmenteri* (fig. 2, *a*, *b*). The two species also differ in the relative distance from anterior end to the nerve ring. When calculated as a percentage of the body length, this distance is 1.5–2 times greater in *K. parmenteri*, than in *K. sharpiloi* sp. n. (tabl. 2).

Pairwise comparison of approximately 2,100 bases of ribosomal DNA sequences (partial 18S, complete ITS1+5.8S+ITS2, partial 28S) obtained from 2 specimens of the new species and 3 specimens of *K. parmenteri* showed 75 nucleotide differences between the two species. Sequences of *Krefftascaris sharpiloi* sp. n. showed no intraspecific variability. Only 4 nucleotide substitutions were found in the ITS region of *K. parmenteri* from two localities in Northern Queensland compared with specimens from Northern Territory and Western Australia, which were identical to one another. These regions and collecting localities are quite distant from one another (fig. 3). For instance, the Ross River Dam in Northern Queensland and the Parry Creek Farm Billabong in the Western Australia are situated more than 1900 km apart. In contrast, sequences of *K. parmenteri* obtained from localities situated only 200–300 km apart did not show any differences. More importantly, sequences of *K. parmenteri* and *K. sharpiloi* sp. n. obtained from specimens collected from the same host species, *C. rugosa*, and at the same site on the Daly

**Table 2. Differences in distance to nerve ring in *Krefftascaris sharpiloi* sp. n. and *Krefftascaris parmenteri* Sprent, 1980**

**Таблица 2. Отличия в расстоянии до нервного кольца у *Krefftascaris sharpiloi* sp. n. и *Krefftascaris parmenteri* Sprent, 1980**

| Species | Distance to nerve ring | |
|---|---|---|
| | in mm | in % of body length |
| *Krefftascaris sharpiloi* sp. n. *: | | |
| males | 361 | 0.92 |
| females | 374 | 0.91 |
| *Krefftascaris parmenteri* (our data)*: | | |
| males | 461 | 1.73 |
| females | 495 | 1.45 |
| *Krefftascaris parmenteri* (after Sprent, 1980)**: | | |
| males | 363 | 1.71 |
| females | 470 | 1.62 |

* Mean values. ** Recalculated from minimum and maximum values.

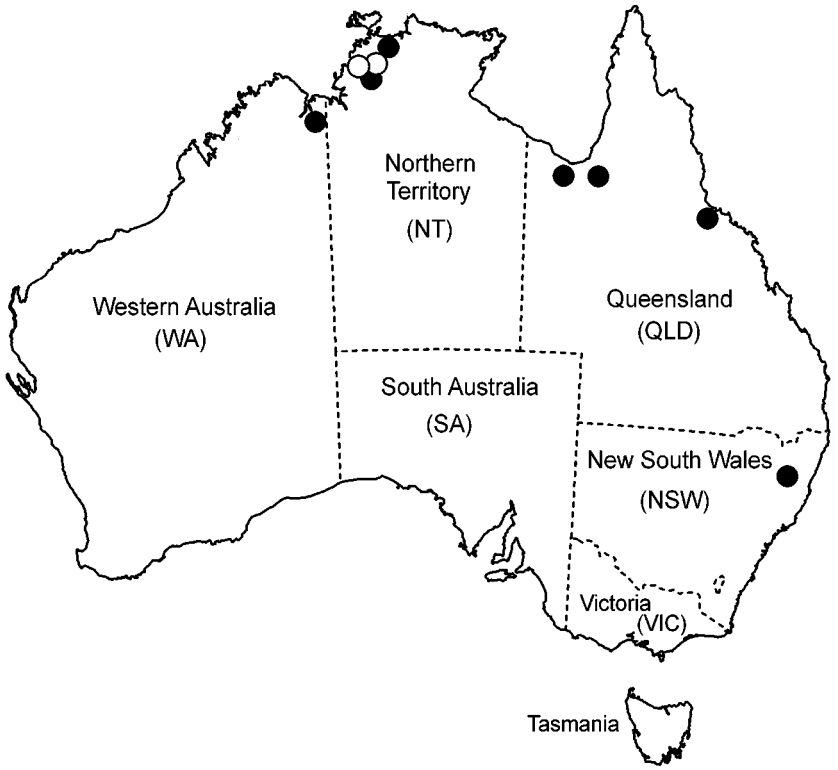


Fig. 3. Distribution of *Krefftascaris* spp. in Australia: ○ — *K. sharpiloi* sp. n.; ● — *K. parmenteri*.
Рис. 3. Распространение видов рода *Krefftascaris* в Австралии: ○ — *K. sharpiloi* sp. n.; ● — *K. parmenteri*.

