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Characterisation of the tongue worm, *Linguatula serrata* (Pentastomida: Linguatulidae), in Australia

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ABSTRACT

We describe adult males and females and a nymph belonging to *Linguatula serrata* in Australia, based on light and scanning electron microscopies. In addition, 18S and Cox1 sequence data have also been provided and were compared with similar sequences in GenBank. Our specimens had identical 18S sequences and limited genetic distance in Cox1 region which fell within the intra-specific range observed for *Linguatula* spp. suggesting that they all belong to one species. Phylogenetic analyses showed that Australian specimens were grouped with *L. serrata* in Europe where the species was first found and described. A number of *L. serrata* from Iran and Bangladesh formed a distinct group. The genetic distance between these *Linguatula* and Australian/European *L. serrata* ranged from 0.46% to 2.21% which is larger than the genetic distance observed between *L. arctica* and Australian/European *L. serrata* (0.12%) suggesting that they belong to a different species. As pointed out previously by several other authors, *L. serrata* comprises more than one species and those from the Palearctic region (including Iran and Bangladesh) should not be automatically named *L. serrata* unless there is enough evidence for the identification. To accurately address the complex taxonomy of *Linguatula* spp. a detailed morphological and genetic characterisation of numerous developmental stages of the parasite is necessary, to ensure morphological differences are not due to development. This however may not be achievable in the near future due to significant reduction in expertise as well as research funding awarded in this area of research to understand the basics of our planet.

1. Introduction

Members of the pentastomid genus *Linguatula* are known to infect canids, such as dogs, foxes and wolves, as their definitive hosts, and herbivores, such as sheep and cows, as their intermediate hosts (Riley, 1986). They are also of zoonotic significance and there are several reports of human infection with these parasites globally (Koehsler et al., 2011; Oluwasina et al., 2014; Tabibian et al., 2012).

The taxonomy and nomenclature history of *L. serrata,* however, is confusing and controversial and is in need of revision (Gjerde, 2013). Christoffersen and De Assis (2013) provided a detailed history of the species, outlining a number of important points that need to be taken into account when researching this, and closely related, species. The original description of the species is based on a larval specimen, collected from the lung of a hare (Frölich, 1789). Although adult pentastomes had been collected from the nasal passages of dogs (Chabert, 1787) prior to the Frölich's description (Frölich, 1789), their external segmented appearance led them to be described as species within the cestode genus *Taenia* (Chabert, 1787). It was not until 70 years after its initial description that the link between the larval stage in the hare and the adult stage in dogs was made (Nicoli, 1963), with confirmation of their identity as *L. serrata*.

Even though *L. serrata* is a cosmopolitan parasite, with records on every inhabited continent (see Christoffersen and De Assis (2013); Gjerde (2013)), research into its systematics has been incredibly poor. It would appear that any pentastomid collected from the nasal passage of a mammalian host, anywhere in the world, has been attributed to *L. serrata*. This has usually been done without any morphological, let alone genetic, assessment to determine actual species identity. Riley et al. (1987) outlined this for *L. arctica*, from the nasal passages of reindeer in Norway, which had traditionally been reported as *L. serrata*, despite being found in a very different host group. Gjerde (2013) recently genetically confirmed *L. arctica* as a separate species, but also highlighted the potential misidentification of a *L. serrata* specimen that they used as a comparison in their molecular analysis. This highlights the need for concurrent morphological and molecular assessment of

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specimens to confirm the identity of known and potentially new species of *Linguatula*.

Similarly in Australia, a review of the literature shows that the nymph or larval stages of the parasite have been referred to under various names. For example, the first report of tongue worms in Australia was of two nymphs in the mesenteric lymph nodes of cattle from Victoria identified as *Linguatula ferox* or *Pentastomum denticulatum* (Ralph, 1865), both of which were later considered junior synonyms of *L. serrata* (Christoffersen and De Assis, 2013; Küchenmeister, 1855). Then an unknown parasitic worm encysted in the viscera of cattle from Queensland was reported (Barnard and Park, 1893). These parasites were concluded to be nymphs of *Pentastomum denticulatum* belonging to adult *L. serrata* by Johnston and Cleland (1910) who also found nymphs in the mesenteric lymph nodes of cattle from the Illawarra region in NSW. Johnson (1910) described the first record of an adult tongue worm in Australia after finding a single female of a species he named *L. dingophila* in the nasal cavity of a dingo examined post-mortem in 1904, assumed, but not confirmed, from South Australia. The *L. serrata* nymphs found in the mesenteric lymph nodes of cattle from various parts of NSW were used to experimentally infect domestic dogs where the nymphs successfully matured into adults (Johnston, 1911). Later, Johnston (1916) considered *L. dingophila* synonymous to *L. serrata* as the differences, such as shorter length, were likely to be a reflection of the original specimen being an immature female. Following finding nymphs in the mesenteric lymph nodes of cattle killed at the Brisbane abattoir, Johnston (1918) reported larvae of *Pentastomum denticulatum* as present in Queensland. The reports since then referred the nymph or adult stage of the parasite to *L. serrata*, except for Heymons (1932), who on the basis of Johnson's perfunctory description, noted that the pentastome from the dingo appeared to have fewer annuli than the cosmopolitan *L. serrata* from dogs, foxes, wolves etc., and suggested that marsupials were the probable intermediate hosts of *L. dingophila*. However, other authors (Riley et al., 1985) concluded that *L. serrata* may infect the dingo and/or feral dog and questioned the validity of *L. dingophila*. At all times, reports were only based on morphology, generally only using body length and annuli counts, with few reports of hook measurements for differentiation.

A recent study in Australia showed that these parasites are more common than previously thought with a 67% infection in wild dogs (Shamsi et al., 2017). However, the knowledge about their specific identity in Australia is still poor. Therefore, the aim of the present study was to morphologically and genetically characterise the adult tongue worms, from the nasal cavity of wild dogs in Australia, and a nymph, from abattoir-killed cattle, to get an insight into the specific identity of the Australian specimens.

