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First record of *Thysanozoon brocchii* (Platyhelminthes: Polycladida) from Indian waters

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Abstract

This work reports the occurrence of *Thysanozoon brocchii* from the rocky intertidal coast of Ratnagiri and Dwarka, the West Coast of India, for the first time. Two morphotypes were found, the first morph has a buff brownish papillate dorsal surface, with few specimens having white spots. The second morph has light coloured papillae which form a distinct cross marking along the dorsal surface. The species possesses double male copulatory apparatus with seminal vesicle, prostatic vesicle and sclerotized stylet. This cosmopolitan species has previously been recorded from Italy and Mediterranean, Japan, South and West Africa, Florida, New Zealand, Brazil, and United Kingdom. Synonymized species and older descriptions have been compared to examine similarities and dissimilarities. Considering the existence of varied colour morphs of this species, a detailed comparative analysis of morphological characters, reproductive histology and molecular framework is recommended.

Keywords: Pseudocerotidae, Colour patterns, Intraspecific variations, Reproductive anatomy, Cosmopolitan, Maharashtra, Gujarat

Introduction

Polyclad flatworms are free living members of the phylum Platyhelminthes. These coral reef and rocky shore inhabitants are more diverse in the tropical environment (Prudhoe, 1985). Cryptic behavior, apparent specificity with food preference, aposematic colouration and mimicry with opisthobranch molluscs and fishes (Ang & Newman 1998; Newman & Canon, 2005) make them potentially significant in intertidal or reef ecology.

Members of the family Pseudocerotidae are peculiar for their brilliant colour patterns. However, colour variation is commonly seen forming species complex in their systematics. Perhaps such complexities arising in the taxonomy of these worms can be resolved by careful observations of external as well as reproductive anatomical features. Newman and Canon (1995) described three species of the genus *Pseudoceros* from the Indo-Pacific region showing remarkable similarity within external appearance. They, too, emphasized the significance of precise documentation of colour and patterns. Litvaitis, et al. (2010) tested the coloration pattern of *Pseudoceros bicolor* complex against molecular evidence and emerging complexities in the taxonomy of the same.

Grube (1840) described the genus *Thysanozoon* considering the papillate dorsal surface, unlike the rest of the Cotylean genera. Type species of the genus was *Thysanozoon diesingii*, which has later been synonymized for *T. brocchii*. This genus includes about 23 species worldwide (Tyler, 2013). Laidlaw (1902) reported *Thysanozoon plehni* from the Laccadive Island of India. However, this species has been reviewed and assigned to *Acanthozoon plehni*. After a gap of about a century, Apte and Pitale (2011) mentioned a member of this genus from Kavaratti, Lakshadweep Island, India. Later, Sreeraj and Raghunathan (2013) reported *Thysanozoon nigropapilosum* from South Andaman.

The present study reports *Thysanozoon brocchii* for the first time from the Indian shoreline. Apart from external morphology and reproductive anatomy, the study encompassed the review of characteristic features of some of the synonyms and a few previous descriptions, and their comparisons with the two different morphs presented herein.

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Materials and methods

Collection was carried out during the daytime low tide by handpicking the worm using a paint brush, within the littoral area of Ratnagiri (Mandvi 16.98758° N 73.27486° E), Maharashtra state and Dwarka (22.240323° N 68.957424° E), Gujarat state, West Coast of India (Fig. 1). Presence of heterogeneous habitat is a common characteristic of both these shores, and pebbles, cobbles and rock pools of various sizes and depths are found at various zones. Luxuriant growth of algae and patches of coral-rubble were observed during surveys.

Photographs were taken in the wild as well as ex situ to record true colour and pattern. Animals were then fixed in 10% frozen buffered formalin and later stored in 70% ethanol for long term preservation (Quiroga et al., 2004). Studies on key anatomical features were carried out using a Stereo Microscope (Leica EZ4 D). The systematic classification system established by Faubel (1984) was followed. Longitudinal serial sections of the reproductive system (6 μm) were obtained by specimen (Pclad-0044) embedding in paraplast and staining with hematoxilin and eosine. Remaining specimens are deposited in collections of the Bombay Natural History Society.

SYSTEMATICS.
Order POLYCLADIDA Lang, 1884
Suborder COTYLEA Lang, 1884
Family Pseudocerotidae Lang, 1884
Genus *Thysanozoon* Grube, 1840
Thysanozoon brocchii Risso, 1818

Material examined

Two specimens (11.49 mm × 8.32 mm and 11.30 mm × 8 mm, preserved) found under rock pebble at Mandavi, Ratnagiri, 9 May 2012 (BNHS_Pclad-0044) as 18 histological slides.

One specimen (10.2 mm × 8.5 mm preserved) found under rock pebble within algae at Dwarka, Gujarat, 12 December 2012 (BNHS_Pclad-0080).