River floodplain in Northern Territory, had 75 nucleotide differences. Thus, molecular data strongly support the status of *K. sharpiloi* sp. n. as a new species.

***Krefftascaris parmenteri*** Sprent, 1980 (fig. 2, *b*)

The species was previously known only from the original description only (Sprent, 1980). We have found *K. parmenteri* from additional localities and host species. Combined data on host and geographic distribution of this species are summarized below.

Taxonomic Summary

Type host: common snake-necked turtle *Chelodina longicollis* Ogilby, 1890.

Other hosts: Northern snapping turtle *Elseya dentata* (reported by Sprent, 1980), sandstone snake-neck turtle *C. burrungandjii*, northern snake-necked turtle *C. rugosa*, Cann's long-necked turtle *C. canni*, northern yellow-faced turtle *Emydura tanybaraga* (our data).

Type specimens: Queensland Museum. Holotype: G12108 (♂). Allotype: G12109 (♀).

Type locality: Gara River, New South Wales.

Other localities: Ross River Dam, Leichardt Lagoon and Armraynald Station in Northern Queensland, Daly River and Mary River in Northern Territory and Parry Creek Farm Billabong in Western Australia (our data, for details see tabl. 1).

Molecular phylogenetic analysis

The alignment was trimmed to the length of the shortest sequence. The total length of the alignment was 1688 bases with the shortest sequence being 1682 bases long and longest being 1685 bases long. Due to the conserved nature of the gene, only 5 sites

were excluded from the analysis. Of the remaining 1683 sites, 168 were variable and 70 were parsimony-informative. Due to the low level of variability and small number of parsimony-informative characters the 18S gene is not the best DNA region for phylogenetic inference at this taxonomic level. Unfortunately, our choice of molecular target for analysis was limited by the sequences currently available in the GenBank. The 18S sequences were best represented for the Ascaridoidea and suitable outgroups.

Both ML and MP analyses yielded almost identical tree topology (fig. 4) with varying levels of bootstrap support. The only difference between the two trees was clustering of *Contracaecum* and *Toxocara* in a weakly (53%) supported clade in MP analysis while in the ML analysis the support for this clade was lower than 50% and thus they are on figure 4 as a polytomy. Several clades were only weakly supported as a result of conserved nature of the analyzed gene. General topology of the branches and terminal taxa was similar to that demonstrated and discussed in detail in the previous phylogenetic treatments of the Ascaridioidea (Nadler, Hudspeth, 1998, 2000; Nadler et al., 2007). *Krefftascaris sharpiloi* clustered together with *Heterocheilus tunicatus*, a member of the Heterocheilidae. This clade received high 96% bootstrap support in the MP analysis and 90% support in the ML analysis.