2. Martials and methods

2.1. Pentastome collection

All collections were performed in accordance with relevant guidelines and regulations. The heads of wild dogs and foxes were provided by professional vertebrate pest control officers of the Australian Capital Territory Parks and Conservation Service, New South Wales Forests, New South Wales Local Lands Services and the Victorian Department of Environment, Land, Water and Planning and donated to our study. These animals were trapped and shot by the vertebrate pest control officers during the normal course of their duties in accordance with the relevant regulations and laws relating to trapping and destruction of vertebrate pests in the Australian Capital Territory, New South Wales and Victoria. The heads of the animals were removed, packed in labelled plastic bags and kept frozen until examined. Adult parasite specimens were collected from the nasal cavity of wild dogs and foxes as detailed in the previous study (Shamsi et al., 2017). To collect nymph stages of the parasite, mesenteric lymph nodes from cattle (*Bos taurus*) were collected by meat inspectors in a local abattoir and examined as outlined in the previous study (Shamsi et al., 2017). Pentastomes were preserved in 70% ethanol for further molecular and morphological examination.

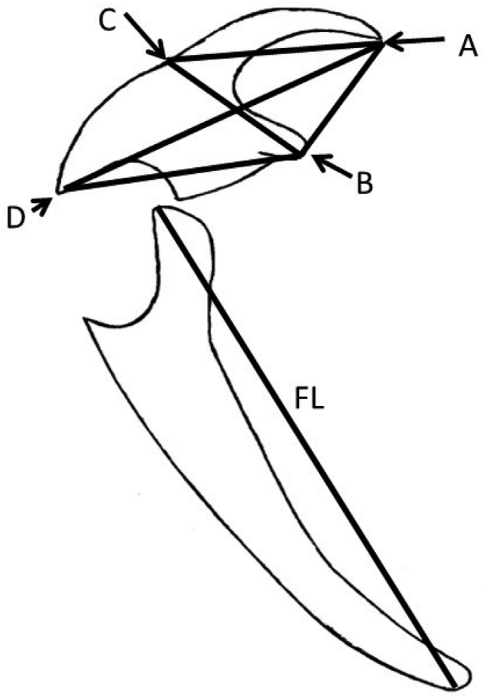

Fig. 1. Template for measurement conventions of the adult hook and fulcrum. AB – hook gape; AC – blade length; AD – hook length; BC – base length; CD – plateau length; BD – hook width; FL – fulcrum length.

2.2. Light microscopy

Adult pentastomes were measured (Body Length, BL, and Body Width, BW; in mm) and the number of annuli were counted. One set of hooks and the oral cadre were dissected from each of a number of individuals and mounted in lactophenol and observed using light microscopy. Hook dimensions measured for all specimens were blade length (AC), hook length (AD), base length (BC), plateau length (CD), and hook gape (AD) (Fig. 1). Buccal cadre measurements were of buccal cadre length and buccal cadre width.

Drawings were made with the use of a drawing tube. Photos of slide mounted specimens were taken using a 9 MP Microscope Digital Camera (AmScope Model MU900).

2.3. Scanning electron microscopy (SEM)

To obtain scanning electron microscopy images, following preservation in 70% ethanol, specimens were dehydrated using a graded series of ethanol. Excess 70% ethanol was removed from around the sample using a transfer pipette before being replaced by 80% ethanol for 20 min. This step was repeated with 90% ethanol and followed by 100% anhydrous ethanol dehydration three times. Samples were then dried in liquid CO_2 in a Tousimis® Autosamdri-931 critical point dryer (USA). A carbon tab was used to attach samples to a stub before gold coating (25 mA for 2 min) in an Emitech K550X Sputter Coater (Quorum Technologies, UK). Samples were examined in a scanning electron microscope (JCM-5000 Benchtop SEM NeoScope, Jeol Ltd, USA) with accelerating voltage set at 10-15kv.

2.4. DNA extraction, PCR, sequencing and phylogenetic analyses

A small piece of the body of the adult pentastomes was cut for DNA

Table 1
Details of sequences obtained from GenBank to build the phylogenetic tree.

Species	GenBank Accessions		locality	Host	Reference
	COXI	18S			
L. serrata	MN893765	MN889436	Australia	Cattle	This study
L. serrata	MN893766	MN889437	Australia	Fox	This study
L. serrata	MN893767	MN889438	Australia	Dog	This study
L. serrata	MN893768	MN889439	Australia	Fox	This study
L. serrata	MN893769	MN889440	Australia	Dog	This study
L. serrata	KF029447	JX088397	Norway	Dog	Gjerde (2013)
L. serrata	KY829108	-	Peru	Vicugna	Gomez-Puerta et al. (2017)
L. serrata	LC150781	-	Bangladesh	Zebu	Mohanta and Itagaki (2017)
L. serrata	LC150782	-	Bangladesh	Zebu	Mohanta and Itagaki (2017)
L. serrata	LC150784	-	Bangladesh	Zebu	Mohanta and Itagaki (2017)
L. serrata	-	KT581433	Iran	Goat	Unpublished
L. serrata	-	KT581431	Iran	Sheep	Unpublished
L. serrata	-	KT581432	Iran	Cattle	Unpublished
L. serrata	-	KP100453	Iran	Cattle	Ghorashi et al. (2016)
L. serrata	KF830138	-	Iran	Sheep	Unpublished
L. serrata	KF830141	-	Iran	Camel	Unpublished
L. serrata	KF830142	-	Iran	Dog	Unpublished
L. serrata	KU234185	-	Iran	Goat	Unpublished
L. serrata	KU240056	-	Iran	Cattle	Unpublished
L. serrata	KU240062	-	Iran	Cattle	Unpublished
L. arctica	KF029443	KF029440	Norway	Reindeer	Gjerde (2013)
L. arctica	KF029444	KF029442	Norway	Reindeer	Gjerde (2013)
L. arctica	KF029445	KF029441	Norway	Reindeer	Gjerde (2013)
L. arctica	KF029446	KF029439	Norway	Reindeer	Gjerde (2013)
Armillifer agkistrodontis	FJ607340	FJ607339	China	Snake	Chen et al. (2010)