Diagnosis

Brown-buff to cream dorsal surface with yellowish brown to dark brown papillae cover, margin with pinkish tint; ends with white dotted line. Papillae from median longitudinal line are lighter thus distinct, transverse line of light coloured papillae about 1/3rd posterior to longitudinal length also present in single

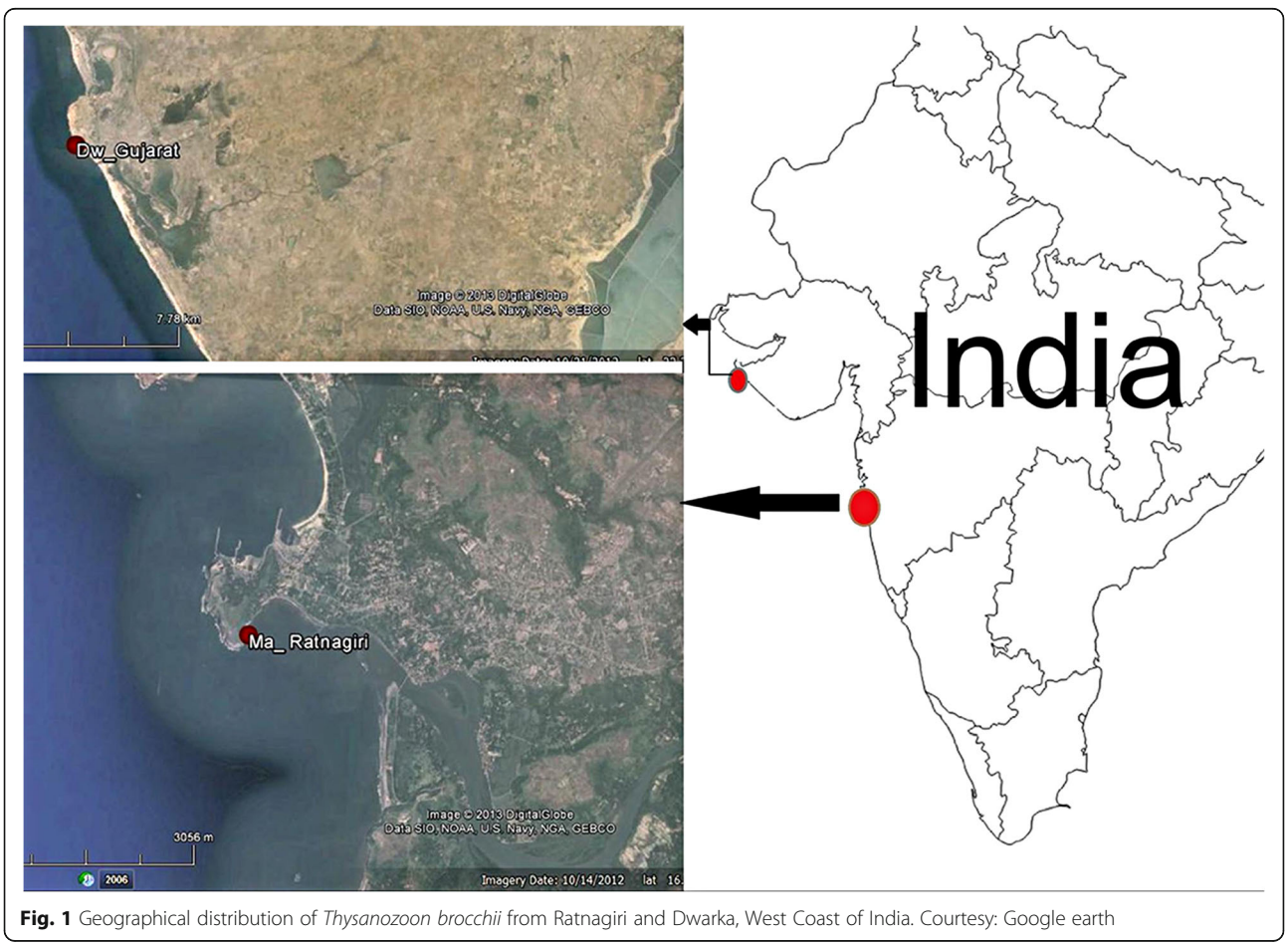

Fig. 1 Geographical distribution of *Thysanozoon brocchii* from Ratnagiri and Dwarka, West Coast of India. Courtesy: Google earth

specimen (Pclad-0080); double male reproductive structures; elongated vagina.

Description

The first morph (BNHS-Pclad-0044) is broadly oval, oblong body and found slightly raised medially. Dorsal surface covered with papillae which are aggregated in median region, diminish in size, and become scarce towards margin and found absent a little above the margin. Papillae are approximately cylindrical (0.3 mm–0.4 mm), knob-like or even tapering in the same specimen.

Dorsal surface, ground colour is buff-brown-creamish with black median longitudinal stripe. Yellowish reticulation of intestinal branches observed prominently towards margin. Pinkish tint found towards periphery, and a dotted white line on the rim. Papillae are buff-brown to dark brown having grayish outline and some possess white spots around the tip. Papillae present over median longitudinal stripe are mostly distinct, tapering, and lack brown pigments. These papillae are creamish or whitish, possess white dots and run antero-posteriorly. Pseudotentacles are held erect; they are ear-like, creamish brown and with white tip. On the inner margin of each pseudotentacle, a fine black line that runs towards the median stripe can be observed. Cerebral eyespots (30–34) arranged in horseshoe-shaped cluster (size 0.27 mm), present within the colourless area just posterior to the pseudotentacles (Fig. 2c). Pseudotentacular eyes are found distributed as four clusters dorsally and two clusters ventrally. Dorsal cluster bears about 40–45 eyespots whereas ventral cluster bears about 50–60 eyespots.

