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Kapsulotaenia chisholmae n. sp. (Cestoda: Proteocephalidae), from *Varanus spenceri* (Reptilia: Varanidae) in Australia

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Abstract: The proteocephalidean cestode *Kapsulotaenia chisholmae* n. sp. (Proteocephalidae: Acanthotaeniinae) is described from the intestine of the monitor lizard *Varanus spenceri* Lucas & Frost, 1903 (Reptilia: Varanidae) in Australia. *Kapsulotaenia chisholmae* n. sp. is compared with its five recognized congeners. The new species differs from *K. sandgroundi* (Carter, 1943), *K. varia* (Beddard, 1913) and *K. tidswelli* (Johnston, 1909) by the anterior position of the vagina to the cirrus-sac. It also differs from *K. varia*, *K. frezei* Schmidt & Kuntz, 1974 and *K. saccifera* (Ratz, 1900) by a different egg number in each clusters (8-13 in *K. chisholmae* versus 12-20, 90-100 and more than 100, respectively), from *K. frezei* and *K. saccifera* by a different cluster shape (spherical to oval versus banana-shaped in the latter two species), from *K. sandgroundi* and *K. tidswelli* by a greater diameter of the embryophore (37-45 µm versus 25-30 µm and 19-32 µm, respectively); from *K. sandgroundi* and *K. varia* (*sensu* Nybelin, 1917), by the absence of a vaginal sphincter. Finally, *K. chisholmae* differs from *K. varia*, *K. tidswelli*, *K. frezei* and *K. saccifera* by a larger size (length of the strobila up to 315 mm versus 30 mm, 27-30 mm, 40 mm, and 10-40 mm, respectively). We consider *K. saccifera* to be a *species inquirenda* due to its very poor description (no illustration, nor description of the scolex, number of testes, cirrus-sac ratio, testis field, uterine branches number, etc.). Specimens redescribed by Nybelin (1917) as *K. varia* (Beddard, 1913) are considered to be another, yet unnamed species of *Kapsulotaenia*.

Keywords: Australia - Queensland - *Kapsulotaenia* - Cestoda - Varanidae - taxonomy.

INTRODUCTION

Varanus spenceri Lucas & Frost, 1903 is a large lizard which grows up 1.2 metres (Cogger, 2014), and is probably the least known of the large Australian varanid lizards (Lemm & Bedford, 2004). It is confined to the black soil country, which is the most fertile soil in Australia, of west Queensland and the Barkly Tablelands of the Northern Territory, Australia, where perennial Mitchell Grass (*Astrebla* spp.) is the dominant vegetation. Apart from report of the nematode *Abbreviata hastaspicula* from a road-killed *V. spenceri* (Wooley, 2010), there have been no reports on the cestodes parasites of this large species. The first author undertook this study to determine whether the helminth fauna differed significantly from that in related species (*V. gouldii*, *V. panoptes*), and to investigate whether its restricted and specialized habitat, and hence its diet, exercised a discernible influence on this fauna. A paper concerning the nematode fauna present in *V. spenceri* has been published recently (Jones, 2013). The cestodes recovered from four *V. spenceri* are considered to belong to a previously undescribed species, and this is described below.

MATERIALS AND METHODS

The worms came from two different sources. The first worms came from dissected gastro-intestinal tracts of 14 preserved *V. spenceri* held in the Queensland Museum. Cestodes were prepared using standard methods; they were washed in water to remove alcohol, stained with basic fuchsin or haematoxylin, washed, fixed in 70% ethanol, destained in acid alcohol, dehydrated through an alcohol series, cleared in xylol, and mounted in DPX (distyrene, tricresyl phosphate and xylene). Alternatively, some were stained following the method described in de Chambrier (2001). The second source consists in one specimen without scolex, which was collected in November 2000 in Nelia (Queensland) by I. Beveridge.

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Mature proglottides were embedded in paraffin wax, sectioned at 12-15 µm, and stained with haematoxylin and eosin. Several proglottides and the scoleces were cleared in lactophenol. Helminths were examined under a BA series Olympus microscope or with a Leica DMLB, and illustrations made with a drawing tube. Scolices for scanning electron microscopy (SEM) were processed as follows: worms were dehydrated in a graded ethanol series (80, 96, twice 100%), then transferred to a graded amyl acetate series, critical point dried in CO_2, sputter-coated with gold and examined with a Zeiss 940A electron microscope. Type of uterine formation defined according to de Chambrier *et al.* (2004). Relative ovarian size (ratio of ovarian size in relation to that of entire proglottis) calculated according to de Chambrier *et al.* (2012).