extraction which was done using DNAeasy Blood & Tissue Kits (Qiagen, Australia) and eluted into 45 μl of water. The Cytochrome oxidase subunit 1 (Cox1) gene and 18S ribosomal RNA (18sRNA) gene were amplified using the primer sets and conditions in accordance with Gjerde (2013). PCR amplicons were sent to the Australian Genome Research Facility (Queensland) for bidirectional sequencing using the same primers. Forward and reverse AB1 trace files were quality checked using Seq Scanner (Applied Biosystems/Thermo Fisher). Cox1 and 18S RNA gene sequences of *Linguatula* spp. were either generated in our current study, or were obtained from GenBank (Table 1). Cox1 and 18sRNA sequences were aligned with Geneious alignment algorithm by using Geneious version 11.1.4 (Kearse et al., 2012), and then were double checked with all variable sites in the original trace files for confirmation. Alignments were then truncated to 533 and 1751 bp, based on the shortest sequence, for Cox1 and 18sRNA sequence, respectively. *Armillifer agkistrodontis* Cox1 (FJ607340.1) and 18sRNA sequences (FJ607339.1) were used as outgroup in the downstream phylogenetic analyses. Phylogenetic relationship among species was inferred by MrBayes (v3.2) using HKY + I and K2P models for Cox1 and 18sRNA alignments as determined by Jmodeltest 2.0, respectively. Number of generations were set as 2,000,000 and 1,000,000 for Cox1and 18sRNA regions, respectively.

3. Results

Materials examined: Six male and six female adult specimens were examined by light microscopy (Table 2). All these specimens were deposited in the Australian Museum (AM) under accession numbers P.104065-P.104076. In addition three adult males and three adult females were subjected to scanning electron microscopy (Table 2). There was only one nymph in good condition available for this study which was subjected to scanning electron microscopy (Table 2). The sequences of the 18S rDNA and Cox1 regions of this nymph were also obtained.

3.1. Description

Females (n = 6): Females were considerably larger than males. Body flattened anteriorly, tapering to a cylindrical form toward posterior part. Females were 48.0–70.0 (mean 59.9) mm long, 7.0–8.5 (7.9) mm wide, with 86–109 (92) annuli. Head region in female was rounded with varied degrees of roundness (Fig. 2 A, H, I). Details of hooks measurements are provided in Table 3. Two pairs of single hooks with externally visible tip were present below the mouth in hook pits; first pair located closer to each other and more anteriorly than the second pair (Fig. 2A, H, I). Hooks without spines or accessory pieces; hook tips sharply pointed (Fig. 5A and B). Mouth rounded surrounded by a buccal cadre (Fig. 2B, H, I, and 5 E). A number of sensillae with variable size and arrangements both within individual and between individuals were present dorso-laterally on each side of the head (Fig. 2D). Two pairs of rounded papillae were present ventrally at the anterior part of the body (Fig. 2H and I). They were elevated with a small centric node. Each annulus had a row of pores (chloride cell caps) located on the upper part of the annuli (Fig. 2E and F). Posterior edge of annulus with scale-like spines; more prominent in anterior part of body. A distinct terminal cleft was present at the posterior tip (Fig. 2J).

Males (n = 6): Body claviform; 15–18 (mean 16.4) mm long; 2.5–4 (3.2) mm wide; with 75–89 (80) annuli throughout the body; head and body indistinct; cranial edge of the head rounded broadly. Mouth subterminal, surrounded by a circular buccal cadre (Figs. 3A and 5F); two pairs of hooks located ventrally on head area, on each side (Fig. 3A). Hooks without spines or accessory pieces; hook tips sharply pointed (Fig. 5C and D). Genital pore located ventrally below the level of hooks, interrupting annuli 1–4 (Fig. 3A). Three sensillae visible in SEM images on the dorso-lateral region of the head adjacent to a small pore (Fig. 3C). Each annulus bearing multiple rows of spines on posterior edge and a row of pores (chloride cell caps) centrally (Fig. 3D). Posterior end of males were grooved with variable morphology (Fig. 3E–G). Copulatory spicules curved with no basal ornamentation (Fig. 5G).

The single nymph examined was approximately 3 mm long and 0.7 wide (Fig. 4A). Two pairs of hooks were present ventrally on the head region (Fig. 4B and D). Hooks tips sharply pointed. Dorsal accessory pieces with blunt tips present on each pair (Fig. 4B and D). Sensillae in head region were variable in size and arrangements (Fig. 4C). First annulus between anterior and posterior sets of hooks; 4th annulus

Table 2
Details of specimens used for light (*) and scanning electron microscopies in the present study.

Parasite code (museum accession number)	Developmental stage	Host	Locality	Collection date
131–1* (P.104065)	Adult female	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
131–2*(P.104066)	Adult female	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
132–1*(P.104067)	Adult female	Wild dog	#3 Tolbar Road, Eastern border of the Kosciusko National Park	10/06/2018
132–2*(P.104068)	Adult female	Wild dog	#3 Tolbar Road, Eastern border of the Kosciusko National Park	10/06/2018
132–4*(P.104069)	Adult female	Wild dog	#3 Tolbar Road, Eastern border of the Kosciusko National Park	10/06/2018
132–5*(P.104070)	Adult female	Wild dog	#3 Tolbar Road, Eastern border of the Kosciusko National Park	10/06/2018
131–3*(P.104071)	Adult male	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
131–4*(P.104072)	Adult male	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
131–5*(P.104073)	Adult male	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
131–6*(P.104074)	Adult male	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
131–7*(P.104075)	Adult male	Wild dog	#1 Tolbar Rd, Eastern border of the Kosciusko National Park	11/06/2018
132–3*(P.104076)	Adult male	Wild dog	#3 Tolbar Road, Eastern border of the Kosciusko National Park	10/06/2018
63	Adult female	Wild dog	Tumbarumba, NSW	18/06/2015
83	Adult female	Wild dog	Tumbarumba, NSW	20/07/2015
92	Adult female	Wild dog	Brindabella, NSW	20/08/2015
82	Adult male	Fox	Tumbarumba, NSW	17/07/2015
94	Adult male	Wild dog	Mullion, NSW	20/08/2015
222	Adult male	Wild dog	NSW	2018
31	Nymph	Cow	NSW	2015

