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FISH PARASITOLOGY - ORIGINAL PAPER

Description and characterisation of *Terranova pectinolabiata* n. sp. (Nematoda: Anisakidae) in great hammerhead shark, *Sphyrna mokarran* (Rüppell, 1837), in Australia

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Abstract

Terranova pectinolabiata n. sp. is described from the great hammerhead, *Sphyrna mokarran*, from Australian waters. This represents the first report of a species of *Terranova* from the host species. The new species is characterised by the morphology of the caudal plates and labia. ITS sequences were obtained for 20 specimens which were identical, despite morphological variation that has traditionally been indicative of separation of species. Additionally, genetic analyses confirmed the identification of the larval *Terranova* Type II previously reported in Australian and New Caledonian waters as *Terranova pectinolabiata* n. sp.

Keywords New species · Ascaridata · Sphyrnidae

Introduction

The genus *Terranova* was established in 1914 by the British explorers Leiper and Atkinson. They named a female nematode specimen collected from the stomach of a gummy shark, *Mustelus antarcticus*, from off New Zealand waters (Bay of Islands), after "Terra Nova", the ship they were sailing on (Leiper and Atkinson 1915), as *Terranova antarctica*. Features of this nematode were large simple labia, presence of an intestinal caecum (slightly longer than the cylindrical ventriculus (Gibson, unpublished observation (Sprent 1979)) and absence of inter-labia. Subsequently, more species with similar characteristics have been described, mainly from elasmobranchs, but also from aquatic reptiles (Baylis 1931; Baylis and Daubney 1922; Diaz-Ungria 1967; Fang and Luo 2006; Linton 1901; Sprent 1979; Thwaite 1927).

According to the World Register of Marine Species, the eight current valid species of the genus are *T. amoyensis* Fang & Luo, 2006, *T. antarctica* Leiper & Atkinson, 1914, *T. brevicapitata* (Linton, 1901) Skrjabin, Schikhobalova & Mozgovoi, 1951, *T. edcaballeroi* Díaz-Ungría, 1970, *T. galeocerdonis* (Thwaite, 1927) Mozgovoi, 1953, *T. ginglymostomae* Olsen, 1952, *T. pristis* (Baylis & Daubney, 1922) Johnston & Mawson, 1945, and *T. scoliodontis* (Baylis, 1931) Johnston & Mawson, 1945. However, *T. ginglymostomae* was considered to be a junior synonym of *T. galeocerdonis* by (Tanzola and Sardella 2006) based on morphological features, and, due to the absence of a male specimen, the full description of *T. antarctica* remains unknown. Table 1 provides geographical localities reported for the *Terranova* spp. The distinction of these species is mainly based on the morphological characters, and there are very limited sequence data for specific identification of these taxa as adult in GenBank.

Of the species listed above, four have been previously reported from Australian waters (Bruce et al. 1994; Bruce and Cannon 1990): *T. galeocerdonis* (including as *T. ginglymostomae*), *T. pristis* and *T. scoliodontis*. There are also several reports of *Terranova* larval types (I and II) in

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Table 1 Geographical locations for the previous reports of the *Terranova* spp.

Taxa	Locality	Reference
T. amoyensis	Taiwan Strait	Fang and Luo (2006)
T. antarctica	Bay of Islands, New Zealand	Leiper and Atkinson (1915)
T. brevicapitata	Woods Hole, Massachusetts, USA	Linton (1901)
T. edcaballeroi	Delta del Orinoco, Venezuela	Diaz-Ungria (1967)
T. galeocerdonis	Hopei, Solomon Islands; Encounter Bay, South Australia	Bruce and Cannon (1990)
	Off Bahia Blanca and Punta Tejada, Province of Buenos Aires, Argentina	Tanzola and Sardella (2006)
	Twynams Paar, Ceylon Pearl Banks	Thwaite (1927)
T. ginglymostomae	Moreton Bay and Caloundra, Queensland, Australia	Bruce and Cannon (1990)
	Tortugas, Florida, USA	Olsen (1952)
T. pristis	Balgal, Queensland, Australia	Bruce and Cannon (1990)
	Ulubaria, River Hughli, India	Baylis and Daubney (1922)

various fish species in Australian waters (Jabbar et al. 2012; Shamsi et al. 2018b; Shamsi and Suthar 2016a) with no current specific identification since larval anisakids cannot be identified to species level due to the lack of taxonomically important features and comparable sequence data from a well-identified adult. In this study, a new species of *Terranova*, collected from hammerhead shark in Australian waters, is described and genetically characterized.

Materials and methods

Hosts

Thirteen sharks were collected from localities off the New South Wales (NSW) coast of eastern Australia. Sharks were collected as part of various other projects and made available for parasite examination. *Sphyrna mokarran* collected from northern New South Wales were caught in five bather-protection gill-nets deployed off Ballina and Evans Head during the austral summer of 2016/2017 (Dec 2016–Jan 2017). Dead sharks were removed from the gill-nets and frozen whole. Sharks were defrosted and the stomach and spiral valve were removed, placed in bags and refrozen.