Museum acronyms used in this text are as follows:

BMNH – Natural History Museum, London

MHNG – Muséum d'histoire naturelle de Genève, Switzerland

QM J (hosts), G (parasites) – Queensland Museum, Brisbane, Australia

RESULTS

Proteocephalidea Mola, 1928
Proteocephalidae La Rue, 1911
Acanthotaeniinae Freze, 1963

***Kapsulotaenia chisholmae* n. sp.**
Figs 1-11

Material examined

Holotype: QM G235015; 5 slides of whole mounted specimen from host QM J47127; Central-west Queensland, Australia; no date recorded.

Paratypes: QM G235016, 5 slides of whole mounted specimen from host QM J41654; Coorabulka Station, SW Queensland, 24°07' S, 140°07'E. – QM G235017, 3 slides of whole mounted specimen; 7 km West of Nelia, Queensland 20°39′0″S, 142°13′0″E, collected 01.11.2000. – QM G235018, 3 slides of cross sections; 7 km West of Nelia, Queensland 20°39′0″S, 142°13′0″E, collected 01.11.2000. – MHNG-PLAT-31201, 3 slides of whole mounted specimen and 12 slides of cross sections; 7 km West of Nelia, Queensland 20°39′0″S, 142°13′0″E, collected 01.11.2000.

Additional specimens: QM G235019; 3 specimens from host QM J15694; no date recorded.

Type locality: Central-west Queensland, Australia.

Type host: *Varanus spenceri* Lucas & Frost, 1903. Reptilia, Varanidae. Queensland Museum accession no. J47127.

Site of infection: Upper small intestine.

Prevalence of infection: Five out of 14 examined hosts were infected (36%).

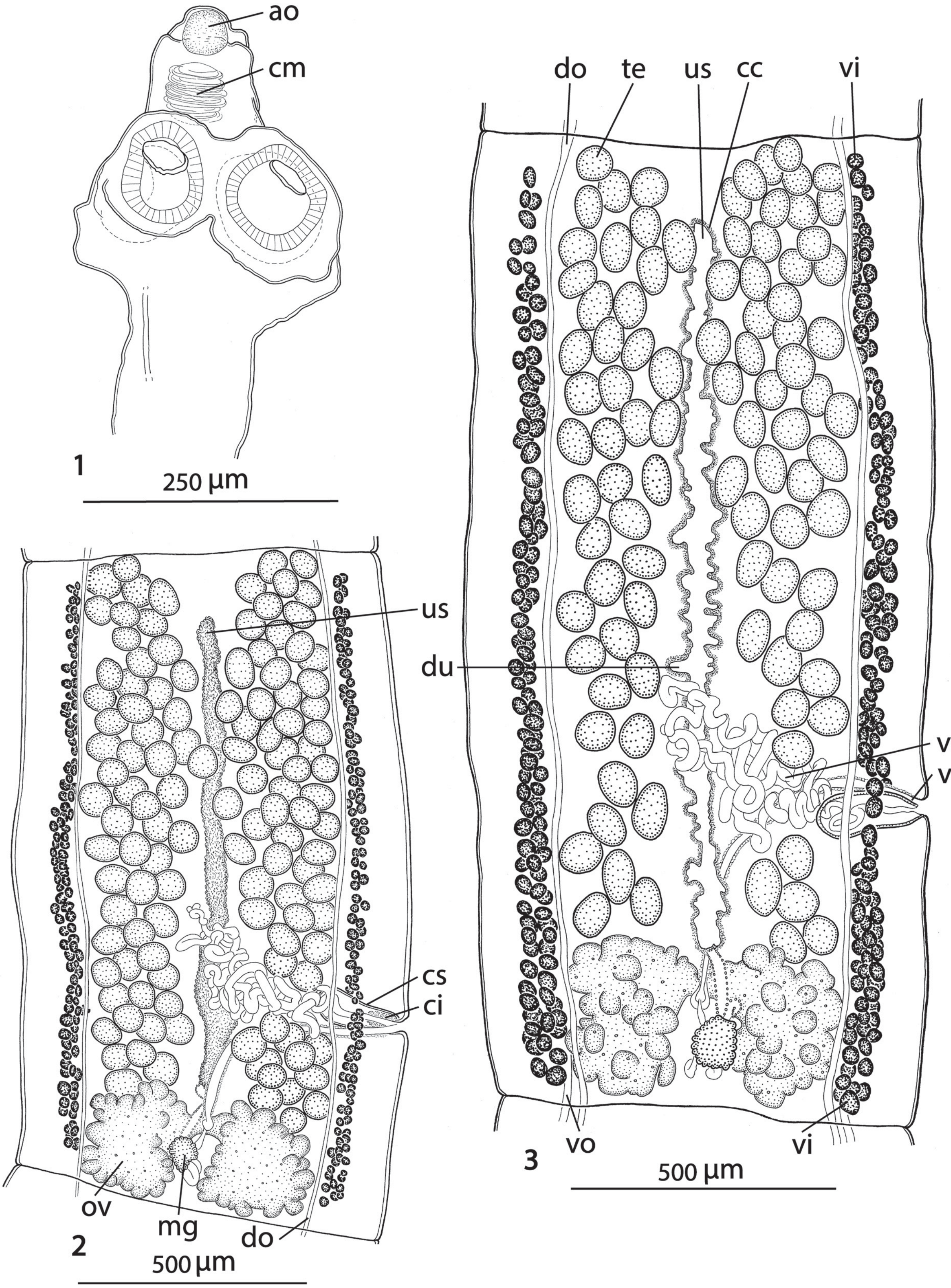
Etymology: The species is dedicated to Leslie Chisholm, South Australian Museum, Australia.