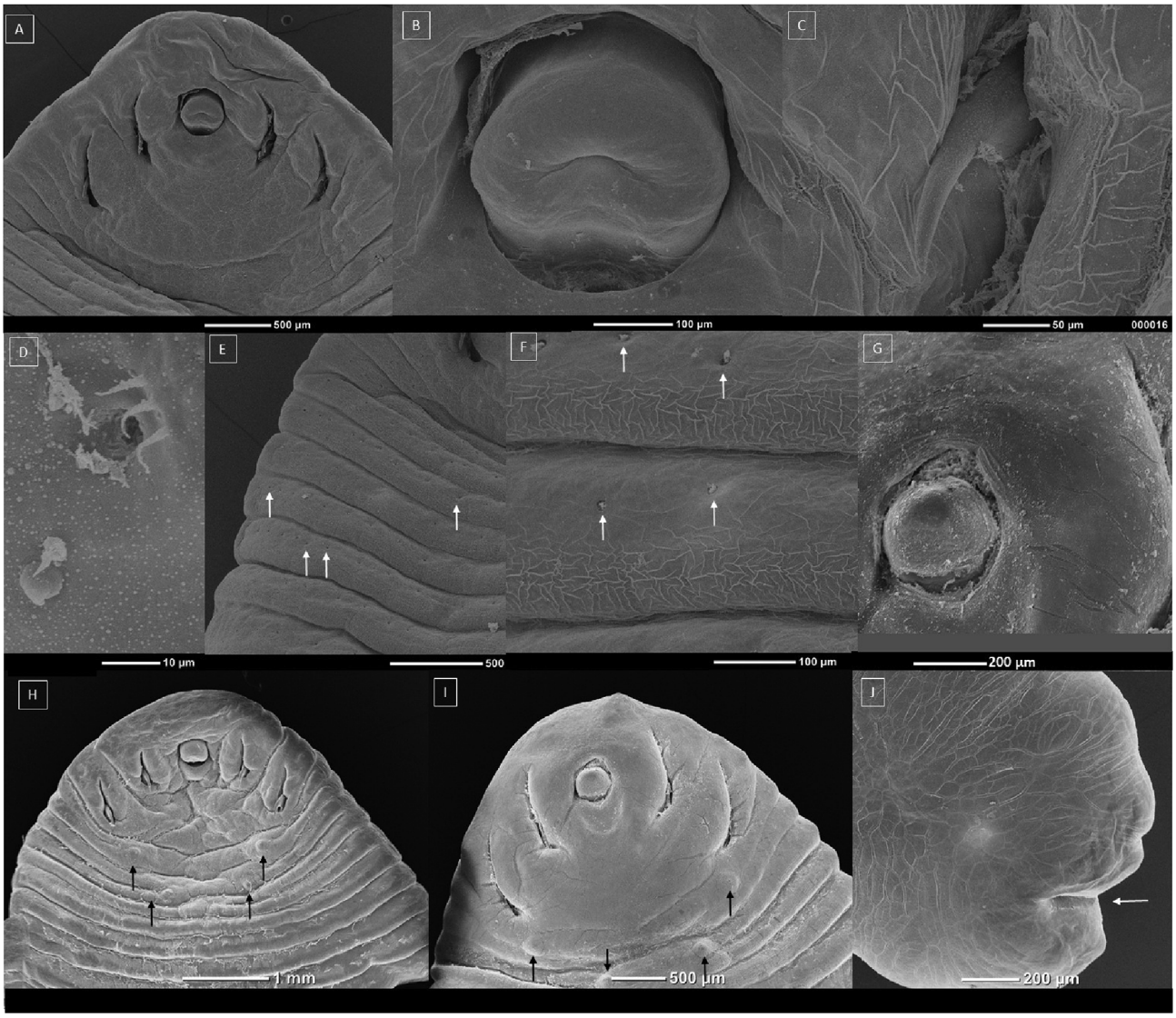

Fig. 2. Scanning electron microscopy of female specimens: A) ventral side of the cranial region of the parasite, showing the mouth, two pairs of hooks and the single row of sensory pores on each annulus; B) magnified view of the mouth; C) magnified view of a hook; D) magnified view of the sensory sensillae; E & F) showing arrangement and structure of the annulus' sensory pores (see arrows); G) mouth in another specimen; H & I) ventral side of the cranial region of two other female specimens showing variations in the morphology of the head. Arrows are pointing at papillae; J) posterior end of the female showing terminal cleft (see the arrow).

Table 3
Comparison of measurements of specimens of *Linguatula serrata* collected in this study with literature records. Measurements are in microns unless otherwise stated. Date is presented as a mean with range in parentheses.