Parasites

The digestive system was separated at the junction of the stomach and the spiral valve. The stomach was opened longitudinally on a tray and examined for dietary components and parasites. In stomachs with a large quantity of food or liquid, the stomach and its contents were placed into a jar and washed. The spiral valve was opened longitudinally, washed and examined for parasites. The washings for each section were searched using a dissecting microscope.

All nematodes were collected, washed in physiological saline and then preserved in 70% ethanol. For a selection of nematodes collected, a small piece of the mid-body of each nematode was excised for molecular study, and the rest of the nematode was cleared in lactophenol for morphological examination.

Morphological examination

Anterior and posterior parts of each nematode were examined under light microscope. Illustrations were made using a microscope equipped with camera lucida. All measurements are given in millimetres, unless stated otherwise. Mean measurements are given, followed by the range in parentheses. Specimens have been deposited in the South Australian Museum, Adelaide (SAM) under AHC48627 to AHC48637, including Holotype (AHC48627) and Allotype (AHC48628).

Two representatives, one male and one female, were subjected to scanning electron microscopy (SEM). They were washed and dehydrated overnight in a series of graded ethanol solutions (70, 80, 90, 95 and 100%). After three additional overnight washes in absolute ethanol, the specimens were critical point dried using a tousimis Autosamdri-931 (USA). Samples were then mounted on a 12 mm carbon tab (ProSci Tech, Austalia) and sputter coated with gold using a K550X Sputter Coater (Quorum Technologies, UK). The specimens were examined under a JEOL (Peabody, Massachusetts, USA) NeoScope SEM with accelerating voltage set at 10 kv.

Genetic characterization

Twenty samples were selected for the sequencing of the ITS region. Genomic DNA (gDNA) was isolated from all individual larvae using Qiagen kit and eluted into 45 μl of water. The ITS region (including ITS1, 5.8 and ITS2) was amplified using the primer sets SS1: 5'-GTTTCCGTAGGTGA ACCTGCG-3′ (forward) and NC2: 5'-TTAGTTTCTTTTCC TCCGCT-3′ (reverse), and cycling conditions in accordance with (Shamsi and Suthar 2016b). An aliquot (4 μl) of each

Table 2 - Samples used for DNA analyses

Nematode species	GenBank (ITS-1)	GenBank (ITS-2)	Host species (scientific name)	Host species (common name)	Geographical locality	Reference
Pulchrascaris chiloscyllii	MF061687	MF061687	*Sphyrna lewini*	Scalloped hammerhead	China	Li et al. (2018)
Pseudoterranova decipiens	KM273067	KM273067	*Gadus morhua*	Atlantic cod	Denmark	Mehrdana et al. (2014)
Terranova sp. Type I	MH190367–8	MH190325–31	*Atule mate*	Yellowtail scad	New Caledonia	Shamsi et al. (2018a)
	MH190371–5	MH190333	*Decapterus macarellus*	Mackerel scad		
			Epinephelus ongus	White-streaked grouper		
			Lutjanus rivulatus	Blubberlip snapper		
			Saurida undosquamis	Brushtooth lizardfish		
			Scomberoides sp.	-		
			Sufflamen fraenatum	Masked triggerfish		
			Variola albimarginata	White-edged lyretail		
	JX848666–7	JX848681-4	*Caesio cuning*	Redbelly yellowtail fusilier	Lizard Island, Australia	Jabbar et al. (2012)
			Cephalopholis cyanostigma	Bluespotted hind		
			Grammatorcynus bicarinatus	Shark mackerel		
			Lethrinus nebulosus	Spangled emperor		
			Plectropomus leopardus	Leopard coral grouper		
			Scomberomorus commerson	Narrow-barred Spanish mackerel		
			Sphyraena forsteri	Bigeye barracuda		
Terranova sp. Type II	LN795828	LN7958711–2	*Abudefduf whitleyi*	Whitley's sergeant	Heron Island, Australia	Shamsi and Suthar (2016a)
	LN795851		*Lutjanus argentimaculatus*	Mangrove red snapper		
			L. carponotatus	Stripey Snapper		
			Grammatorcynus bicarinatus	Shark mackerel		
	JX848668–72	JX848686–7	*Atherinomorus endrachtensis*	Eendracht Land silverside	Lizard Island, Australia	Jabbar et al. (2012)
			Caesio cuning	Redbelly yellowtail fusilier		
			Caranx papuensis	Brassy trevally		
			Cephalopholis cyanostigma	Bluespotted hind		
			Chaetodon citrinellus	Speckled butterflyfish		
			Cheilodipterus intermedius	Intermediate cardinalfish		
			Grammatorcynus bicarinatus	Shark mackerel		
			Lethrinus nebulosus	Spangled emperor		
			Lutjanus carponotatus	Spanish flag snapper		
			Paracirrhites forsteri	Blackside hawkfish		
			Plectropomus leopardus	Leopard coral grouper		
			Scomberomorus commerson	Narrow-barred Spanish mackerel		
			Scolopsis monogramma	Monogrammed monocle bream		
			Sphyraena forsteri	Bigeye barracuda		
	MG594306–7	MG594330-1	*Tripodichthys angustifrons*	Black-flag tripodfish	Morton Bay, Australia	Shamsi et al. (2018b)
	MH190376–84	MH190334-41	*Atule mate*	Yellowtail scad	New Caledonia	Shamsi et al. (2018a)
			Carangoides cf. *orthogrammus*	Island trevally		
			Echeneis naucrates	Live sharksucker		
			Epinephelus areolatus	Areolate grouper		
			Nemipterus furcosus	Fork-tailed threadfin bream		
			Saurida undosquamis	Brushtooth lizardfish		
			Scomberoides sp	-		
			Scomberomorus commerson	Narrow-barred Spanish mackerel		
			Sphyraena qunie	Blackfin barracuda		
Terranova pectinolabiata n. sp.	MK542878–97	MK542878–97	*Sphyrna mokarran*	Great hammerhead shark	New South wales, Australia	This study