Description (based on holotype and two paratypes; measurements in micrometres unless otherwise stated): Strobila acraspedote, anapolytic, total length 180-315 mm, maximum width 840, body dorsoventrally flattened, early proglottides short and wide, immature proglottides wider than long to longer than wide, mature proglottides approximately twice as long as wide, 1.30 to 1.95 mm long, 715-840 wide, with pregravid and gravid proglottides longer than wide (1.8-2.3 mm long × 755-995 wide and 2.5-3.6 mm long × 875-1090 wide, respectively).

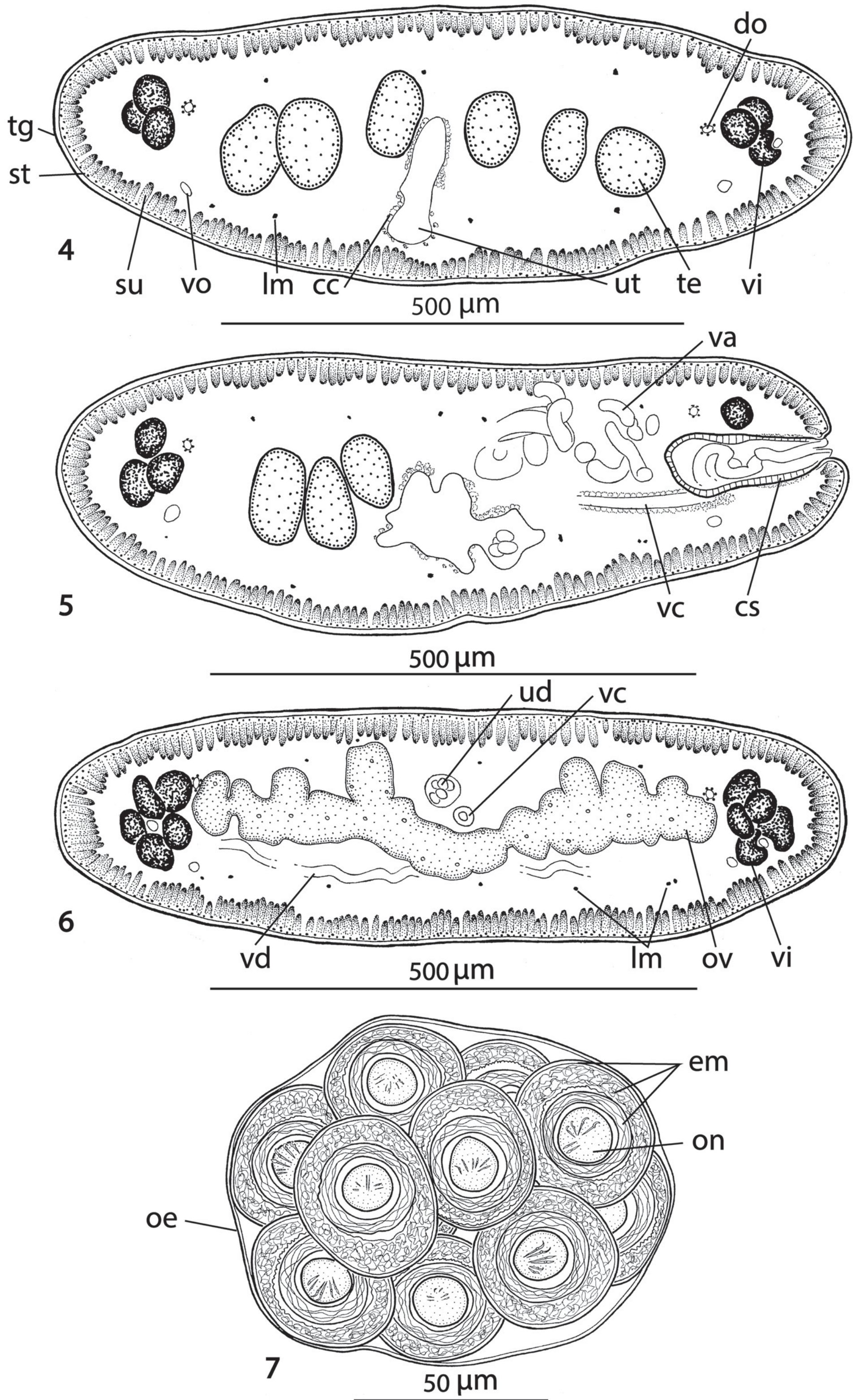
Scolex 340-450 wide, with pyramidal anterior retractile apex, almost twice as long as wide when totally everted, with apical organ 70-90 in diameter, and width at base about 130 (Figs 1, 8-11). Four circular suckers, appear to face anteriorly, 90-160 in diameter. Rostellum-like retractable (Fig. 11). Small gladiate spinitriches on neck and proglottides. Proliferation zone about 140 µm wide. Very fine layer of subtegumentary longitudinal muscles, with well-stained subtegumentary cells. Internal longitudinal muscles formed by 8-10 bundles of muscle fibres; bundles absent on lateral sides of proglottides (Figs 4-6). Two pairs of osmoregulatory canals situated between testes and vitelline follicles, with dorsal canals slightly medial to ventral ones. Ventral canals thin-walled, 15-18 µm wide, sometimes overlapping vitelline follicles. Dorsal canals thick-walled (surrounded by thin muscle fibres), about 5-10 µm wide (Figs 4-6).

