

***Empruthotrema quindecima* sp. n. (Monogenea: Monocotylidae) from the nasal fossae of *Taeniura lymma* (Dasyatididae) from Heron Island and Lizard Island, Great Barrier Reef, Queensland, Australia**

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Abstract. *Empruthotrema quindecima* sp. n. (Monogenea: Monocotylidae) is described from the nasal fossae of the blue-spotted fantail ray *Taeniura lymma* (Forsskål, 1775) collected from the reef flats of Heron Island and Lizard Island located at the southern and northern sections, respectively, of the Great Barrier Reef in Queensland, Australia. *Empruthotrema quindecima* has 15 marginal loculi on the haptor which distinguishes it from the other five members of the genus which have either 13 or 14 marginal loculi. The generic diagnosis of *Empruthotrema* Johnston et Tiegs, 1922 is amended to accommodate the new species, a key to species is provided and the evolution of the different configurations of the haptor loculi within the genus is discussed.

Since *Empruthotrema* was first proposed by Johnston and Tiegs (1922), a total of five species have been described (see Chisholm and Whittington 1999). *Empruthotrema* was distinguished by Chisholm and Whittington (1999) from the other four genera in the Merizocotylinea Johnston et Tiegs, 1922 by the presence of 13 or 14 marginal loculi and by the absence of hamuli. During an on-going study of the monogeneans of elasmobranchs from Australia (1995-present), a new species of *Empruthotrema* was discovered in the nasal fossae of the blue-spotted fantail ray collected both at Heron Island and Lizard Island in Queensland, Australia. The generic diagnosis of *Empruthotrema* is revised to accommodate this new species and a key to species is given. We also discuss how the marginal haptor loculi may have evolved in the new species with respect to the scheme proposed by Chisholm and Whittington (1999) for other species of *Empruthotrema*.

MATERIALS AND METHODS

A single specimen of *Taeniura lymma* (Forsskål, 1775) was collected by spearfishing off Wistari Reef near Heron Island at the southern end of the Great Barrier Reef, Queensland, Australia (23°27'S; 151°55'E) in July 1995. Ten *T. lymma* were caught with a seine net in Shark Bay, Heron Island in between July 1996 and June 1998. One specimen of *T. lymma* was collected by seine net off the beach rock at Trawler Beach and two specimens at Casuarina Beach at Lizard Island on the northern part of the Great Barrier Reef (14°40'S; 145°28'E), in June 1998. The rays were identified from Last and Stevens (1994) and were killed by pithing. The

nasal fossae were excised, put into a Petri dish of fresh filtered seawater, scraped gently using a scalpel blade and the scrapings were examined under a stereomicroscope using transmitted light. Mono-geneans were fixed flattened under coverslip pressure in 10% buffered neutral formalin (BNF) at room temperature. Flattened specimens were either stained with Semichon's carmine or left unstained. All specimens were dehydrated in an ethanol series, cleared in cedarwood oil and mounted in Canada balsam.

Preserved adult specimens were examined using a compound photomicroscope equipped with phase contrast optics and drawings were made with the aid of a drawing tube. Measurements were made using a computerised digitising system similar to that described by Roff and Hopcroft (1986). All measurements are given in micrometres as the mean followed in parentheses by the range and the number of structures measured. Measurements of the male copulatory organ were made from the proximal end, following the curve of the organ, to the distal end. Haptor terminology follows that of Chisholm et al. (1995). Numbering of hooklets follows Llewellyn (1963).

Type material of *Empruthotrema* (see Chisholm and Whittington [1999] for the list of accession numbers) was obtained from the following institutions for comparative purposes: The Natural History Museum BM(NH), Cromwell Road, London, SW7 5BD, UK; The Queensland Museum (QM), P.O. Box 3300, South Brisbane, Queensland 4101, Australia; The United States National Parasite Collection (USNPC), Beltsville, MD 20705, USA. Type material of *E. quindecima* sp. n. is deposited in the QM and at the Institute of Parasitology (IPCR), Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic.