Table 3 Collection and host data for individual sharks found infected with *Terranova pectinolabiata* n. sp. Geographical coordinates are indicated where known

Host number	Host species	Location caught	Date caught	Sex	TL (mm)
16	*Sphyrna mokarran*	Ballina, NSW (− 28.836167, 153,611,380)	09/01/2017	M	3170
26	*Sphyrna mokarran*	Ballina, NSW	26/12/2016	M	2018
30	*Sphyrna mokarran*	Evans Head, NSW	30/01/2017	F	1903
37	*Sphyrna mokarran*	Ballina, NSW (− 28.873050, 153.596450)	08/01/2017	F	3255
22	*Sphyrna mokarran*	Ballina, NSW	23/01/2017	M	2718
25	*Sphyrna mokarran*	Ballina, NSW (− 28.787166, 153.601583)	05/01/2017	M	2950
29	*Sphyrna mokarran*	Ballina, NSW (− 28.873050, 153.596450)	20/12/2016	M	3560
31	*Sphyrna mokarran*	Ballina, NSW (− 28.873050, 153.596450)	08/01/2017	M	2868
39	*Sphyrna mokarran*	Ballina, NSW (− 28.867100, 153.600480)	08/01/2017	M	3310

TL total length

amplicon was examined on a 1.5% *w/v* agarose gel, stained with GelRed™ and photographed using a gel documentation system. PCR amplicons were sent to Australian Genome Research Facility (Queensland) for sequencing. The sequencing data resulted from this study were deposited in GenBank under accession numbers MK542878 to MK542897.

Apart from the 20 samples sequenced in this study, additional sequences were selected from GenBank and included in the phylogenetic analysis (Table 2). A majority of GenBank Sequences of closely related species have ITS1 and ITS2 separated. We have concatenated the ITS1 and ITS2 sequences that are generated from the same samples from previous studies (Shamsi et al. 2015, 2018a, b; Shamsi and Suthar 2016a, b). Sequences from this study and the concatenated sequence were aligned using ClustalW (Thompson et al. 1997) in BioEdit (Hall 1999), followed by manual adjustment. The phylogenetic relationships among species were inferred using Bayesian and maximum likelihood method using the software MrBayes v 3.2 (Ronquist and Huelsenbeck 2003) and MEGA-X (Kumar et al. 2018), respectively. Indels were excluded for analysis. *Pseudoterranova decipiens* was used as outgroup. The HKY model was applied as suggested by Jmodeltest2 (Darriba et al. 2012). For the Bayesian analysis, sample frequency was set at 1000, and the number of generation was set at 4,000,000. All other parameters were set as default. After the mcmc run, the first 25% samples were discarded, and the sumt command was used to summarize the phylogenetic tree. For the maximum likelihood analysis, tree topology and branch lengths were tested by 10,000 bootstrap replications. Figtree v 1.4.3 was used to visualize the phylogenetic trees (Rambaut 2014).

Results

A total of 13 sharks were examined for nematodes of which nine (69.2%) were infected with specimens of *T. pectinolabiata* n. sp. (Table 3). Microscopy revealed that most specimens were immature or a larval stage of the parasite (Table 4). Additionally, most adult specimens were not in good morphological condition; therefore, the morphological description below is based on three females and five males

Table 4 Number of nematodes collected and examined in the present study

Shark number	No. of nematodes found	No. collected for morphological and molecular work (MM, IM, MF, IF, L)	No. suitable for morphology (M and F)	No. for sequencing
16	133	10 (4,0,6,0,0)	2 and 3	6
22	17	10 (1,1,2,5,1)	1 and 0	1
25	9	3 (0,0,0,1,2)	–	–
26	16	12 (1,0,8,0,3)	1 and 0	6
29	2	2 (0,0,0,0,2)	–	–
30	3	3 (0,0,1,1,1)	–	3
31	4	2 (Unable to verify due to poor condition)	–	–
37	26	11 (5,0,6,0,0)	1 and 0	4
39	20	10 (0,0,4,4,2)	–	–
42	18 (+ 78 ex intestine – vial 50)			1