Testes medullary (Figs 4, 5), spherical to oval, often slightly elongated (40 × 24), in one or two layers, in two almost separated lateral fields (Figs 2, 3) total number 89-132, ($\bar{x}$=108, n=10); 36-52 preporal testes, 5-12 postporal testes and 44-76 aporal testes. Vas deferens strongly coiled, directed anteriorly, reaching to midline of proglottis, often crossing it (Figs 2, 3). Cirrus-sac thick-walled, 175-200 long and 70-85 wide, representing 20-27% ($\bar{x}$=24%, n=12) of proglottis width. Cirrus occupies about 40% of length of cirrus-sac. Genital atrium narrow, deep; genital pores irregularly alternating, post-equatorial, situated at 66-77% ($\bar{x}$=71%, n=12) of proglottis length (Figs 2, 3).

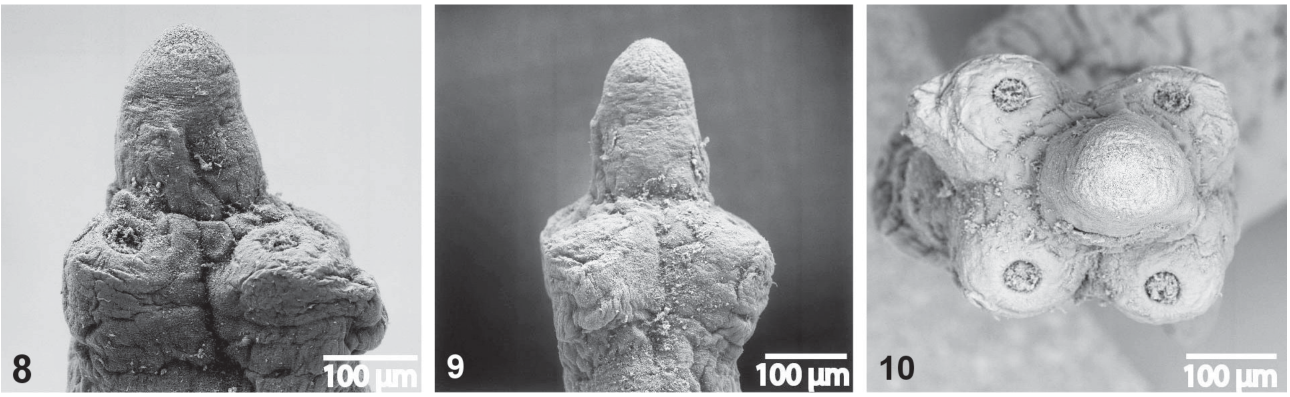
Ovary medullary, bilobed, with dorsal lobes penetrating to cortex (Fig. 6), with narrow isthmus and follicles on dorsal side; ovary 385-630 wide, occupying 57-65% ($\bar{x}$=62%, n=12) of proglottis width (Figs 2, 3). Relative ovarian size (ratio of ovarian size in relation to that of entire proglottis – see de Chambrier *et al.*, 2012 for methods of measuring) 6.9-7.5% of proglottis size. Vagina anterior (93%) or posterior (7%, n=55) to cirrus-sac, lined with stained cells in its terminal (distal) part, without observable vaginal sphincter. Mehlis' gland 50-95 in diameter, representing 7-10% of proglottis width. Vitelline follicles oval, small, arranged in two lateral bands on each side of proglottis (Figs 2, 3), interrupted