Another morph (BNHS-Pclad-0080) has creamish dorsal surface and yellowish reticulation noticed especially towards margin (Fig. 2b). Dotted white line delineates the margin. Brown coloured papillae are cylindrical, pointed, with white spots around the tip. Papillae present over

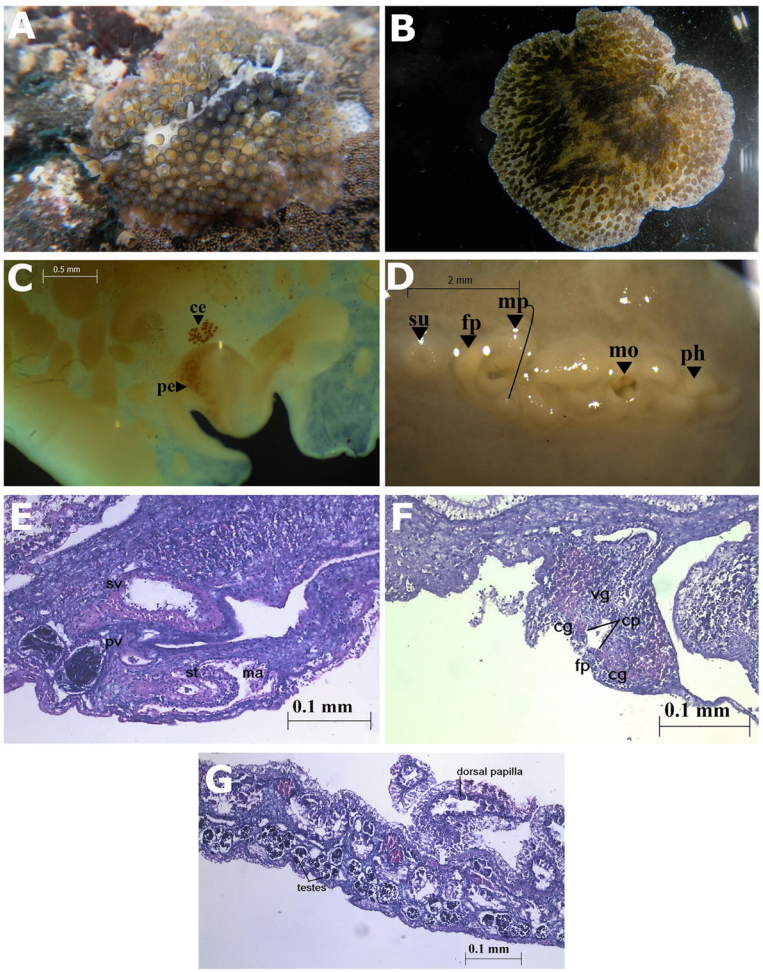

Fig. 2 *Thysanozoon brocchii* Risso, 1818: **a** (1st morph) under pebble within the rock pool (**b**) 2nd morph (**c**) anterior end with cerebral eyespots (ce), pseudotentacular eyespots (pe); (**d**) ventral surface showing pharynx (ph), Mouth (mo) male gonopore (mp), female gonopore (fp), sucker (su); (**e**) Sagittal section of male reproductive system with male antrum (ma), prostatic vesicle (pv), stylet (st); (**f**) female reproductive structure with vagina (vg), cement pouch (cp), cement gland (cg), Intestine (it); (**g**) ventral testes and dorsal papillae

median longitudinal and transverse line are creamish, possess white spots and form a cross-like marking. Black-brown pigments of the median stripe can be seen over anterior region and on the pseudotentacles.

The two morphs described above are similar in terms papillae arrangement, median lighter papillae and margin colouration. The papillae shape in second morph is cylindrical and white dots present over each papillae are more numerous than the first morph. Ground colour is creamish and papillae colour is darker and colour tone is more even in second morph as in the first one. Considering the distinctive colour pattern of second morph the single specimen has been kept intact and not been sectioned for histology.

Semi-transparent whitish ventral surface, with a mouth, opening medially within pharynx and about 3.01 mm distant from anterior margin (Fig. 2d). Pharynx is in the form of 4–5 simple and shallow folds and about 4.68 mm long. Two male gonopores are present immediately behind the pharynx, on either side of the median line and about 1.24 mm distant from each other. Medially placed, female gonopore is present 0.71 mm posterior to the male gonopore followed by sucker (0.46 mm from fp).

Double male copulatory system with numerous testes (0.02 mm to 0.04 mm in length) arranged in 2–3 rows located ventrally. Seminal vesicle (0.14 mm × 0.064 mm) is muscular, slightly oval, bent and pointed at its anterior end (Fig. 2e). Vas deference arranged laterally, visible through ventral side and runs posteriorly. Small and oval prostatic vesicle (0.0435 mm × 0.0342 mm) found ventral to the seminal vesicle. Penis with stylet found further down to the seminal vesicle, probably contracted or bent during fixation, as it is visible only in the form of a rounded structure (Fig. 2e). Male atrium is shallow (0.045 mm).

Female reproductive system consists of ovaries which are found scattered dorsally. Female antrum is narrow, with minute lateral invagination for cement pouches. Vagina elongated backward (0.2 mm) and cement glands are seen spread around vagina (Fig. 2f).

Taxonomic remarks

In taxonomic study of pseudocerotidae, the genus *Thysanozoon* is difficult, probably due to the unavailability of enough details in the older literature. Body colour pattern, papillae shape, their colour and distribution are important features in the taxonomic study of this genus (Brusa et al., 2009). The specimens (BNHS_Pclad-0044 and 0080) somewhat fit with the original description of Risso, 1818 and specimens described later (Palombi, 1928; Pearse, 1938; Marcus and Marcus, 1968; Brusa et al., 2009 and Bahia et al., 2012, 2014) from different parts of the world.

About twenty species described earlier have later been synonymized for *T. brocchii* (Faubel, 1984). Table 1 gives the review of the characters noted from these synonyms.