MM mature male, *IM* immature male, *MF* mature female, *IF* immature female, *L* larva

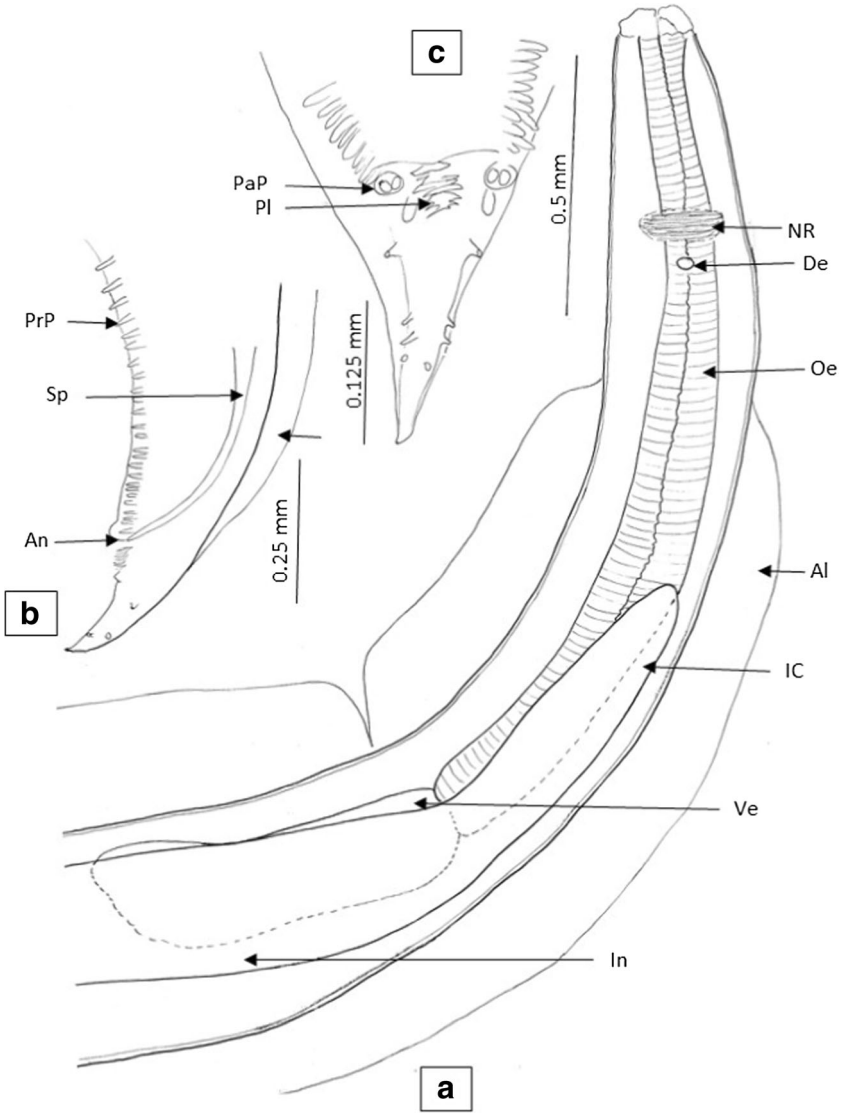

Fig. 1 Drawing of *Terranova pectinolabiata* n.sp. a) anterior end showing beginning of alae (al), position of nerve ring (NR) and deirid (De), morphology of the digestive system, including oesophagus (Oe), ventriculus (Ve), intestinal caecum (IC) and intestine (In); b) lateral view of male tail, including spicule (sp), precloacal papillae (PrP) and anus (An); c) ventral view of mail tail, including a pair of large paraclaocal double papillae (PaP) and caudal plates or plectanes (Pl)

which were suitable for light microscopy, of which one male and one female were subjected to scanning electron microscopy.

Superfamily Ascaridoidea

Family Anisakidae

***Terranova pectinolabiata* n. sp.** (Figs. 1–3)

Description (based on 8 specimens):

Cuticle annulated. Alae present, starting close to deirids on one or both sides, run along entire length of body, terminating at or near the tail. Labia small with comb-like structure on their inner cranial side. Inter-labia absent. Conspicuous cervical papillae or deirids present near nerve ring. Ventriculus cylindrical, 2.6–6.5 times as long as wide, shorter in males. Ventricular appendix absent. Intestinal caecum present. Gubernaculum absent. Spicules sub-equal in length, with blunt origin (Fig. 3i) and sharp and pointy end (Fig. 3g). Pre-cloacal papillae usually forming ridges, with elongate pulp that has stem-like appearance, arranged irregularly and posteriorly in two rows on the ventral surface of males (Fig. 2f). Cuticular plates or plectanes in males present posterior to cloaca (Figs. 1c, 2g, 3f), irregular in shape and size, with the smallest pelctane located posteriorly. Large medioventral pre-cloacal papilla present in males but not very much projected (Fig. 2g). Tail short and alated in both sexes, ventrally curved in males (Fig. 3d) but simple and conical in females (Fig. 3g, h). The biometrical data for males and females are presented below. The measurements have been performed on ethanol-fixed specimens.