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RESULTS

Merizocotylinae Johnston et Tiegs, 1922

Empruthotrema Johnston et Tiegs, 1922

Amended generic diagnosis. Merizocotylinae (*sensu* Chisholm and Whittington 1999). Haptor with one central, five peripheral and 13, 14 or 15 marginal loculi. Hamuli absent. Eyespots present (*E. dasyatidis* and *E. quindecima*) or absent. Male copulatory organ a sclerotised tube; accessory piece present in *E. tasmaniensis*. Descending limb of oötype present.

Type species: *Empruthotrema raiae* (MacCallum, 1916) Johnston et Tiegs, 1922.

Additional species: *Empruthotrema dasyatidis* Whittington et Kearns, 1992; *Empruthotrema kearni* Whittington, 1990; *Empruthotrema quindecima* sp. n.; *Empruthotrema tasmaniensis* Chisholm et Whittington, 1999; *Empruthotrema torpedinis* Kearns, 1976.

Empruthotrema quindecima sp. n. Figs. 1, 2, 3D

Description. Description and measurements based on 14 flattened specimens. Body (excluding haptor) 660 (499-823, $n = 14$) long, 277 (219-364, $n = 14$) wide at level of testis. Haptor oval, 219 (192-264, $n = 10$) long, 191 (166-209, $n = 10$) wide. Haptor with one central, five peripheral, 15 marginal loculi (Figs. 1, 3D). Hooklets (Fig. 2A), 13 (12-15, $n = 14$) long, distributed in haptor as illustrated (Fig. 1).

Three apertures containing what appears to be needle-like secretion on each side of ventrolateral margin of anterior end (Fig. 1). Glands and ducts associated with these apertures not seen. Anterior glands, ducts and openings containing granular secretion not observed. Pharynx small 64 (52-68, $n = 14$) long, 39 (33-47, $n = 14$) wide; pharyngeal glands prominent. Oesophagus absent. Two pairs of eyespots dorsolateral to pharynx (Fig. 1).

Testis single, indented anteriorly (Fig. 1). Vas deferens arises from left side of testis, runs anteriorly dorsal to left vaginal duct and transverse vitelline duct. Vas deferens inflates to form seminal vesicle, curves to right side of body anterior to ejaculatory bulb. Seminal vesicle runs posteriorly, narrows, enters posterior part of ejaculatory bulb (Fig. 1). Ejaculatory bulb slightly oval, 72 (55-90, $n = 14$) long, 63 (46-78, $n = 14$) wide. Male accessory glands enter posterior portion of ejaculatory bulb. Male copulatory organ slightly curved tube 76 (67-94, $n = 14$) long, tapering distally (Fig. 2B).

Ovary loops right intestinal caecum dorsoventrally, narrows to form oviduct (Fig. 1). Oviduct becomes ovovitelline duct, runs anteriorly to join oötype. Oötype 105 (81-119, $n = 10$) long. Mehlis' glands not seen. Vaginal pores intercaecal opening at level just anterior to common genital pore; vaginal ducts wide, forming V-shape (Fig. 1). Narrow duct from vaginae runs