Figs 1-3. *Kapsulotaenia chisholmae* n. sp. (1) Total lateral view of a scolex (holotype – QM G235015). (2) Dorsal view of a mature proglottis (paratype MHNG-PLAT-31201). (3) Dorsal view of a pregravid proglottis (paratype MHNG-PLAT-31201). Abbreviations: ao – apical organ; cc – chromophilic cells; ci – cirrus; cm – circular musculature; cs – cirrus-sac; do – dorsal osmoregulatory canal; du – uterine diverticula; mg – Mehlis' glands; ov – ovary; te – testes; us – uterine stem; va – vas deferens; vc – vaginal canal; vi – vitelline follicles; vo – ventral osmoregulatory canal.



Figs 4-7. *Kapsulotaenia chisholmae* n. sp. (4) Transversal section of a proglottis at level of its anterior part (paratype MHNG-PLAT-31201). (5) Transversal section of a proglottis at level of cirrus-sac (paratype MHNG-PLAT-31201). (6) Transversal section of a proglottis at level of the ovary (paratype QM G235018). (7) Egg cluster. Abbreviations: ci – cirrus; cs – cirrus-sac; do – dorsal osmoregulatory canal; em – tri-layered embryophore; lm – internal longitudinal musculature; oe – outer envelope; on – oncosphere; ov – ovary; st – subtegumental muscle fibres; su – subtegumental cells; te – testes; tg – tegument; ud – uteroduct; ut – uterus; va – vas deferens; vc – vaginal canal; vd – vitelloduct; vi – vitelline follicles; vo – ventral osmoregulatory canal.



Figs 8-10. *Kapsulotaenia chisholmae* n. sp. Figs. 8-10, QM G235019. Scanning electron micrographs. (8) Dorsoventral view. (9) Lateral view. (10) Apical view.