Tergipes dicquemare, *Planaria dicquemari* and *Thysanozoon dicquemaris* although very poorly described, seems to be similar in terms of dorsal and ventral colour pattern. *Planaria tuberculata*, *Planaria verrucosa* are similar in terms of colour and pattern. *Eolidiceros panormis* and *Thysanozoon diesingii* are nearly identical due to possession of yellow and purple pigment on dorsal surface as well as the purple dash lines on the margin. The colour pattern of *Eolidiceros brochii* is slightly different from the other species due to possession of reddish-brown papillae and purple dots all over the dorsal surface. Four of the synonyms viz. *E. panormis*, *T. diesingii*, *T. lagidium* and *T. fockei* are found possessing purple pigmentation on dorsal surface. Transverse bands of lighter papillae forming a cross were observed within *E. panormis*, *T. brochii* var. *cruciatum* and *T. lagidium*. The remarks section of Table 1 elaborates the comments from the authors about the resemblance of their respective specimens, either with *T. brocchii* or previously described allied species. Diesing (1850) reports about *Thysanozoon tuberculatum* and synonymizes three previously known species viz. *Planaria tuberculata, Thysanozoon dicquemaris* and *Planaria dicquemari*. *Planaria brocchi* and *Eolidiceros brocchi* are synonymised for the species *Thysanozoon brocchii* and *Eolidiceros panormus* for *Thysanozoon panormus* respectively. Further, Diesing (1862) while separately mentioning the genus *Thysanozoon*, synonymizes species which are described earlier under the varied genera viz. Planariae spec. by Delle Chiaje, Stylochi spec. by Diesing Eolidiceri spec. by Quatrefages.

Table 2 gives an account of characters mentioned from some of the older descriptions of *Thysanozoon brocchii*. The descriptions by Quatrefage (1845) and Lang (1884) are unvarying and collected from type locality of the concerned species. Material obtained from Japan (Yeri and Kaburaki, 1916; Kato, 1944) was found resembling the above two descriptions, especially in terms of possessing purple pigment and papillae colour pattern. Palombi (1928) differs with Lang (1884), Quatrefage (1845), Yeri & Kaburaki (1916), and Marcus and Marcus (1968) due to having exclusive brown dorsal pigment and ventral eyespot arrangements. Meanwhile, Pearse (1938) recorded the three morphs (pigmentation- brown, purple and intermediate) of the species from Crooked Island. Marcus and Marcus (1968) elaborated the reason for presence of broader female antrum in Marcus (1949) material of *T. lagidium*. They also discussed the external and internal anatomical, intraspecific variations within this species and finally merged *T. lagidium* with *T. brocchii*. Faubel (1984) while reviewing the polyclad systematics followed the same criteria. However, Prudhoe (1985)

Table 1 Review of the characters from synonymized taxa of *Thysanozoon brocchii*

Synonymized taxa	Body form	Colour -pattern	Eye spots/arrangement	Habitat/Locality	Remarks
Tergipes dicquemare Risso, 1818	oblong, flattened, Dorsal: covered with many sessile papillae serving as respiratory organ, tentacles earlike, Ventral: greyish	Dorsal White- yellowish Intestinal canal - white –reddish	-	Under stone Nice sea, France	-
Planaria tuberculata Delle chiaje, 1828	compressed, ovate, wide Dorsal: papillate; absent towards margin, longitudinal median line present	Dorsal Papillae: few with white dots, median line white colour Margin: white band even on pseudotentacles	-	Algae dweller Naples, Italy	Not identical to *Planaria brocchii* described by Risso 1818, although has certain similar characters in body colouration
Thysanozoon tuberculatum Delle Chiaje, 1828	Elliptical Dorsal: long tentacles, median papillae with conical tip	Dorsal: Blackish Papillae: median with white tip and bluish at the base	-	Found among algae	-
Planaria verrucosa Delle chiaje, 1829	Dorsal: papillate; more on back and smaller towards margin	Dorsal Margin: milky white and black	-	Naples, Italy	differs from *P. tuberculata* being small, colourful margin without papillae and papillae bears white dots at the tip
Stylochus papillosus Diesing, 1836	½ - 1-1/2 in. narrows ovate flattened Tentacles: filament like, medially raised (keel on the middle) Papillae: numerous papillae present on the keel area	Dorsal: light yellow or reddish-brown keel: white	Indefinite number on the disc shape bright spot	Adriatic Sea	-
Thysanozoon diesingii Grube, 1840	flat, leaf like Dorsal: covered with papillae (pedicels)	Dorsal: longitudinal bright yellowish line Margin: white with purple dash lines	Eye spots 31–28	Palermo-Italy east coast of Ceylon (Sri-Lanka) Indian Ocean	compared with Mediterranean *P. brochii* and *P. tuberculata* and mentioned the similarity with *P. brochii* except for the absence of papillae opening
Planaria dicquemari Delle Chiaje, 1841	Papillae: larger in the middle and smaller towards margin	Dorsal: Yellow, blackish speckled Margin: whitish	In two groups at the base of the tentacles		differs from *P. tuberculata* (Delle Chiaje, 1828) in terms of size, papillae and colour
Thysanozoon diequemaris (Delle Chiaje, 1841) Diesing, 1850	Elliptical, Papillae: long, conical, tentacles somewhat sickle shape	Dorsal: Body greyish Papillae: yellowish white Ventral: Grey	-	In stones- Mediterranean Sea	-
Eolidiceros brochii Quatrafages, 1845	regularly elliptical, slightly curved in the middle Tentacles: triangular, Papillae	Dorsal: light yellowish brown, edges are transparent and bluish Upper side of the head region is white; tinted	Numerous CE: 20–25 TE: group of 5–6 eyespots between the tentacles	In *Fucus* Naples	May be similar to *Planaria tuberculate* (Delle Chiaje, 1828)