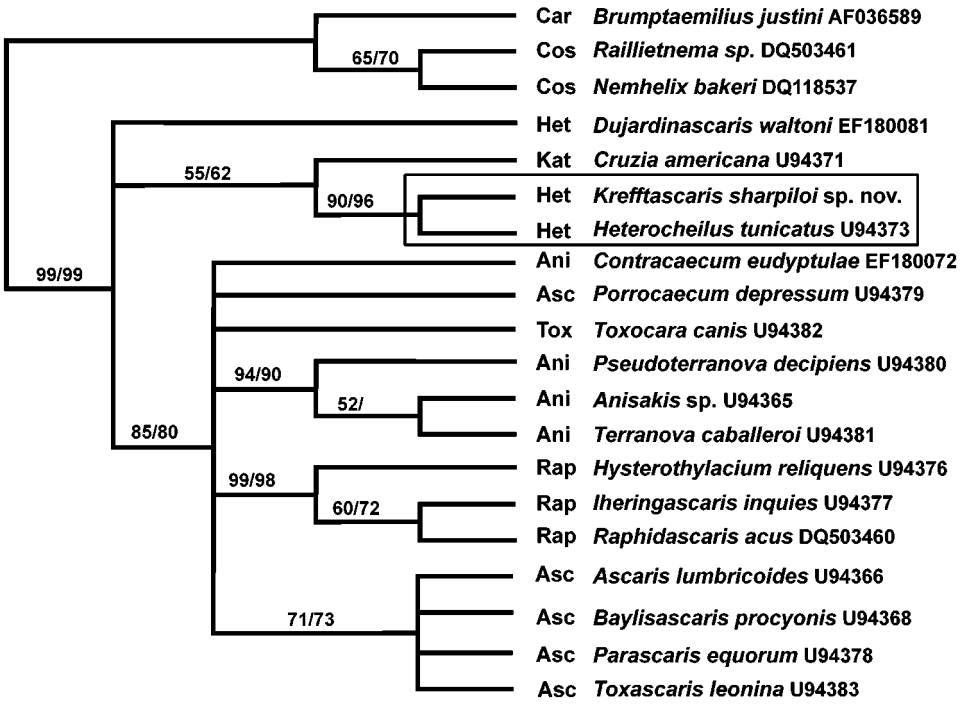


Fig. 4. Phylogenetic tree based on analysis of partial sequences of 18S rDNA gene using Maximum Likelihood and Maximum Parsimony algorithms in PAUP*. Higher than 50% bootstrap support values for ML/MP analyses are shown above internodes. Rectangle indicates close relationship between *Krefftascaris* and Heterocheilidae. GenBank accession numbers are shown next to species names. Abbreviations: Car — Carnoyidae, Cos — Cosmocercidae, Het — Heterocheilidae, Kat — Kathlaniidae, Asc — Ascarididae, Tox — Toxocaridae, Ani — Anisakidae, Rap — Raphidascarididae.

Рис. 4. Филогенетическое древо по данным анализа последовательностей фрагмента гена 18S рДНК с использованием алгоритмов максимального подобия и максимальной парсимонии в программе PAUP*. Прямоугольник указывает на филогенетическую близость *Krefftascaris* и Heterocheilidae. Сокращения: Car — Carnoyidae, Cos — Cosmocercidae, Het — Heterocheilidae, Kat — Kathlaniidae, Asc — Ascarididae, Tox — Toxocaridae, Ani — Anisakidae, Rap — Raphidascarididae.

## Discussion

J. F. A. Sprent (1980) reported a short cuticular needle at the tip of the tail in young female specimens of *K. parmenteri*. However, we observed this structure in both young and adult females of *K. parmenteri* and *K. sharpiloi* sp. n. It seems possible that this fragile feature may be lost in fixed specimens depending on the fixation procedure.

Metric characters in nematodes parasites of animals usually display a high degree of variability due to continued growth of these parasites during the adult stage. This variability frequently prevents efficient use of metric characters in the differentiation of closely related species. However, if the same measurement (e. g. the distance between the anterior end and the nerve ring) differs remarkably and consistently among groups of similarly sized specimens this character may be useful for species differentiation. Additionally, relative metric characters in nematodes demonstrate lower limits of variability than do absolute measurements (Baker, 1978). This line of evidence gives us cause to consider the difference in relative distance from the anterior end to the nerve ring a valid character for species differentiation between *K. sharpiloi* sp. n. and *K. parmenteri*.

Although we have collected *Chelodina* throughout the range of these turtles, the new species, *K. sharpiloi*, was found only in *C. rugosa* collected in Northern Territory. *Kreftascaris parmenteri*, in contrast, was observed in three *Chelodina* species, *Emydura tanybaraga* and *Elseya dentata* from a much broader geographical range: New South Wales (Sprent, 1980), Queensland, Northern Territory and Western Australia (fig. 3). Our findings represent first records of *Krefftascaris* in *Chelodina rugosa*, *C. burrungandjii*, *C. canni* and *Emydura tanybaraga* and is also the first report of this genus in the Northern Territory, Queensland and Western Australia. *Krefftascaris parmenteri* is only second endoparasite species known from *Ch. burrungandjii*.