	This study		Riley et al. (1985)	Sambon (1922)		Rezaei et al. (2016)	
Characteristic	Male	Female	Male	Male	Female	Male	Female
No. specimens	6	6	1	-	-	20	20
Total Length (mm)	16.4 (15-18)	59.9 (48-70)	14	18–20	80–130	18 (14-22)	68 (51-92)
Max Width (mm)	3.2 (2.5–4)	7.9 (7–8.5)	-	3–4	80–10	3 (2.6–3.5)	9 (6.5–12)
No. annuli	80 (75-89)	92 (86-109)	84	~90	-	87 (84-89)	88 (85-93)
Anterior Hook							
AC	129.6 (113-150)	160.6 (145-175)	-	-	-	-	-
AD	211 (195-235)	252.5 (235-280)	395, 400[a]	-	-	214 (200-228)[b]	423 (396-468)[b]
BC	101.7 (90-115)	142.5 (105-165)	160, 170[a]	-	-	-	-
CD	100.8 (75-130)	132.5 (105-165)	-	-	-	-	-
AB	82.6 (75-100)	92 (75-105)	-	-	-	-	-
FL	265	510 (500-520)	-	-	-	-	-
Posterior Hook							
AC	136 (120-150)	150 (140-160)	-	-	-	-	-
AD	212 (200-230)	270 (250-190)	-	-	-	-	-
BC	108.3 (100-120)	145 (110-160)	-	-	-	-	-
CD	103.3 (90-130)	145 (110-180)	-	-	-	-	-
AB	94 (80-130)	100 (90-110)	-	-	-	-	-
FL	145 (130-160)	470	-	-	-	-	-
Buccal Cadre L	180	227.5 (130-300)	-	-	-	329 (300-360)	540 (504-576)
Buccal Cadre W	180	197.5 (90-270)	-	-	-	414 (199-228)[c]	417 (396-432)
Copulatory Spicule L	448.5 (430-460)	-	-	-	-	-	-
Copulatory Spicule W	162.5 (140-175)	-	-	-	-	-	-
Host species	*Canis familiaris*		*Canis familiaris dingo*	-	-	*Canis familiaris*	
Geographical Location	Tolbar Road, Kosciuszko National Park, New South Wales, Australia		Head of Dart River, Victorian Alps	-	-	Iran	

[a] Measurement template provided in Riley and Self (1981). No differentiation into anterior or posterior hooks.
[b] Measurement given as hook width and presumed to be equivalent to AD in this study. No differentiation into anterior or posterior hooks.
[c] Data is as presented in Table from Rezaei et al. (2016).

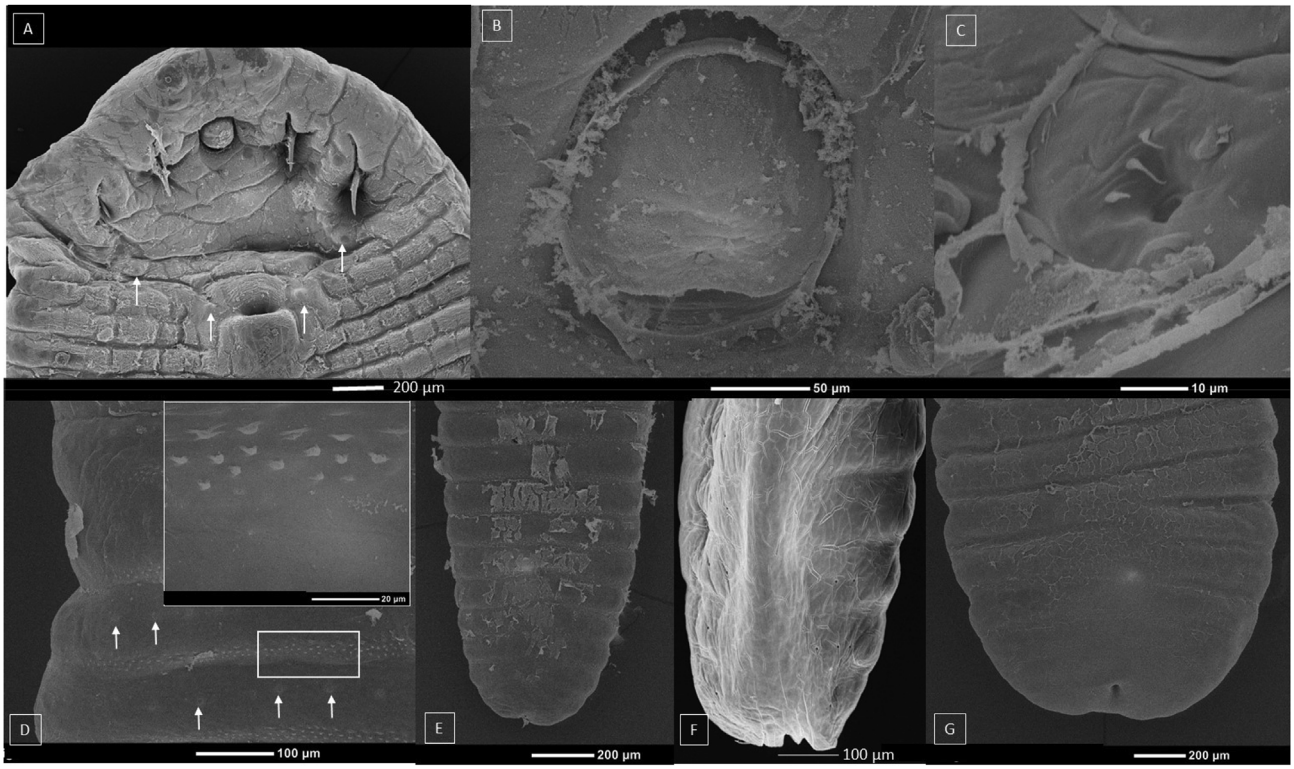

Fig. 3. Scanning electron microscopy of the male specimen: A) ventral side of the cranial region of the parasite, showing the mouth, two pairs of hooks and the two pairs of papillae (arrows), with the genital pore interrupting the annular rows midline; B) magnified view of the mouth; C) magnified view of the sensory sensillae; D) showing arrangement and structure of the annulus' sensory pores (arrows) and spines (within the square); E to G) variation in the posterior end of the male specimens.