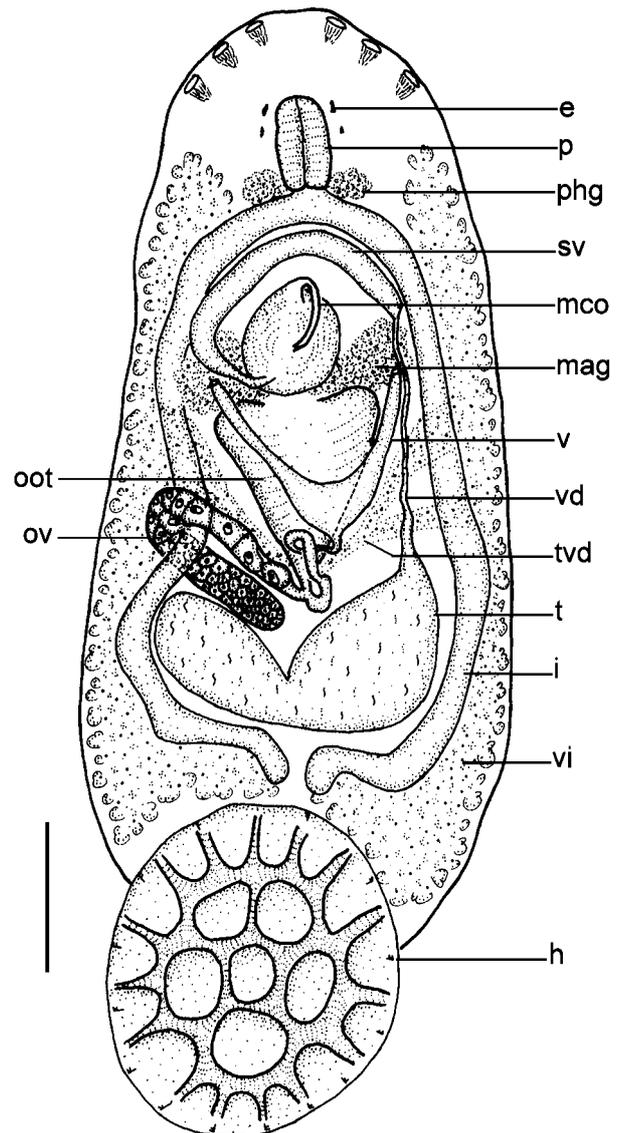


Fig. 1. *Empruthotrema quindecima* sp. n. Full-body drawing, ventral view. Abbreviations: e – eyespots; h – hooklet; i – intestinal caecum; mag – male accessory glands; mco – male copulatory organ; oot – oötype; ov – ovary; p – pharynx; phg – pharyngeal glands; sv – seminal vesicle; t – testis; tvd – transverse vitelline duct; v – vagina; vd – vas deferens; vi – vitellarium. Scale bar = 100µm.

posteriorly, loops once then runs anteriorly to join oviduct. Seminal receptacle not observed. Vitellarium extending from level of mid-part of pharynx to posterior portion of body proper. Transverse vitelline duct at level of anterior portion of ovary; common vitelline duct enters oviduct dorsally. Egg (Fig. 2C) tetrahedral, side 65 (60-72, $n = 3$) (measured within oötype).

Type host: *Taeniura lymma* (Forsskål, 1775).

Type locality: Heron Island, Great Barrier Reef, Queensland, Australia.

Additional locality: Lizard Island, Great Barrier Reef, Queensland, Australia.

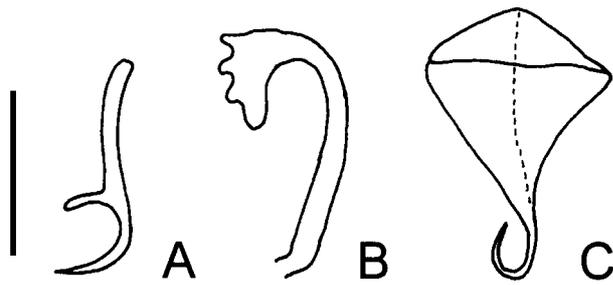


Fig. 2. *Empruthotrema quindecima* sp. n. **A** – Hooklet. **B** – Male copulatory organ. **C** – Egg. Scale bar: A = 10µm; B, C = 50µm.

Site on host: Nasal fossae.

Infection details: Five of 11 rays at Heron Island infected. Three of three rays at Lizard Island infected.

Etymology: From the Latin word *quindecim* meaning 15 which refers to the number of marginal haptor loculi in this species.

Material examined: Holotype QM G216017, 13 paratypes QM G216018-30; 2 paratypes IPCR M-352.