Table 1 Review of the characters from synonymized taxa of *Thysanozoon brocchii (Continued)*

	(appendages): fusiform, longer and bigger and smaller towards edges Size: 18 mm	with greenish, sides are light brown Papillae: reddish-brown but tip is yellowish white; blackish purple dots all over the surface			
Eolidiceros panormis Quatrefage, 1845	elliptical, triangular head and straight large tentacles Dorsal: papillate (cylindrical appendages) Size: 6 mm × 3 mm	Dorsal: mid region yellow-greenish speckled with brown, head region white, anterior end with purple and brown pigments, transverse pale yellow band present 1/3 posterior of the body Papillae: similar colour like body but less numerous than *E. brocchii* Margin: brown speckled and purple dashes	Dorsal: 3 large eyespots surrounded by smaller ones; one is posterior and two are at the base of the pseudotentacles Ventral: arranged between and at the base of the pseudotentacles	on rock Palermo, Italy	Compared with *E. brocchii* for eyespots, body shape, colour pattern and arrangement of dorsal papillae
Thysanozoon fockei Diesing, 1850	Sub elliptical Dorsal: papillated	Papillae: yellow-purple papillae	-	Tergesti, Italy	-
Planeolis panormus, Stimpson 1857	Papillae: scattered over the body; head larger and with large tentacles	-	ocelli on the tentacles at the base of the tentacles and between the tentacles	-	information about genus is given but refers to *Eolidiceros panormus* Quatrefage, 1845 for the species description
Thysanozoon sp. (*Eolidiceros quatrefages*) Moseley, 1877	Size: when expanded 10 cm × 6 cm	Dorsal: dark purple, tubercles with white tip	-	Zamboangan, Philippines	
Thysanozoon brochii var. *cruciatum* Laidlaw, 1906	Size: 8–16 mm	Dorsal: yellow-dark gray, narrow longitudinal white stripe and 1/3 posterior transverse band present	-	under rock Porto Praya, Cape Verde Island	-
Thysanozoon lagidium Marcus, 1949	Dorsal: papillate Size: 13x7mm	Dorsal: Grayish brown-purplish Papillae: darker, few with white tip light coloured papillae forming longitudinal stripe and medial transverse stripe present	-	Brazil	-
Thysazonoon cf. *lagidium* Quiroga et al. 2004 Based on image presented in the paper	-	Dorsal Papillae: black- brown midline papillae whitish; whitish papillae forming cross are present around 1/3rd of body length		Coloumbia; Brazil	-

Table 2 Review of the characters noted from older literature of *Thysanozoon brocchii*

Author	Body form	Colour -Pattern	Eye spots/arrangement	Habitat/Locality	Remark
Tergipes brocchii Risso, 1818	Oval, oblong, tubercles on the dorsal surface; tentacles ear shaped	Dorsal: violet brown; Papillae: white dots at the end of the tubercles Ventral: transparent	small, black	Under stones Nice sea, France and Naples, Italy	Original description
Quatrefages, 1845	Elliptical, raised medially Papillae: dense in the middle surface and scarce towards margin longitudinal mid-line papillae are fusiform and longer; small tubers towards margin Size: 16 mm × 8-9 mm	Dorsal: yellowish-brown reddish; purple pigments Papillae: brown reddish with yellowish white tip Margin: dark brown ends with white line Cerebral area is colourless triangular portion between pseudotentacles	CE: two groups 20–25; 5–6 PE: 5–6 smaller eyes near the edges of the pseudotentacles Ventral- 5-6 eyes placed on each tentacle; line of 7–8 large eyespots	Naples, Italy	-
Diesing, 1850	Elongated plane Papillae: fusiform Tentacles- thick and tuberculate	Dorsal: yellowish Margin: white-blue Papillae: red-brown to black -blue	Beneath, above and at the base of the tentacles	In rocks Nice Sea; Toulouse	-
Lang, 1884	Compressed, broadly oval, oblong Dorsal: papillate (tubers or tubes) Papillae: bulgy at the base and tapers further; small and sparse in the longitudinal and transverse midline Pseudotentacles: Bead shaped, sharp	Dorsal: dark brown-violet with white margin; lighter than the papillae Papillae: whitish spots on the darker papillae, lighter in transverse and longitudinal bands, pore at the tip might be the intestinal diverticulum leading outside Pseudotentacles: Whitish grey or dark brown-black with light colour spots Cerebral area is lighter and clearly demarcated within pseudotentacular area Ventral: dirty gray-blue-brownish or yellowish	CE: Horseshoe shape PE: two roundish groups each on either side of mid-line	shallow water in association with ascidian and sabellids, Naples and coast of Posilipo, Italy	wide variations observed within same individuals for various characters (papillae shape, number, colour and size vary greatly), *Eolidiceros panormus* is young specimen of *T. brochii*; sexual maturation occurs at different size
Yeri and Kaburaki, 1916	Broadly Oval, frilled margin Size: 35 mm × 21 mm Papillae: slender-conical, all over the dorsal surface, longer in the mid region; smaller and sparse towards the margin	Dorsal: purplish gray or yellowish purple, yellowish-whitish; longitudinal median stripe Papillae: dark gray with purple tint, base colourless, few with whitish tip Some specimens with whitish papillae forming transverse line Ventral: Lighter and comparatively darker at the margin	CE: two clusters above the brain in colourless area PE: numerous	Misaki and Matsuwa, Japan	Internal anatomy same as Mediterranean specimens, intestinal branches extending in to the papillae