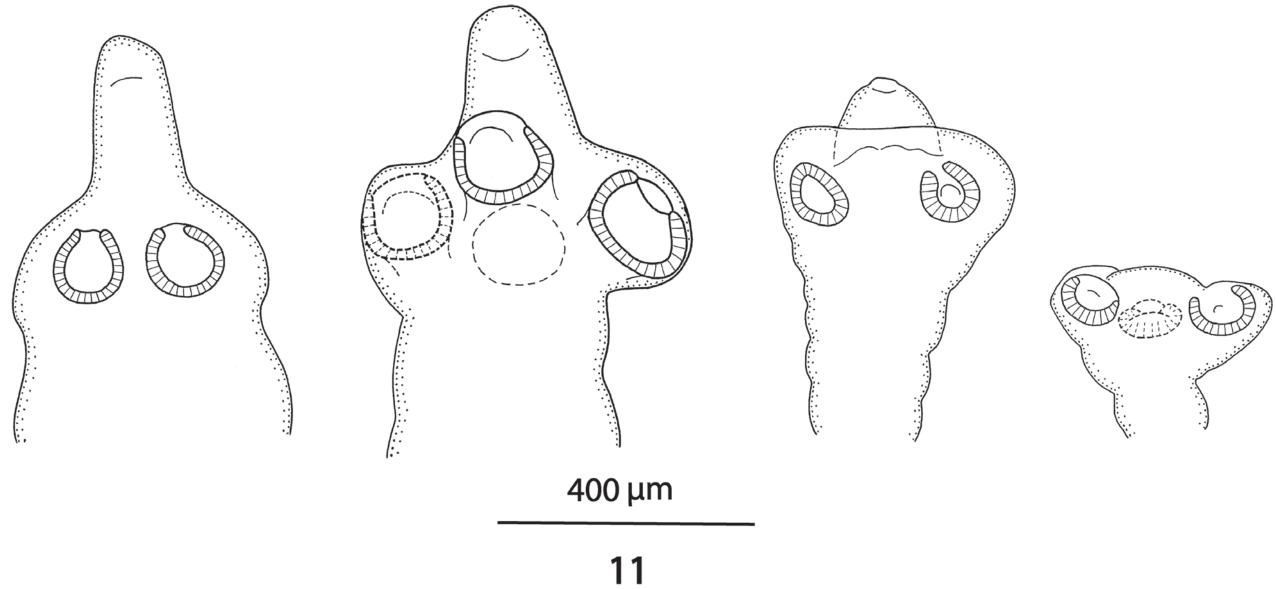


Fig 11. *Kapsulotaenia chisholmae* n. sp. Schematic view of the scoleces, showing the different shape of contraction, with rostellum extended, partially and fully retracted.

on poral side ventrally at level of terminal genitalia (cirrus-sac and vagina). Vitelline follicles do not reach anterior or posterior margin of proglottides, occupying 92-98% of proglottis length on poral side and 92-97% on aporal side (Figs 2-3).

Primordium of uterine stem medullary, already present in immature proglottides as an elongated structure formed by a thick layer of chromophilic cells. Formation of uterus of type 1 according to de Chambrier *et al.* (2004): uterine stem tipped with conspicuous concentration of numerous intensely stained cells (Fig. 2); lumen appears in first mature proglottides. Formation of lateral diverticula begins before appearance of eggs in uterus. Thin-walled lateral diverticula grow in pregravid and gravid proglottides, occupying up to 75% of proglottis width. Gravid proglottides with 300-350 egg clusters. In gravid proglottides, ovaries, Mehlis' gland, testes and vas deferens diminished in size, whereas cirrus-sac still visible. Lateral uterine branches difficult to observe.

8-13 eggs in spherical to oval clusters, 100-125 x 95-110, surrounded by smooth, thin membrane (outer envelope) closely apposed to eggs (Fig. 7). Eggs spherical, with a three-layered embryophore, with thick external layer, 37-45 in diameter, intermediate layer nucleate of irregular shape 35-40 in diameter, internal layer envelope well developed, 23-28 in diameter. Oncosphere spherical, 12-15 in diameter, with three pairs of embryonic hooks, 6-7 long (Fig. 7).

REMARKS

Due to the fact that the majority of cestodes were recovered from preserved lizards, most specimens of *K. chisholmae* were not in optimal condition: longitudinal excretory canals were sometimes difficult to distinguish, the uterine lateral diverticula in gravid proglottides were unclear, and the irregular shape and size of the testes may have been an artifact of poor preservation. The specimen collected from a living host in Nelia is better preserved, but does not have the scolex. Nonetheless, several constant features could be identified which readily differentiate this species from the other species of *Kapsulotaenia* (Table 1).

de Chambrier (2006) recognized five species which have been formally described, viz. *Kapsulotaenia tidswelli* (Johnston, 1909) from *Varanus varius* in eastern Australia (Johnston, 1909), *K. sandgroundi* (Carter, 1943) from the Komodo dragon, *V. komodoensis* (Carter, 1943), *K. frezei* Schmidt & Kuntz, 1974 from *V. salvator* (= *V. palawanensis* Koch, Gaulke & Böhme, 2010) in Palawan, Philippines (see Koch *et al.*, 2010; Schmidt & Kuntz, 1974), *K. saccifera* (Ratz, 1900), from *Varanus* sp. in New Guinea (Ratz, 1900a, b), and *K. varia* (Beddard, 1913) from *V. varius* in Australia (Beddard, 1913). Yamaguti (1959) however considered *K. varia* to be a synonym of *K. sandgroundi*, an opinion that the present authors do not share. Johnston (1909) commented that the scolex in *K. saccifera* is very similar to that in *K. tidswelli*.