Remarks. There are now six valid species in *Empruthotrema*, including *E. quindecima* sp. n. As noted by Chisholm and Whittington (1999), there are few sclerotised structures that can be used to distinguish between species of *Empruthotrema* and the morphology of the male copulatory organ is relatively uniform across the genus. *Empruthotrema quindecima* has 15 marginal loculi distinguishing it clearly from all of the other species in the genus which have 13 or 14 marginal loculi. *Empruthotrema quindecima* also has two distinct pairs of eyespots; *E. dasyatidis* is the only other species with eyespots.

Key to Species

- 1 Haptor with 15 marginal loculi (Figs. 1, 3D); eyespots present *E. quindecima*
- Haptor with 13 or 14 marginal loculi (Fig. 3A-C); eyespots present or absent 2
- 2 Haptor with 13 marginal loculi (Fig. 3B, 3C) 3
- Haptor with 14 marginal loculi (Fig. 3A); vaginae parallel to transverse vitelline duct *E. raiae*
- 3 Hooklet pairs I and II associated with two posteriormost marginal loculi (Fig. 3C) *E. torpedinis*
- Hooklet pairs I and II associated with single posteriormost marginal loculus (Fig. 3B) 4
- 4 Accessory piece of male copulatory organ present *E. tasmaniensis*
- Accessory piece of male copulatory organ absent ... 5
- 5 Eyespots present in form of dispersed pigment granules; vaginae form U-shape *E. dasyatidis*
- Eyespots absent; vaginae parallel to transverse vitelline duct *E. kearni*

DISCUSSION

Members of *Empruthotrema* have now been recorded from the nasal fossae of elasmobranch hosts representing six different families. *Empruthotrema dasyatidis* has been recorded from the Dasyatidae, Orectolobidae and Triakidae (see Whittington and Kearns 1992, Chisholm and Whittington 1999) and *E. quindecima* is found on a dasyatid (present study). *Empruthotrema kearni* and *E. tasmaniensis* have both been described from the Myliobatidae (see Whittington 1990, Chisholm and Whittington 1999). *Empruthotrema raiae* and *E. torpedinis* are found on the Rajidae and Torpedinidae, respectively (see Kearns 1976, Chisholm and Whittington 1999). Members of the closely related *Merizocotyle* Cerfontaine, 1894 are also found on a wide diversity of hosts (see Chisholm and Whittington 1999).

With the proposal of *E. quindecima*, four distinct haptor types, which we designate here as Types 1-4 (see below), are now known in the genus. All species have one central and five peripheral loculi but the number and/or distribution of the marginal loculi differs. *Empruthotrema raiae* is the only species with 14 marginal loculi (Fig. 3A; Type 1). *Empruthotrema dasyatidis*, *E. kearni*, *E. tasmaniensis* and *E. torpedinis* all have 13 marginal loculi (Fig. 3B, C) but their distribution differs. Chisholm and Whittington (1999) used the location of the hooklets as a guide, and postulated that the two posteriormost marginal loculi seen in *E. raiae* (Fig. 3A) could become fused resulting in a haptor with marginal loculi as seen in *E. dasyatidis*, *E. kearni* and *E. tasmaniensis* (Fig. 3B; Type 2). They further postulated that the presence of 13 marginal loculi in *E. torpedinis* may be a result of the fusion of the anteriormost two loculi (Fig. 3C; Type 3). With the discovery of *E. quindecima*, however, the evolution of the different configurations of the marginal loculi may not be as suggested by Chisholm and Whittington (1999). The assumption inherent in the hypothesis of Chisholm and Whittington (1999) is that the Type 1 haptor of *E. raiae* is plesiomorphic in the genus. This assumption is based on the conclusions of Timofeeva (1985) who postulated, from a functional perspective, that the initial appearance of numerous marginal loculi (as seen in the sister genus *Merizocotyle*) is followed by the progressive reduction of marginal loculi and on the phylogenetic interpretation of Chisholm et al. (1995) that a greater number of marginal loculi is plesiomorphic and a reduction in number is apomorphic in the Merizocotylineae. If indeed greater numbers of marginal loculi are plesiomorphic as hypothesised by Timofeeva (1985) and Chisholm et al. (1995), then the haptor of *E. quindecima* represents the plesiomorphic state and the other haptor types in *Empruthotrema* are the result of the loss or fusion of the loculi in Type 4 (Fig. 3D).