Table 2 Review of the characters noted from older literature of *Thysanozoon brocchii (Continued)*

Palombi, 1928	Flatten with free wavy margin	Dorsal: surface chestnut brown except for the marginal area, space between the pseudotentacles lighter	CE: two clusters placed centrally within colourless area PE: irregular arrangement ventral: present within each pseudotentacles two groups of eye clusters, further adjoining row of eyes run along the anterior margin	Suez Canal	Specimen observed matches Lang's description of *T. brochii*. *T. dissingii* Grube, 1840 can be considered as *T. brochii*
Pearse, 1938	Dorsal: Papillate Size: 28-33 × 8-12 mm	Dorsal: cream with light yellow reticulum, black pigmentation, median longitudinal dark stripe with light line through it, anterior tentacular region blackish with unpigmented cerebral patch containing eyespots Papillae: light brown Papillae: purplish-brown become light brown towards margin, some with white spots and dark tips and forms 'T' Margin: brown-purple redial bands	-	Eel grass, Crooked Island, near Florida	Three colour morphs were collected, immature specimens
Palombi, 1939	-	-	-	Shelley beach (East London) South Africa	compare with Palombi, 1938 and Kato's Japanese specimen and mentioned cosmopolitan distribution
Kato, 1944	Size: 50–60 mm	Dorsal: Brown colour Papillae: brown with whitish yellow especially at median line and spread otherwise Margin: bluish	-	Misaki, Susaki near Simoda, Sugasima, Sima, Seto- Japan	gives cross section of male reproductive system and papillae showing intestinal branch
Hyman, 1952	-	Dorsal: purplish maroon Papillae: brown some with white spots Margin: white spots	-	Under stones county causeway/ Biscayne Bay; Florida	immature specimen thus not sectioned for reproductive histology however, intestinal branches going to papillae is observed uncertainty about the identification
Marcus & Marcus, 1968	Dorsal: Papillate, scares towards margin Size: 15 mm × 5 mm and 24 mm × 16 mm	Dorsal: papillae are darker and become lighter towards the margin, white spots on the papillae	-	Algae and mangroves from Piscadera bay, Curacao and Florida	Broader cement pouches resulting in complex female antrum can be considered as usual intraspecific variation thus *T. lagidium* can be merged with *T. brocchii*. Review of Lang,1884; Marcus, 1949 and Pearse, 1938 and Hyman, 1952 descriptions were made

Table 2 Review of the characters noted from older literature of *Thysanozoon brocchii (Continued)*

Vera et al. 2008	elongated	Dorsal: darker to light brown ground colour, mid-dorsal longitudinal line and perpendicular white line forming cross	-	Canary Islands (Eastern Atlantic Ocean)	active swimmer compared with Quiroga et al. 2004 *Thysanozoon* cf. *lagidum* from Colombia
Brusa et al. 2009	Oval, papillate, slightly undulated margins Papillae: Mid-line papillae are longer and decreases towards the periphery Pseudotentacles: pointed ear like Size: 25 mm × 15 mm	Dorsal: yellowish brown, margin with discontinuous black line Pseudotentacles: dark brown with white tip	CE: Horseshoe shape PE: four dorsal and two ventral clusters	Puerto Pirámides and Puerto Madryn, Argentina	Followed the Marcus and Marcus, 1968 and considered the *T. lagidium* as synonym of *T. brocchii*
Bulnes et al. 2011	Oval Pseudotentacles: enlarged distally and no pigmentation Papillae: big bulky conical, longer and dense around dorsal bulge; declines towards margin (length and number); no papillae on the margin Size: 26 mm × 15 mm	Dorsal: overall light brown but yellowish brown in the central became transparent towards margin; round black spots over the surface especially towards margin	CE: two separated triangular clusters \TE: numerous single row dorsally Ventral: scattered	In mussel and ascidians community Mar del Plata harbour, Argentina	Hypothesised papillae on surface for gas exchange and digestion as also mentioned by Prodhoe.
Bahia et al., 2012	Oval, elongated Size: 12-17 mm × 10-14 mm Papillae: dorsal surface with smaller towards the margin Pseudotentacles: ear like	Dorsal: Whitish with dark brown to yellow brown pigmentation median longitudinal whitish line, some specimens with transverse line also forming cross Some papillae with white spot Ventral: white	CE- Horseshoe shape, 30–50 eyespots PE- four dorsal(20–25) and two ventral clusters (65–70)	rocky and reef-flat areas Santa Rita and Búzios, Brazil	Differ in eye spots arrangement with Palombi, 1928; Considered the presence of complex female antrum as intraspecific variation; Emphasis on the revision of species considering the varied colour pattern and its cosmopolitan occurrence
Noreña et al. 2014	oval, oblong Papillae: acorn-like	Colour photo reference is also available		Atlantic coast of the Iberian Peninsula	
Bahia et al. 2014	Papillae: size decrease towards margin Size: 25 × 19 mm to 7 × 6 mm	Dorsal: brown to yellowish brown with longitudinal median line cream coloured, cream transversal line, some papillae with white spots	-	Praia das Conchas, Cabo Frio, Brazil	-

considered *T. lagidium* as a separate species. Two recent findings (Brusa et al., 2009 & Bahia et al., 2012, 2014) from Argentina and Brazil respectively show absence of purple pigments mentioned in most of the older records. Current specimens (First morph) found intermediate of all the older descriptions, but shows approximate resemblance with Bahia et al., 2012, 2014 in terms of colour-pattern and eyespots arrangement. Second morph shows close similarity with Pearse (1938) (brown morph) Quiroga et al., 2004 and Brusa et al., 2009.