Recent publications of nematodes from Australian freshwater turtles (Ferguson, Smales, 1998, 2006; Zelmer, Platt, 2005) did not report *Krefftascaris*, although some of the authors (Zelmer, Platt, 2005) have examined the same turtle species from localities close to the sites of our own collections. This may reflect the fact that members of *Krefftascaris*, despite broad distribution on the continent, are somewhat rare parasites of Australian freshwater turtles; the general prevalence of *Krefftascaris* spp. in all turtles examined in our study was 5.9%. On the other hand, the collection efforts to date do not account for seasonal fluctuations in *Kreftascaris* populations that may cause these worms to be overlooked.

Hundreds of *Emydura* (short-necked turtles) have been examined as part of our research and by others from a number of localities throughout the range of this genus. Nevertheless, only a single individual of *E. tanybaraga* collected from a billabong near Daly River in Northern Territory NT infected with *K. parmenteri* was found in. Thus, *Krefftascaris* have been found in representatives of all genera of Australian side-necked turtles other than monotypical *Pseudemydura* Siebenrock, 1901. Its only species *Pseudemydura umbrina* Siebenrock, 1901 is critically endangered and thus could not be examined for parasites.

Available prevalence data suggest that *Chelodina* is probably the preferred host group of *Krefftascaris*.

Previous molecular phylogenetic works (Nadler, Hudspeth, 1998, 2000; Nadler et al., 2007) have significantly improved our knowledge of the phylogenetic interrelationships within the Ascaridoidea. However, previous studies did not include sequences of *Krefftascaris*. In our analysis, *K. sharpiloi* clustered together with a member of the Heterocheilidae with high bootstrap support in both ML and MP searches. *Krefftascaris* also shows morphological similarity (structure of the head end, presence of labial pulp and 4 pairs of precloacal/genital papillae, etc.) with some heterocheilids, especially

those from crocodilians (Sprent, 1983, 1999). Therefore, we provisionally place *Krefftascaris* in the Heterocheilidae. Members of the Heterocheilidae and particularly Heterocheilinae include several parasites of Australian crocodiles (Sprent, 1983, 1999). We hypothesize that *Krefftascaris* or its ancestor may have been acquired in the course of evolution from crocodilians. However, any definite statement on this matter would be premature with limited currently available data. Inclusion of additional ascaridoid taxa and especially those from crocodilians into future phylogenetic studies will provide a better basis for discussion of this hypothesis.

The level of intraspecific sequence variability in the sequenced ITS−28S region among populations of *K. parmenteri* separated by nearly 2000 kilometres was only four nucleotide substitutions. This is nearly 20 times lower than the observed level of interspecific variability between *K. parmenteri* and *K. sharpiloi* sp. n. (75 bases). Although the two species are easily differentiated genetically, they are very similar morphologically. The observed morphological differences were stable, but relatively minor. This is reminiscent of the situation observed in other groups of Australian freshwater turtle parasites such as digenean genera *Aptorchis* Nicoll, 1914, *Choanocotyle* Jue Sue and Platt, 1998, *Buckarootrema* Platt and Brooks, 2001 and nematode genus *Camallanus* Railliet et Henry, 1915. All of these genera have been shown to circumscribe two or more morphologically very similar species that, nevertheless, possess very distinct genetic differences (Platt, Tkach, 2003; Snyder, Tkach, 2006; Tkach, Snyder, 2006, 2007 a, b, 2008; Kuzmin et al., 2009). This pattern suggests rather recent and active speciation processes resulting from the disruption of previously uninterrupted turtle distributions by progressive aridization of the climate and a consequent separation of major river drainages. Further accumulation of faunistic, morphological and sequence data will allow better assessment of the trends in the evolution of parasites of Australian freshwater turtles.

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