The cestodes recovered from five out of fourteen *V. spenceri* in the present study resemble *K. sandgroundi*, as described by Carter (1943) and redescribed by de Chambrier (2006). However, *Kapsulotaenia chisholmae* n. sp. differs from this species and from *K. varia* and *K. tidswelli* by the anterior position of the vagina to cirrus-sac. It also differs from *K. varia*, *K. frezei* and *K. saccifera* by a different egg number in each cluster (8-13 in *K. chisholmae* versus 12-20, 90-100 and more than 100, respectively), from *K. frezei* and *K. saccifera* by a different cluster shape (spherical to oval versus banana-shaped in the two latter), from *K. sandgroundi* and *K. tidswelli* by a greater diameter of the embryophore (37-45 µm versus respectively 25-30 µm and 19-32 µm); from *K. sandgroundi* and *K. varia* (*sensu* Nybelin, 1917 – see below), by the absence of a vaginal sphincter. Finally, *K. chisholmae* differs from *K. varia, K. tidswelli, K. frezei* and *K. saccifera* by a larger length of the strobila (up to 315 mm versus 30 mm, 27-30 mm, 40 mm, and 10-40 mm, respectively) (Table 1).

DISCUSSION

Within the cestode order Proteocephalidea, Freze (1963) erected the subfamily Acanthotaeniinae, with three genera *Acanthotaenia* Linstow, 1903, *Rostellotaenia* Freze, 1963 and *Kapsulotaenia* Freze, 1963, all of which occur in monitors (*Varanus* spp.) (Freze, 1965). de Chambrier & de Chambrier (2010) described another acanthotaenine genus, *Vandiermenia*, a parasite of the red-bellied snake *Pseudechis porphyriacus* from Australia. *Kapsulotaenia* is characterized principally by the presence of eggs in clusters in the gravid proglottides. Based on molecular data, de Chambrier *et al*. (2004) separated four species in this genus in Australia, with one species in *V. varius* (White, 1790), two in *V. gouldii* (Gray, 1838) and one in *V. rosenbergi* Mertens, 1957. There is also one species in *V. indicus* (Daudin, 1802) (A. de Chambrier, unpublished). Species of *Kapsulotaenia* are believed to exhibit oioxenous specificity for their host (*sensu* Euzet & Combes, 1980). The main problem in the study of the *Kapsulotaenia* species is that most descriptions are incomplete. Ratz (1900a, b) gave an identical, very poor description of *Ichthyotaenia saccifera* (= *K. saccifera*) in two different publications, one in French and the other in German. Schwarz (1908) redescribed this taxon, but both descriptions are incomplete (missing a description of the scolex, number of testes, cirrus-sac ratio, testis field, uterine branches number, etc.) and no illustration was presented. Furthermore, the host was not identified to species. But Schwarz (1908) described for *K. saccifera* egg clusters elongated, bag-shaped (banana-shaped), each proglottis containing 2 to 11 clusters, which allows us to differentiate this taxa from our new species. Pending a revision of the genus, we consider *K. saccifera* as a *species inquirenda*.

Beddard (1913) described *Acanthotaenia varia* (= *Kapsulotaenia varia*) from *Varanus varius* in a "Zoological Garden". Nybelin (1917) collected some cestode specimens from *Varanus gouldii* that he identified as *Acanthotaenia varia* Beddard, 1913. However, the description of these worms differs from the ones incompletely described by Beddard (1913) in the position of the vagina relative to the cirrus-sac (posterior in Beddard's description and anterior/posterior in Nybelin's) and in the absence of a vaginal sphincter in the former specimens but present in the latter (Table 1). Therefore, the specimens identified as *Kapsulotaenia varia* (Beddard, 1913) described by Nybelin (1917) are considered as *Kapsulotaenia* sp. Furthermore, de Chambrier *et al*. (2004, 2015) observed a strict (oioxenous) specificity for the members of the monophyletic *Kapsulotaenia* and described differences between *Kapsulotaenia* sp. 2 from *Varanus gouldii* and *Kapsulotaenia* sp. 4 from *V. varius*. Johnston (1909) described *K. tidswelli* (as *Acanthotaenia tidswelli*) from *Varanus varius*, but the same author (Johnston, 1912) listed this species also as parasite of *Varanus gouldii*. However, this host record should be confirmed (voucher specimens are not available). It is obvious that the genus *Kapsulotaenia* is pending taxonomic revision.