Overall, compilation and comparison certainly represents the combinations of colour patterns exist between the *T. brocchii* species complex (Table 3). Perhaps these combinations are accountable for the current taxonomic confusion. Nonetheless, several currently synonymized species have also been synonymized previously and their progress (from genera - *Tergipes*, *Eolidiceros* or *Planeolis*) towards the genus *Thysanozoon* (after 1840) is clearly evident as one approaches from Risso (1818) to Marcus and Marcus (1968). But, several older descriptions are not even and do not cover all the criteria which can be commonly used to compare species within the complex. Faubel (1984) while revising polyclad systematics includes twenty such species as synonyms of *T. brocchii*. Prudhoe (1985) proposed the *T. lagidium* as different species based on the transfer line forming 'T' shape colour pattern of papillae. However, with reference to the information presented in the Table1 few more species exist with similar character and not found stated by Prodhoe (1985). Thus, for a time being *T. lagidium* should be consider as the synonym for *T. brocchii* as mentioned by Faubel (1984).

Currently, this species is known from Naples, Italy (type locality), other parts of the Mediterranean, Algeria, Suez Canal (Palombi, 1928), South and West Africa, Florida, Brazil, Argentina, Borneo, Japan, Vietnam, New Zealand (Prudhoe, 1989) and with this report, extend to the Indian Coast.

Table 3 Colour variation on each part of the colour pattern observed in the literature cited

Major colour and pattern combinations observed	
Dorsal ground colour	whitish; cream; light yellow; brown; light brown; grey; dark gray; blackish; dark purple; chestnut brown; white-yellowish; yellowish brown; yellow-greenish; yellowish-brown-reddish; yellowish purple, reddish-brown; grayish-brown-purple; violet-brown; purplish gray; purplish maroon;
Ground colour-pattern	
Speckled	Black; brown
Pigments	Purple; brown; dark brown to yellow brown
Reticulation	light yellow
Median stripe	dark
Round spots	black
Papillae colour	whitish; dark gray; yellowish-white; yellow-purple, reddish-brown; black-brown; brown reddish; black-blue; purplish-brown, brown; Bluish base
Papillae colour - pattern	
Dots	White; blackish purple
Tip	White; yellowish white
Base	colourless
Tints	purple
Margin Colour	white; Milky white to black; white-purple; white-blue; bluish; dark brown; brown-purple; transparent
Margin colour-pattern	
Band	White; radial
Dash	purple
Speckled	brown
Spots	white
Line	discontinuous black

Discussion

Comparative examinations clearly indicate and support the fact of existence of variable colour morphs of this species. Certainly, usual intraspecific variation and two or more species sharing similar colour patterns are two contended facts that exist, particularly in the pseudocerotid polyclads. Thus, the allocation of all presently noted morphs for *T. brocchii* is only convincing when external colour patterns, characters of reproductive anatomy and molecular framework reveal the similarity. In this context, revision of this species is urgently required. Perhaps fresh collection of specimens from the similar or adjacent places of previously described region can contribute to resolve mystery of colour patterns. Cladistical analysis using external characters and molecular data techniques are beneficial. Bulnes et al., 2011 raised important query about the cosmopolitan distribution and temperature as limiting factor in polyclad species distribution. Thus, ecological data inferring the habitat and food preference should be encouraged.

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Authors' contributions

RP first collected, identified and reported species. RP drafted the manuscript. DA guided and provided funds required for the study and carried out final editing of the manuscript. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

Ang HP, Newman LJ. Warning colouration in pseudocerotid flatworms (Platyhelminthes, Polycladida), a preliminary study. Hydrobiologia. 1998;383: 29–33.

Apte D, Pitale R. New records of Polyclad flatworms (Platyhelminthes: Turbellaria) from coral reefs of Lakshadweep Island, India. J Bombay Nat Hist Soc. 2011;108(2):109–13.

Bahia J, Padula V, Delgado M. Five new records and morphological data of polyclad species (Platyhelminthes: Turbellaria) from Rio Grande do Norte, northeastern Brazil. Zootaxa. 2012;3170:31–44.

Bahia J, Padula V, Lavrado HP, Quiroga S. Taxonomy of Cotylea (Platyhelminthes: Polycladida) from Cabo Frio, southeastern Brazil, with the description of a new species. Zootaxa. 2014;3873(5):495–525.

Brusa F, Damborenea C, Quiroga S. First records of Pseudocerotidae (Platyhelminthes: Polycladida: Cotylea) from Patagonia, Argentina. Zootaxa. 2009;2283:51–9.

Bulnes VN, Albano MJ, Obenat SM, Cazzaniga NJ. Three pseudocerotid (Platyhelminthes, Polycladida, Cotylea) species from the Argentinian coast. Zootaxa. 2011;2990:30–44.

Delle CS. Descrizione e notomia degli animali invertebrati della Sicilia citeriore osservati vivi negli anni. Naples: Batteli & Co.; 1841. p. 1–8.

Delle Chiaje S. (1822–1829) Memorie sulla storia e notomia degli animali senza vertebre dell regno di Napoli. Napoli 1822–1829. [the atlas with 109 pl. appeared 1822; the 4 vols of text appeared as Vol 2; 1828 Vol 3; 1830 117–214. Fratelli Fernand.

Diesing CM. Helminthologische Beiträge. Nova Acta Acad Leopoldina. 1836;18:316.