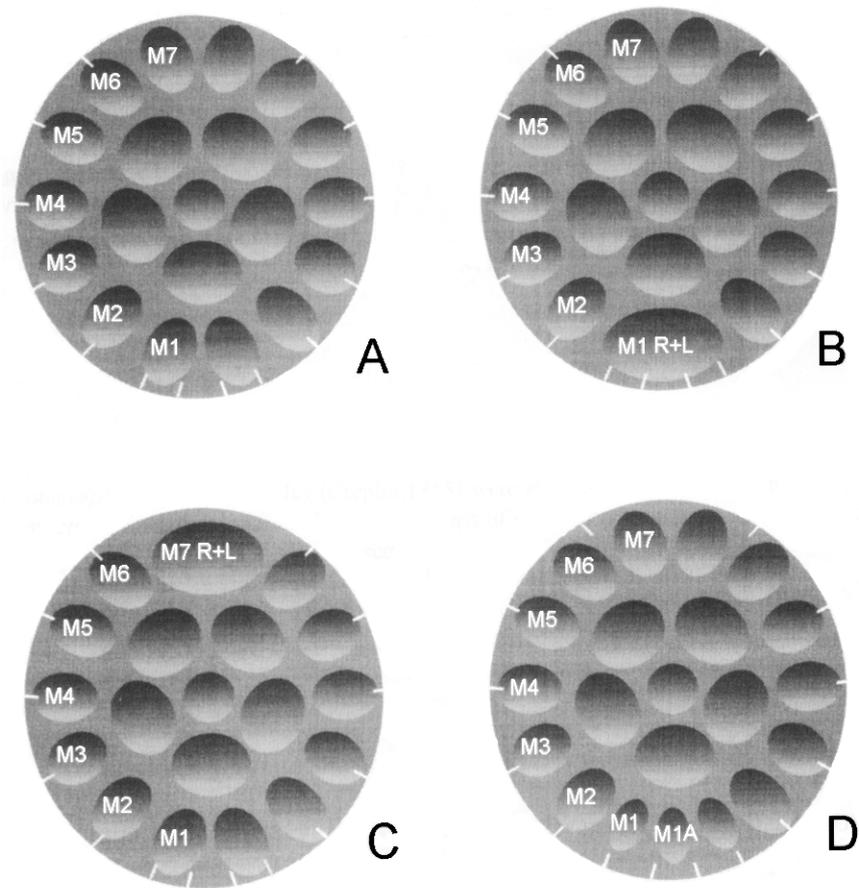


Fig. 3. Diagrammatic representation of the haptors of *Empruthotrema* species. **A** – “Type 1” haptor seen in *E. raiae* with 14 marginal (M) loculi. **B** – “Type 2” haptor seen in *E. dasyatidis*, *E. kearni* and *E. tasmaniensis* with 13 marginal loculi which, according to Chisholm and Whittington (1999), may result from fusion of the right (R) and left (L) posteriormost loculi of the Type 1 haptor. **C** – “Type 3” haptor of *E. torpedinis* also with 13 marginal loculi which, according to Chisholm and Whittington (1999), may result from fusion of the right and left anteriormost loculi of the Type 1 haptor. **D** – “Type 4” haptor seen in *E. quindecima* with 15 marginal loculi; the possible derivation is unclear. Distribution of hooklets indicated by white lines.

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It is with very much regret that we report the death of Professor Bozena Grabda-Kazubska of the W. Stefanski Institute of Parasitology, Polish Academy of Sciences, Warsaw, reviewer for *Folia Parasitologica* for many years and member of the Editorial Board since 1999, who suddenly died on 18 October 1999. She was 63 years old.

We are grateful for all her work. She will be missed.