Females: Body length 52.19 (47.00–57.38); body width 1.20 (0.90–1.50); dorsal labium width and height 0.15 × 0.06 (0.14–0.15 × 0.05–0.08); sub-ventral labium equal in size, 0.11 × 0.06 (0.10–0.11 × 0.06–0.06); deirids 0.77 (0.68–0.85) and 0.80 (0.75–0.85) from the anterior end; oesophagus 5.18 (5.00–5.35) long, 0.10 (0.09–0.11) of body length; ventriculus 1.75 (1.50–2.00) long; intestinal caecum 2.90 (2.55–3.25) long, 1.66 (1.63–1.7) of ventriculus and 0.56

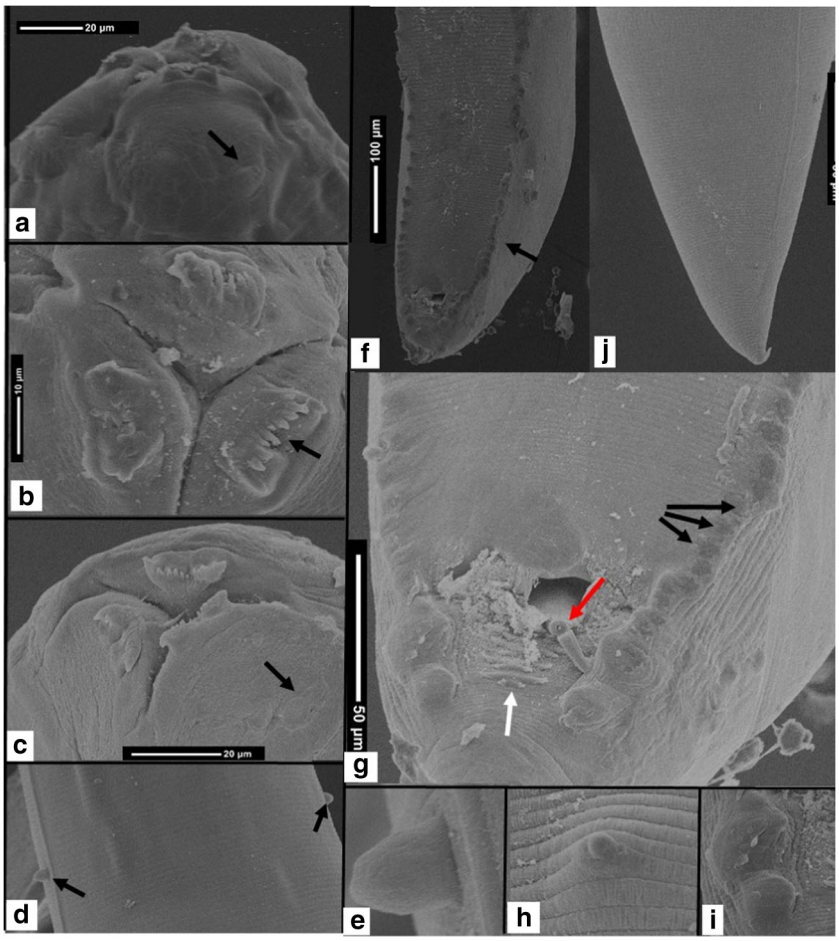

Fig. 2 Scanning electron microscopy of the *Terranova pectinolabiata* n. sp. **a** subventral labium (arrow indicating the labial papilla), **b** apical view of labia (arrow pointing at the comb-like structure), **c** comb-like structures on each labium (arrow indicating the labial papilla), **d** pair of deirids (see arrows), **e** close up of a deirid, **f** ventral view of male tail (arrow is pointing at the row of pre-cloacal papillae), **g** ventral view of cloacal area showing plectanes (white arrow) and large para-cloacal papillae, spicule (red arrow) and partial pre-cloacal papillae (black arrows), **h** pre-cloacal papilla, **i** large paracloacal papillae with double papillae at the top and a large post-cloacal papilla under it, adjacent to plectanes

(0.51–0.61) of oesophageal lengths; tail 0.45 (0.40–0.50) long and 0.32 (0.28–0.35) wide; eggs 0.033 (0.030–0.036) × 0.034 (0.032–0.036).