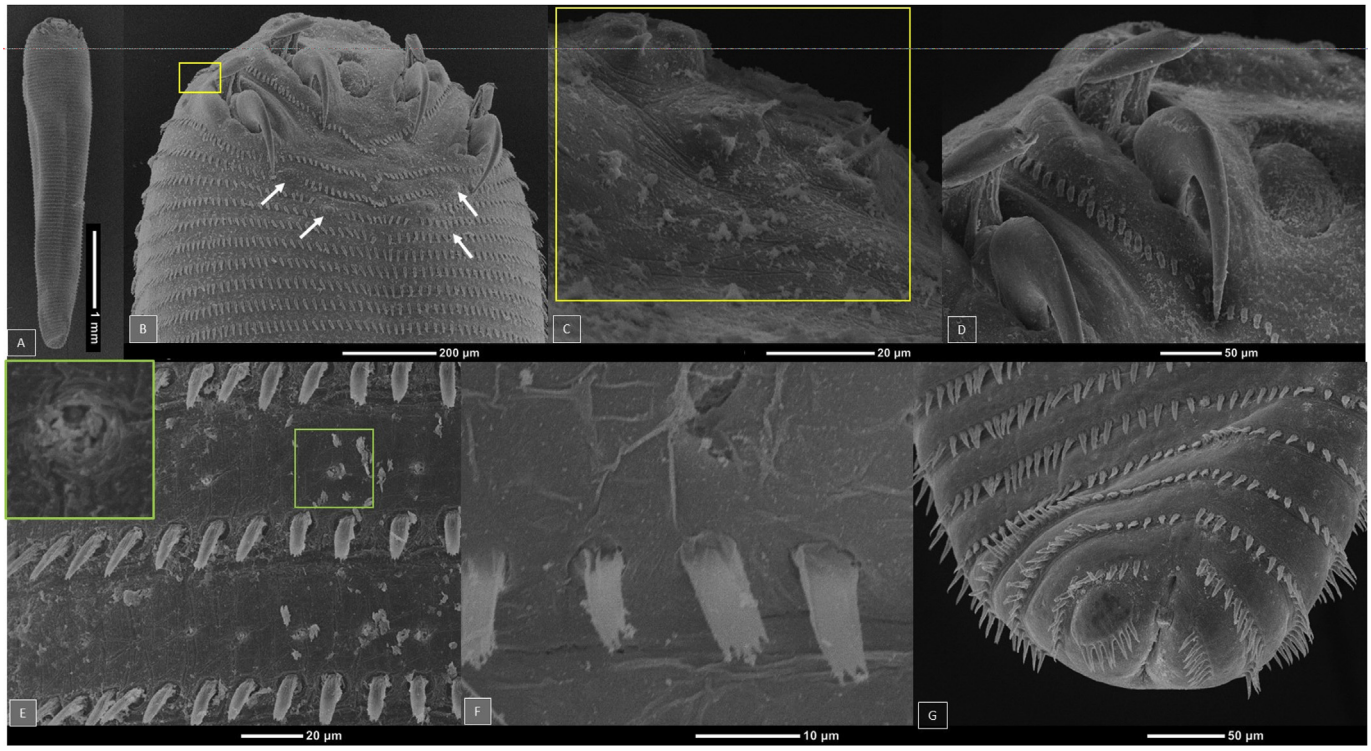

Fig. 4. Scanning electron microscopy of the nymph: A) ventral view of the full body; B) ventral view of the anterior part of the nymph showing two pairs of hooks with dorsal accessory pieces, mouth, sensory papillae (white arrows) and sensillae (within yellow square); C) magnified view of the sensillae; D) magnified view of hooks and dorsal accessory pieces from one side of the body; E) magnified view of the annulus, showing sensory pores (green square) and row of well-developed annular spines; F) magnified view of the annular spines; G) posterior end of the nymph.

interrupted. Annuli were ornamented with a row of prominent spines on posterior edge. Most spines had three to six denticles at the tip but some, in particular those on the last annuli and tip of the tail, had fewer denticles or none. A row of pores were present centrally in each annulus (Fig. 4E and F). Posterior end with terminal cleft and lobed with a plate on one lobe (Fig. 4G).

3.2. Molecular findings

Sequences of 18sRNA region were identical among all specimens collected in Australia in this study and the phylogenetic tree (Fig. 6B) showed that they were grouped with other *Linguatula serrata* reported from Norway. By contrast, the Cox1 region of *Linguatula* species is highly diverse with 11 haplotypes found in the worldwide samples. In Australia, four haplotypes were found among the Cox1 region. The pairwise genetic distance among worldwide *L. serrata* Cox1 sequences ranged from 0% to 1.7%, while the genetic distance among Australian samples ranged from 0% to 0.56%. The limited diversity among Australian samples in mitochondrial Cox1 and nuclear 18S regions indicated the Australian samples likely belong to the same species, which also indicated by the phylogenetic tree in Fig. 6. Sequences obtained from this study have been deposited in GenBank with accession number MN893765-MN893769 (Cox1) and MN889436-MN889440 (18S).

4. Discussion

Taxonomy and identification of *Linguatula* spp. which are solely based on morphological characteristics are known to be problematic due to high level of variation in body features (Riley, 1986). Our male and female specimens were morphologically different within each sex, however they had identical 18S sequence and relatively limited genetic distance in Cox1 region (0–0.56% which was within the intra-specific range observed for *Linguatula* spp. (Gjerde, 2013)), suggesting that they all belong to the same species. Combining morphological and genetic characterisation for Australian specimens was useful for their identification, irrespective of their sex and developmental stage. The greatest morphological variation observed in our specimens was total body length for females which was up to 15 cm long in some samples that were not included in this study (due to damage). The other highly variable features observed in our study were the number and arrangement of sensillae, morphology of the annular spines and the arrangement and location of chloride cells. A search of the literature shows that there are more publications on tongue worm nymphs than on the adults. Descriptions of nymphs assigned to *L. serrata* are highly variable in the literature, with very few providing detailed descriptions. It is known the there are nine nymph stages for the parasite in its intermediate host (Riley et al., 1987) and there is a possibility that morphological differences are due to describing different nymph stage of the parasite.