Diesing C. M. (1850) Systema Helminthum. I. *Vindobonae*, 679.

Diesing K. Revision Der Turebellarien. IN COMMISSION BEI KARI, GEROLD'S SOHN, BÜCHHÄNDLER DER KAISERL. AKADEMIE DER WISSENSCHAFTEN. 1862;2:485–578.

Faubel A. The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea. Mitteilungen des Hamburgischenzoologischen Museums und Instituts. 1984;81:89–259.

Grube AE. (1840) Actinien, Echinodermen und Wuermer des adriatischen und Mittelmeers, nach eigenen Sammlungen beschreiben. Koenigsberg. 92.

Hyman LH. Further notes of the turbellarian fauna of the Atlantic Coast of the United States. Biol Bull. 1952;103:195–200.

Kato K. Polycladida of Japan. *J Sigenkagaku Kenkyusyo* I. 1944:257–318.

Laidlaw F. The marine Turbellaria, with an account of the anatomy of some species. Fauna Geology Maldive Laccadive Archipelagoes. 1902;1:282–312.

Laidlaw, F. (1906) On the marine fauna of the Cape Verde Islands, from collections made in 1904 by Mr C. Crossland. The polyclad Turbellaria. Proceedings of Zoological Society London, 705–719.

Lang A. Die Polycladen des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. *Fauna Flora des Golfesv*. Neapel, Leipzig. 1884;11:1–688.

Litvaitis M, Bolaños M, Quiroga S. When names are wrong and colours deceive: unravelling the Pseudoceros Bicolor species complex (Turbellaria: Polycladida). J Nat Hist. 2010;44(13):829–45. doi:10.1080/00222930903537074.

Marcus E. Turbellaria brasileiros (7). Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo Zoologia. 1949;14:7–155.

Marcus E, Marcus E. Polycladida from Curaçao and faunistically related regions. Stud Fauna Curaçao Caribb Isl. 1968;101:1–133.

Moseley HN. On *Stylochus Pelagicus*, an new species of pelagic planarian, with notes on other pelagic species, on the larval forms of Thysanozoon, and of a Gymnosomatous Pteropod. Mciroscopical J. 1877;17:23–32.

Newman LJ, Cannon LRG. Color pattern variation in tropical flatworm, *Pseudoceros* (Platyhelminthes: Polycladida) with description of three new species. Raffles Bull Zool. 1995;43:435–46.

Newman LJ, Cannon LRG. Fabulous flatworms: a guide to marine polyclads. Version 1. Canberra and Melbourne, Australia: ABRS and CSIRO Publishing, CD-ROM; 2005.

Noreña C, Marquina D, Pérez J, Almon B. First records of Cotylea (Polycladida, Platyhelminthes) for the Atlantic coast of the Iberian peninsula. ZooKeys. 2014;404:1–22. doi:10.3897/zookeys.404.7122.

Palombi A. Report on the Turbellaria (Cambridge expedition to the Suez Canal, 1924). Transact Zool Soc London. 1928;22:579–632.

Palombi A. Turbellari del sud Africa. Secondo contributo. Arch Zool Ital. 1938;25:124–49.

Palombi A. Turbellaria del Sud Africa. Policladi di East London. Terzo contributo Arch zool Italiano. 1939;28:123–49.

Pearse A. Polyclads of the east coast of North America. Proc U S Nat Mus. 1938;86:67–98.

Prudhoe S. A monograph on Polyclad Turbellaria. Oxford: Oxford University Press; 1985. p. 259.

Prudhoe S. Polyclad turbellarians recorded from African waters. Bull Br MusnatHist (zool). 1989;55(1):47–96.

Quatrefages De A. Études sur les types inférieurs de l'embranchement des annelés: mémoire sur quelques planairées marines appartenant aux genres Tricelis (Ehr.), Polycelis (Ehr.), Prosthiostomum (Nob.), Proceros (Nob.), Eolidiceros (Nob.), et Stylochus (Ehr). Annales des Sciences Naturelles, (3) Zool. 1845;4:129–84.

Quiroga S, Bolaños M, Litvaitis M. A checklist of polyclad flatworms (Platyhelminthes: Polycladida) from the Caribbean coast of Colombia, South America. Zootaxa. 2004;633:1–12.

Risso A. Mémoire sur quelques Gastéropodes nouveaux Nudibranches et Tectibranches observés dans la mer de Nice. J Physique, Chimie, d'Hist natt et Arts. 1818;87:272–376.

Sreeraj C, Raghunathan C. Pseudocerotid polyclads (Platyhelminthes, Turbellaria, Polycladida) from Andaman and Nicobar Islands, India. Proc Int Acad Ecol Environ Sci. 2013;3(1):36–41.

Stimpson W. Prodromus descriptionis anumalium evertebratorum quea in Expeditione as Oceanum, Pacificum Septentrionalem a Republica Federata missa, Johanne Rodgers Duce, observavit et descripsit. Part I Turbellaria Dendrocoela. Proc Acad Natl Sci Phila. 1857;9:19–31.

Tyler S. (2013) Thysanozoon Grube, 1840. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=142242 on 2013–10-01.

Vera A, Moro L, Bacallado JJ, Hernández F. Contribución al conocimiento de la biodiversidad de políclados (Platyhelminthes, Turbellaria) em las Islas Canarias. Revista de la Academia Canaria de Ciencias. 2008;20(4):45–59.

Yeri M, Kaburaki T. (1916) Description of some Japenese polyclad turbellaria. J Coll Sci Tokyo Imperial Univ. XXXIX, 1–54.