Males: body length 25.53 (18.95–34.13); body width 0.94 (0.65–1.50); dorsal labium 0.13 × 0.05 ($n = 1$); sub-ventral labia 0.08 (0.008–0.009 × 0.06 (0.05–0.08); deirids 0.57 (0.25–0.75) and 0.59 (0.25–0.77) from the anterior end; oesophagus 2.19 (1.40–2.85) long, 0.08 (0.07–0.08) of body length; ventriculus 1.15 (0.60–2.00) long; intestinal caecum 1.40 (1.15–1.55) long, 1.50 (0.75–1.92) of ventriculus and 0.74 (0.57–0.83) of oesophageal lengths; tail 0.25 (0.20–0.29) long and 0.20 (0.15–0.25) wide; spicules 0.63 (0.40–0.75) and 0.70 (0.63–0.75) long and 0.02 (0.02–0.03) and 0.03 (0.02–0.03) of body length; pre-cloacal papillae 51 (36–59).

Etymology: *pectinolabiata* refers to the comb-like structure on the cranial surface of the labia.

Remarks: The closest related species to *T. pectinolabiata* is *T. galeocerdonis*. The latter has been redescribed by Tanzola and Sardella (2006) based on materials from *Carcharias taurus* off Bahia Blanca and Punta Tejada, in Argentina. Our specimens are clearly distinct based on morphology of caudal plates. There is relatively more gap between plates in *T. galeocerdonis*, and they look orderly whereas plates in *T. pectinolabiata* are situated closer to each other and due to the variance in their size they look to be arranged out of orders. In addition, there is a significantly smaller plate in both species that is located centrally as the third plate in the former but as the last plate in the latter. The position of the comb-like structure on the labia is also different between *T. galeocerdonis* and *T. pectinolabiata*, being situated at the most cranial part of the labia from apical view and being more projected in the former (Fig. 6 in Tanzola and Sardella (2006)) but lower in the new species (see Fig. 2b). Labia are more triangular in the new species than it is in *T. galeocerdonis*.

Genetic characterization: Twenty specimens were selected from males, females, larval and immature specimens and were subjected to sequence analyses of the ITS region. ITS sequences were identical among all specimens. The alignment among species was 935 bp in length. Small genetic differences were found between the samples collected in this study and the sequences of *Terranova* sp. type II from GenBank, with a maximum pairwise genetic distance at 0.468% between *T. pectinolabiata* n. sp. and *Terranova* sp. type II. By contrast, *Terranova* sp. type I appeared to be a distinct group with a minimum among species pairwise genetic distance at 6.58%.