The ecology and distribution of *Varanus komodoensis* (host of *K. sandgroundi*) and *V. spenceri* (host of *K. chisholmae*) differ. *V. komodoensis* only occurs in the

Lesser Sunda islands, Indonesia, principally Komodo and Flores, where it inhabits deciduous monsoon rain forest, savannah and mangrove (Ciofi, 2004). As well as feeding on invertebrates and smaller vertebrates, adult *V. komodoensis* can kill and consume large vertebrates such as deer, wild boar and goats; decomposing carcasses form a significant part of its diet (Auffenberg, 1981). In contrast, *V. spenceri* is a burrowing species confined to relatively treeless grasslands with *Astrebla* spp. in central Queensland and the adjacent Northern Territory, and feeds on other smaller reptiles, small mammals such as *Rattus villosisimus,* and invertebrates such as orthopterans including plague locust species (Pengilley, 1981; Valentic & Turner, 1997; Wooley, 2010). It is interesting to note that no cestodes were recovered from 12 *V. gouldii* and 10 *V. panoptes* in Queensland (H.I. Jones, unpublished).

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Table 1. Summary of diagnostic traits in *Kapsulotaenia* species.
Measurements in micrometres (µm) unless otherwise stated. PC = proportion of the length of cirrus-sac to the width of the proglottis; PP = position of the genital pore (cirrus pore) as % of the proglottis length; OV = percentage of the width of the ovary in relation to the width of the proglottis; SL = supplementary layer within the embryophore (see de Chambrier, 2006, p. 91);* see de Chambrier *et al.* (2012); D = taken from the original drawings; B = measurements taken from cotypes of Beddard (BMNH 1915.5.14.2); R = measurements taken from type specimens of Ratz (MHNG-PLAT-36037).

Species	*K. chisholmae* n. sp.	*K. frezei* Schmidt & Kuntz, 1974	*K. sangroundi* (Carter, 1943)
Reference	This paper	Schmidt & Kuntz, 1974	de Chambrier, 2006
Host	*V. spenceri* Lucas & Frost, 1903	*V. salvator* (Laurenti, 1768) = *V. palawanensis* Koch, Gaulke & Böhme, 2010	*Varanus komodoensis* Ouwens 1912
Type locality	Australia, Nelia, Qld	Philippines, Palawan	Indonesia
Total length (mm)	180-315	40	92-300
Proglottis width	750-840	700	1400-2000
Scolex width	340-450	360-465	640-900
Sucker width	90-160	145-165	180-250
Apical organ width	70-90	40-50	200-250
Testis number	89-132	54-60	92-147
Testis diameter	24x40	50-90	20-45
Testis field	2	2	2 converging anteriorly
PC	20-27%	26% (D)	17-27%
PP	66-77%	43% (D)	61-73%
OV	57-65%	61% (D)	42-59%
Ovary surface ratio*	6.9-7.5%	7.2%	3.4-4.2%
Vagina	mainly anterior (93%)	mainly anterior	posterior
Vaginal sphincter	absent	absent	present
Uterine branches	not visible	absent	21-31 on each side
Embryophore diameter	37-45	34-36	25-30
Oncosphere diameter	12-15	?	10-11
No. eggs in cluster	8-13	90-100	7-13
Cluster shape	rounded	banana-shaped	rounded
SL	Yes	Yes	Yes
Type of uterine formation	Type 1	Type 1	Type 1

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K. tidswelli (Johnston, 1909)	*K. varia* (Beddard, 1913)	*K. varia* (Beddard, 1913) *sensu* Nybelin (1917)**	*K. saccifera* (Ratz, 1900) sp. inq.
Johnston, 1909; Freze, 1965	Beddard, 1913	Nybelin, 1917; Freze, 1965	Ratz, 1900a, b; Freze, 1965
V. varius (White, 1790)	*V. varius* (White, 1790)	*V. gouldii* (Gray, 1838)	*Varanus* sp.
Australia, Bathurst, NSW	"Zoological Garden"	Mt Tamborine, Queensland	New Guinea
27 to 30	more than 30	230, possibly 300	10-40
200-350	? (B = 835)	1500-2000	750
320-400	?	400-420	470
80	?	200-220	150
?	?	130	?
about 90	? (B = 104)	96-150 ($\bar{x}$=120)	?
13-22	?	74-110	36-42
2	2 converging anteriorly	2	?
21% (D)	17-20% (B = 23%)	17-20% (D)	?
about 75%(D=59-70%)	about 50% (B = 69%)	about 50%	about 50%
54%(D)	? (B = 59%)	60% (D)	?
4.6% (D)	? (B = 5.1%)	4.0%	?
mainly posterior	posterior	anterior/posterior	anterior/posterior
?	absent	present	?
not visible	?	?	?
19-32	?	?	?
?	?	?	?
?	12-20	?	(R = much more than 100)
?	rounded	?	(R = banana-shaped)
?	Yes	?	(R=Yes)
Type 1	Type 1	Type 1	Type 1