Another interesting finding based on the 18S sequence data in the present study was grouping of specimens assigned to *L. serrata* (Table 1) in which four Iranian *Linguatula* (GenBank accession numbers: KP100453 and KT58143-3) were distinct from *L. serrata* collected from Australia and Norway (Fig. 6B). The genetic distance between these four Iranian *Linguatula* and other 18S sequences obtained from Australian and Norwegian *L. serrata* ranged from 0.46% to 2.21% which is larger than the genetic distance of 18S sequences between *L. arctica* and *L. serrata* collected from Australia and Norway (0.12%). This finding suggests that the Iranian specimens were erroneously identified as *L. serrata*. The grouping of *Linguatula* spp. based on Cox1 region (Fig. 6A) was different from grouping based on 18S region (Fig. 6B) which is because sequences from Iran and Bangladesh used for Cox1 tree are from different studies than those in the 18S phylogenetic tree (Table 1). Disappointingly the sequences were not trackable to a morphological description, and even if it was provided, the description was limited, therefore a comparison of morphological descriptions between studies

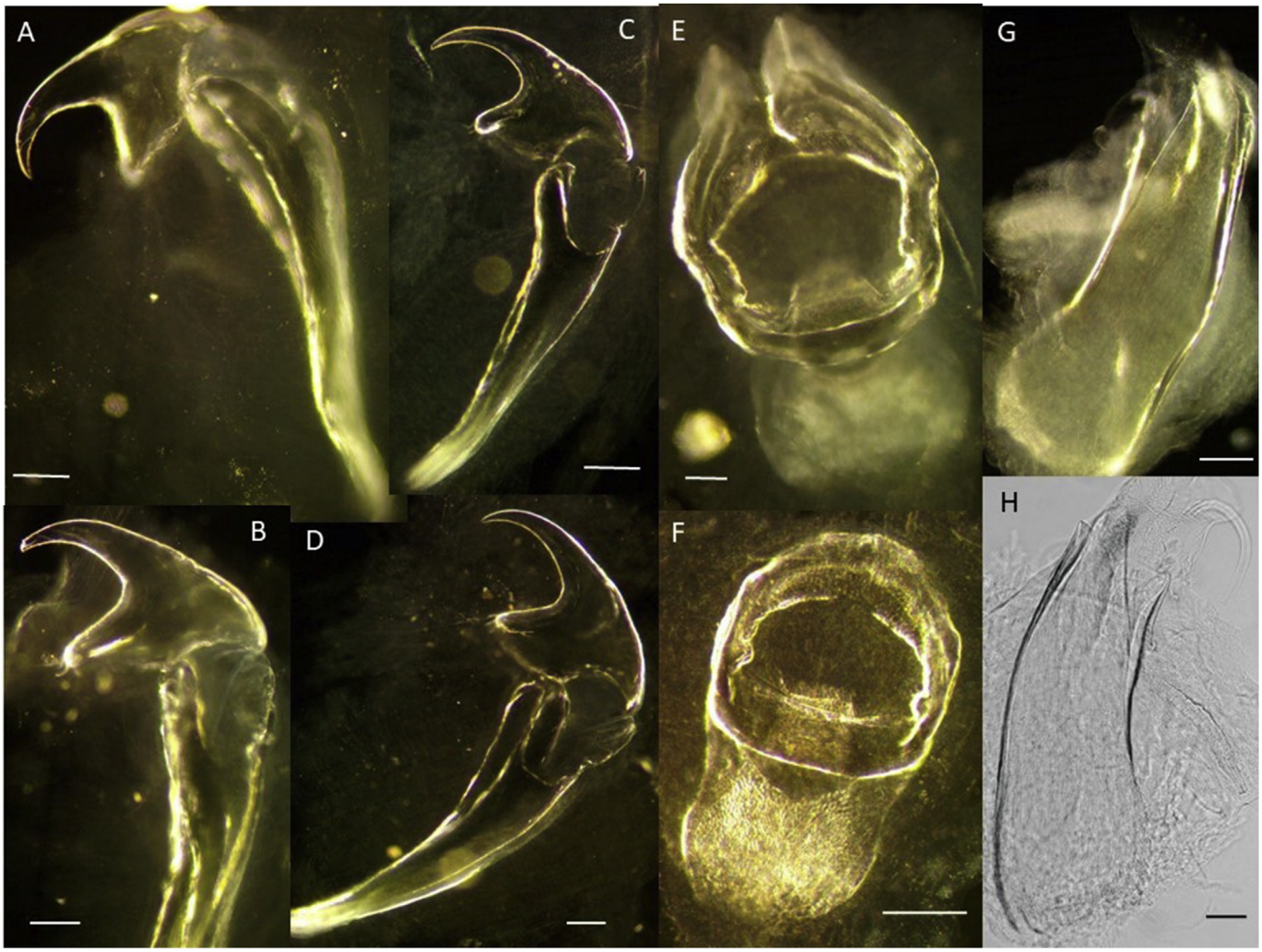

Fig. 5. Light microscopy of adult specimens collected in this study: A, anterior and B, posterior hooks of a female (132-1); C, anterior and D, posterior hooks of male specimens (131-7 and 132-3, respectively); buccal cadre of E, a female (132-1) and F, a male (132-3); G and H, copulatory spicule of males (131-7 and 131-5, respectively). Scale bars = 50μm.

was not possible. Moreover, most publications did not deposit a voucher specimen. The grouping of Australian specimens found in the present study with *L. serrata* from Europe correlate with the history of human movements and white settlement in the country which led to the entry of numerous exotic species to Australia, including cattle, dog and fox. The life cycle of *L. serrata* is completed between carnivores, such as dogs and foxes, and herbivores, such as cattle. All these animals (dog, fox and cattle) are the hosts found in the present study. Hence it is not surprising that specimens found in the present study are found to be the European *L. serrata*. Indeed, *L. serrata* was first described and reported from Europe. Herein, we consider Australian specimens to be *L. serrata* and taxa from Iran and Bangladesh in Fig. 6B, as a distinct but an unknown *Linguatula*. Most of publications from Iran, Bangladesh and the broader region in Asia report nymph stages. Infective nymphs are assumed to be those with rows of annular spines, developed hooks, obvious external segmentation and encystment in the intermediate host (Riley, 1986), as was found with the nymph in this study. Unfortunately, few authors state the stage of development of their nymphs. Although comparable morphological data from those regions are limited it seems that some of those assigned as *L. serrata* from Asia is morphologically different from *L. serrata* in Europe and Australia. For example, Banaja (1983) described the nymph of *L. serrata* as having its head bent ventrally, and long ventral annular spines reaching the posterior portion of the next annulus whereas in our specimen the head region was indistinguishable from the rest of the body and was recognisable only based on the presence of mouth and hooks with annular spines being significantly shorter on the ventral surface. A detailed morphological examination of members of *Linguatula* combined with their genetic characterisation is necessary to determine the specific identity of these parasites in Iran, Bangladesh and the broader Asian region.