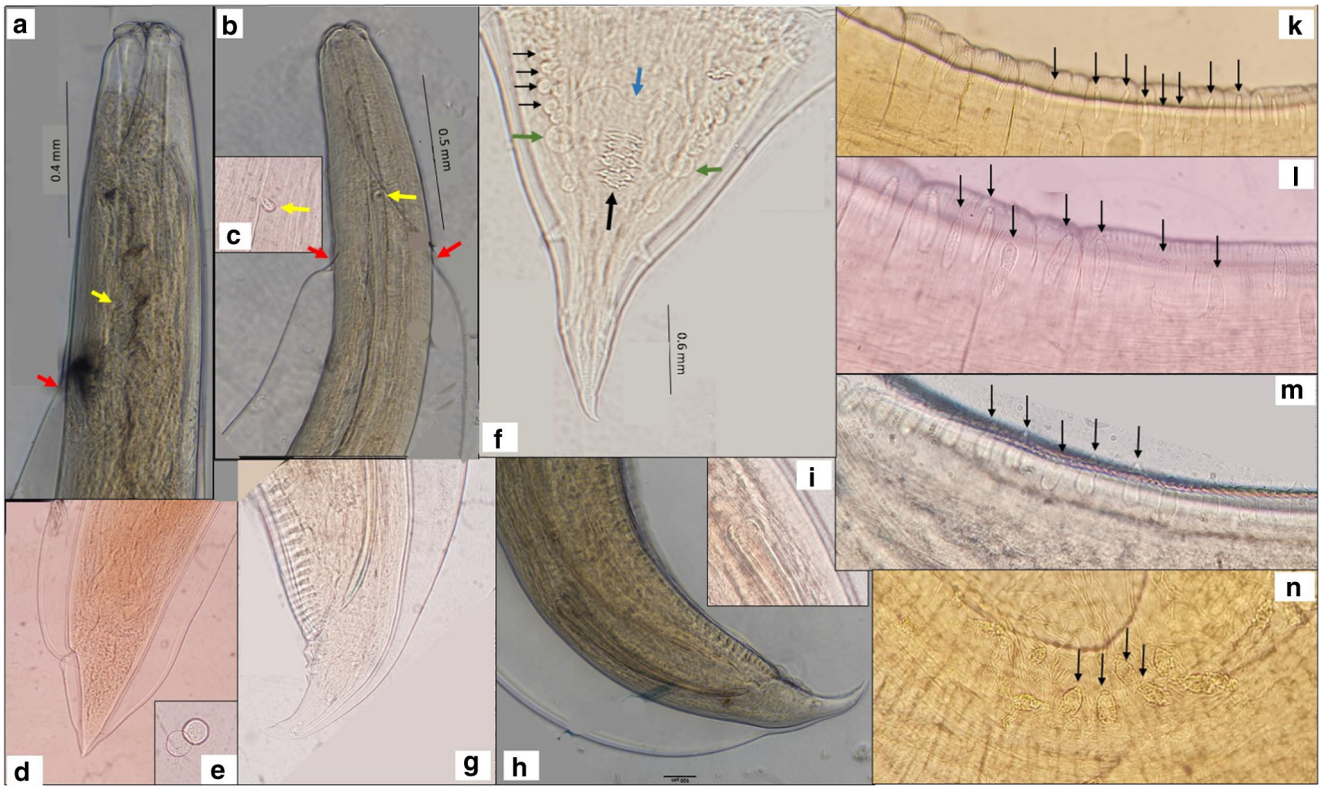

Fig. 3 Light microscopy of *Terranova pectinolabiata* n. sp. **a** and **b** anterior of the parasite showing variable beginning of alae on each side of one specimen as well as variety in different specimens (red arrow); **c** deirid pointed out by a yellow arrow which corresponds to yellow arrows in **a** and **b**; **d** posterior end of a female; **e** eggs; **f** ventral view of a male tail showing precloacal papillae (light black arrows), anus (blue arrow), large paracloacal papillae (green arrows), and plectanes (bold black arrow); **g** and **h** lateral view of two male specimens showing variation in ending of alae; **i** origin of the spicule; **k**–**n** showing variation in morphology of precloacal papillae (shown by arrows) in different specimens

The Bayesian inference phylogenetic tree also clustered 20 *T. pectinolabiata* n. sp. samples with *Terranova* sp. type II with all the *Terranova* sp. type I as a separate clade (Fig. 4) with 100% posterior probability. Similar relationships were also found using maximum likelihood method (Fig. S1).

Discussion

This is the first record of a species of *Terranova* infecting the great hammerhead, *S. mokarran*.

The following identification key can be used to morphologically distinguish between the new species in the present study and previous valid species:

1) No cuticular plate (Deardorff 1987) ..... ***T. brevicapitata***
Cuticular plates present.....**2**
2) Labia prominently bilobed, with wide row of fine teeth; cervical alae fine, inconspicuous; male plectanes laterally truncate (Bruce and Cannon 1990), four simple ventricular plates (Deardorff 1987) ..... ***T. scoliodontis***
Morphology of labia and caudal plates different.....**3**
3) Gubernaculum absent **4**
Gubernaculum present (Bruce and Cannon 1990).....**5**
4) Mid-ventral plectanes bear 5 pairs of sharp marginal spines, median of which is smallest (Tanzola and Sardella 2006); comb-like structures on the labia at the tip of the labia..... ***T. galeocerdonis***
Mid-ventral plectanes with sharp marginal spines but irregular in size and arrangement, the most posterior plate being the smallest; comb-like structures on the side of the labia .....***T. pectinolabiata* n. sp.**
5) Ventriculus long, pre-cloacal papillae 54 pairs, spicules 2% of body length..... .....***T. pristis***
Ventriculus spherical, pre-cloacal papillae 12–13 pairs, spicules 4.3 & 3.9% of body length..... .....***T. amoyensis***

Although the number of specimens that were morphologically examined in the present study were limited, a number of morphological variations have been observed. The start and

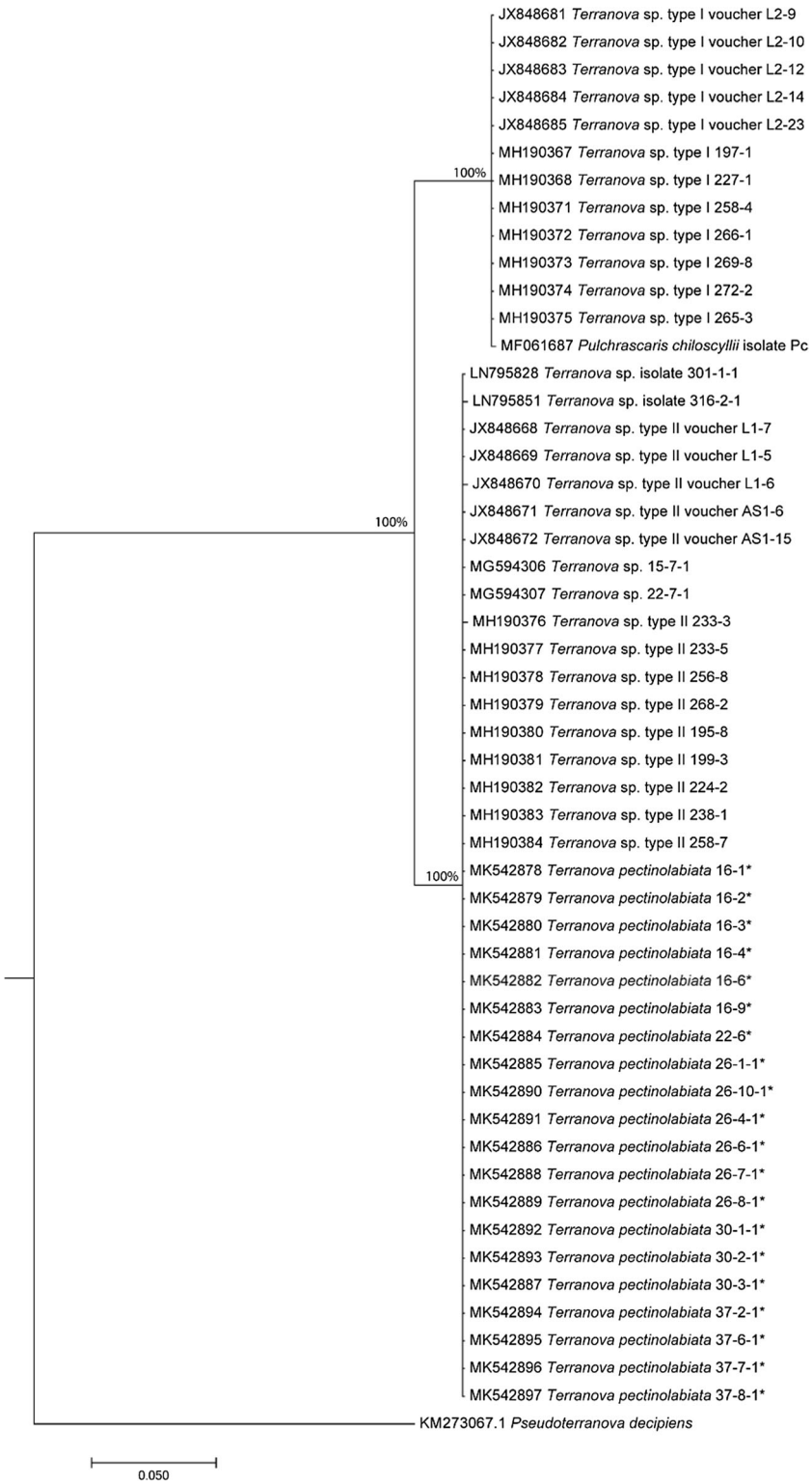

Fig. 4 Bayesian inference phylogenetic relationship among the specimens found in this study and those closely related species from GenBank (see Table 2 for details) using concatenated the ITS1 and ITS2 sequences. Posterior probabilities were shown on the node. *indicates sequences generated from this study

the endpoints of alae were variable among our specimens. While in some specimens, the alae started symmetrically (Fig. 3b), in others the start point was asymmetrical, with one side more towards the anterior than the other (Fig. 3a). Similarly, the distance of deirids from the anterior end was variable, and they were also located symmetrically in some specimens and asymmetrically in others. Another variable feature was morphology of pre-cloacal papillae in males: narrower with a longer stem in immature specimens (Fig. 3k), originating deeper in the cuticle, compared to shorter

and thicker in mature/thicker specimens (Figs. 3l, m), originating more superficially in the cuticle. Indeed, pre-cloacal papillae in the specimens with the widest body width looked like a leaf (Fig. 3n) located on the ventral surface. We also noted that oesophageal and ventriculus lengths, and relative length to body size, were shorter in males than in females. This was also recorded for other species but not pointed out by authors, for example in *T. pristis* (Bruce and Cannon 1990), *T. amoyenmsis* (Fang and Luo 2006) and *T. galeocerdonis* (Bruce and Cannon 1990). Assuming larger oesophagus and ventriculus are more efficient in absorption of nutrients from host, the larger size observed in these studies could be due to the females' role in egg production and their need for higher nutrients, and therefore this is possibly a true intraspecific morphological difference between male and female *Terranova* spp. Given that alae start location and length, deirid location and morphology of the intestinal system and preanal papillae are important taxonomic features, it is fortunate that the sequence data obtained for specimens in the present study established that the abovementioned observations are just morphological variation among individuals of the same taxon. However, this also highlights the need for future studies to include sequence data to ensure species identifications.

Previously, a range of *Terranova* larval type II were found in several fish species caught from Victoria to the Northern Territory, in Australia, as well as in New Caledonian waters (Jabbar et al. 2012; Shamsi et al. 2015, 2018a, b; Shamsi and Suthar 2016a). As evident in the phylogenetic tree and based on the identical ITS-1 and ITS-2 sequence data with the new species found in the present study, it is now known that those *Terranova* larval types reported previously in Australian waters are larval stage of the *Terranova pectinolabiata* n. sp. found in the present study.

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Compliance with ethical standards

All applicable institutional national and international guidelines for the care and use of animals were followed. Approval to deploy the bather-protection nets was granted under section 158 of the Australian Commonwealth Environment Protection and Biodiversity Conservation Act 1999, from the application of sections 18, 18A, 20, 20A, 23 and 24A of Part 3 and Parts 7 to 9 of Chapter 4 of the Act, by the Commonwealth Minister for the Environment and Energy.

Conflict of interest The authors declare that they have no conflict of interest.

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