Our phylogenetic tree based on the Cox1 (Fig. 6A) suggests there may be at least two species of *Linguatula* in Iran which is not surprising given that the country is influenced by at least three faunal regions: Ponto-Caspian, Mesopotamian and Oriental regions. Considering that Africa has multiple species of *Linguatula* and Norway has at least two species, it is erroneous that everything that is found in the recent literature in broader regions such as Palearctic (including Iran and Bangladesh) is automatically designated as *L. serrata* and should be avoided in the future unless there is a strong evidence to justify the identification as *L. serrata*.

For Australia with its unique fauna and ecosystem, understanding the specific identity of these parasites is of significance for a number of other reasons beyond just the interest of taxonomists to address some important questions. Due to its strict boundaries from the rest of the world, the Australian continent provides an excellent place to study the interactions between endemic/exotic hosts/parasites. The hosts examined in the present study were also involved in the entry of several

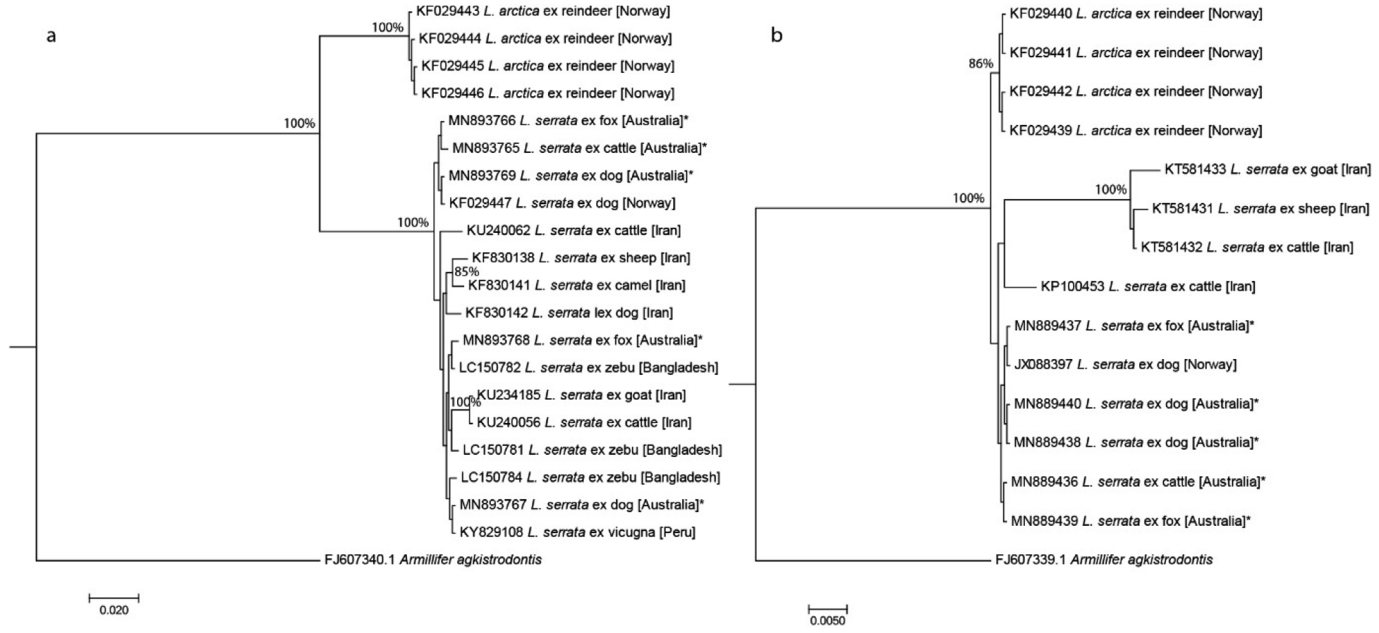

Fig. 6. Phylogenetic trees based on the Cox1 (a) and 18sRNA (b) sequences for *Linguatula* spp., *with Armillifer agkistrodontis* as an outgroup for Cox1 and 18sRNA sequences. Bayesian posterior probabilities values over 85% are indicated above the branches.

other parasites, such as *Babesia* spp., *Echinococcus granulosus* and ticks. Some of the interesting areas for future studies would be to investigate: the extent of infection of the Australian native species to *L. serrata*; the health impact of *L. serrata* on naïve endemic wildlife, such as marsupials, and; presence of other species of *Linguatula* in the country and the possibility of dingoes as entry routes for *Linguatula*?

These and many other questions remain unanswered at this stage. Sambon (1922) in 1922 wrote: "The taxonomic rank of the Linguatulidae has ever been a puzzle to zoologists". This has not changed in the 21st century. Indeed, modern taxonomy relying on publishing single partial sequences and claiming to elucidate taxonomic status, overlooking all other biological and anatomical aspects of members of these taxa, only add to the confusion. With the increasing shortage in the expertise in the field, the confusion over their taxonomy may continue for many years to come. This can impact our ability to deal with zoonotic parasites such as *Linguatula* spp. Members of *Linguatula* are of zoonotic significance (Bhende et al., 2014; Yazdani et al., 2014). They also have significant adverse health impact for their hosts and in particular their intermediate hosts (Godara et al., 2013; Hajipour et al., 2018; Shamsi et al., 2018; Yakhchali and Tehrani, 2013). It is known that the nymphs are highly migratory and do this using their annular spines by elevating them by muscles (Haffner et al., 1969). However, literature review shows pentastomids reported from humans were also mostly assumed to be *L. serrata*!

Declaration of competing interest

The authors declare no competing interests.

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