Morphological characterization of *Podocotyloides parupenei* (Manter, 1963) Pritchard, 1966 (Digenea: Opecoelidae) from *Nemipterus furcosus* (Perciformes: Nemipteridae) of the South China Sea

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Submission data: 20 .10.2024 Acceptance data: 29. 1 .2025 Electronic publishing data: 30.1.2025

Abstract: In the present study, the morphological and morphometric characterization of one species of digenetic trematodes infecting the intestines of the fork-tailed threadfin bream *Nemipterus furcosus* (Perciformes: Nemipteridae) was described by means of light microscopy as a re-description. 93 out of 369 (25.2 %) of this fish species were found to be naturally infected with *Podocotyloides parupenei* (Trematoda: Opecoelidae). This parasite possesses a body that is elongate and widest at the level of the acetabulum, with an oral sucker smaller than the ventral sucker. The prevalence of digenean from the present study did not agree with the previous study. The present paper revealed to the record digenetic parasite, *Podocotyloides parupenei* in the small intestine of the fork-tailed threadfin bream *Nemipterus furcosus* for the first time in Malaysia and the South China Sea.

Key words: Digenea - Opecoelidae- *Podocotyloides* - Light microscopy- *Nemipterus furcosus*- South China Sea.

Introduction

The digenetic trematode helminths are a major group of internal parasitic Platyhelminthes in marine fish [1]. They are one of the most commonly important parasites affecting wild fishes, but uncommon in cultured fishes [2]. Moreover, digenea are mainly parasitic in all freshwater fish. The parasite prevalence and mean intensity are closely associated with several determinant factors, such as parasite species and their life developmental, host and their feeding habits and the physical properties of water body where the fish live. One further factor is the presence of intermediate hosts such as molluscs, and fish-eating birds which help to the onward transmission of parasites infection to other hosts [3]. Fish can be infected with helminth parasites through feeding habits, via an intermediate or a final host. The invasions lead to high mortality when these fish are intermediate hosts for helminth parasites [4]. Both adult and the metacercarial larval stage can be found in the fish host. Very little of adult-stage digenetic trematodes are known to cause considerable damage to the fish host [1]. Fish infection with metacercaria is the major reason of disease, with subsequent economic loss. Metacercariae may affect growth and survival, or disfigure fish so that they lose their market value as a food or ornamental product [5,6]. Some metacercariae in fish are the cause of infections in humans and domestic animals [7,8]. According to previous records by Ngo *et al.*, there are 498 species of parasites were recorded from 225 marine fish species in Vietnam [9]. The most diverse group was the Digenetic trematode, which consider for 43% of the total fish parasite species biodiversity, followed by Monogenea (23.5%), Crustacea (11.6%), Acanthocephala, and Nematoda (8.0% each). In addtion, tow digenean trematodes was described from a Chinese sea snake, Laticauda semifasciata, as the new fauna in the Republic of Korea [10].

Podocotyloides is a genus of digenetic trematode in the family Opecoelidae (Ozaki, 1925). This genus was erected by Yamaguti for *Podocotyloides petalophallus*, a species resembling those in *Podocotyle* Dujardin, 1845, from the reef fish *Plectorhinchus pictus* in the Inland Sea, Japan [11]. On the other hand, *Podocotyloides* (Yamaguti, 1934) has been considered a junior synonym of *Podocotyle* by Park [12]. In addition, the key generic features for genera *Podocotyloides* are the elongate body and without ecsoma, widest at level of acetabulum, rounded to tapering posteriorly, bulging around the ventral sucker; tegument unarmed; acetabulum bigger than an oral sucker, and pedunculate to surmounted by puckered margin of peduncle, in anterior quarter of body; genital pore on left body margin at level of posterior part of pharynx; and vitellaria fields extend from close to posterior extremity to level of posterior part of acetabulum [11]. This paper is one of the first studies to provide data on the helminths and their infection indices in fish *Nemipterus furcosus* from the Terengganu coastal water (in Malaysia) which facing the South China Sea. The specific objective of the study are to study the morphological description of one digenean trematode in one fish species from the Terengganu coastal water.

Materials and methods

Host fish and digenetic trematode collection:

The collected fish, *N. furcosus* obtained from Terengganu-Malaysia coast bordering the South China Sea, have been regularly examined for a period of one year. A total number of 369 fish samples collected using gillnets from the following sites around Terengganu City: (05° 39.7' N, 103° 07.2' E), (05° 38.3' N, 103° 08.3' E), (05° 33.8' N, 103° 05.7' E), (05° 31.0' N, 103° 05.5' E), (05° 30.7' N, 103° 04.7' E), (05° 30.3' N, 103° 03.7' E), (05°

29.6' N, 103° 04.4' E) and (05° 31.4' N, 103° 05.7' E). Marine fish were collected live, immediately transferred to parasitology laboratory at the University Malaysia Terengganu. In the laboratory and prior to the examination, the samples were sorted into taxonomic groups and pulled based on the scientific name. All samples of host species were dissected fresh in order to collect live digenean for the morphological study. This is very important given that the digeneans are brittle and can degrade rapidly after death. Thus, the study of live digenean leads to appropriate fixation for subsequent morphological study. The gastrointestinal tract was examined for parasites by the gut-wash approach [13]. The contents of stomach and intestine were placed in separate petri dishes containing a solution of approximately 0.85% NaCl (Normal Saline) [13]. These contents were examined by a dissecting microscope. Digenetic trematode helminthes were collected live, immediately fixed in saline and then transferred to 70% ethanol.

Morphological samples of digeneans

Preserved digenetic trematode specimens for morphological analysis were placed in alum-carmine for staining. The specimens were overstained and then destained by placing them in acid alcohol solution. Stained digeneans were then dehydrated through a graded series of ethanol (70%, 80%, 90%, 100%) for at least half an hour at each dehydration step cleared in clove oil, and mounted permanently in Canada balsam on microscopic slides [14,15]. A morphological description of the digenean was carried out based on a scheme provided by Jones *et al*., and Manter [16, 20]. Drawings were made with the aid of a drawing tube (camera Lucida) on a Leica microscope (DM750), using a calibrated ocular micrometer which was fitted to the compound microscope. The measurements of the good specimens were made by advanced Nikon microscope (Eclipse 80i) equipped with a digital camera (in micrometers). Metrical data, when incorporated in the descriptions, are given as ranges followed by the mean in parentheses.

Results

Prevalence of digenetic trematode occurrence in *Nemipterus furcosus*:

The current study revealed that one known intestinal digenean (*Podocotyloides parupenei*) from the host fish *N. furcosus* which were collected from Terengganu coastal water. This parasite was registered with low prevalence rate of infection of this marine fish. Out of the 369 examined specimens *of N. furcosus* for parasites, only 93 fish were found infected (25.2%).

Taxonomy of digenean from *Nemipterus furcosus*:

Family: Family Opecoelidae (Ozaki, 1925)

Genus: *Podocotyloides* (Yamaguti , 1934)

Species: *Podocotyloides parupenei* (Manter, 1963; Pritchard, 1966) (Fig. 1, 2).

Description of *Podocotyloides parupenei* (Manter, 1963) Pritchard, 1966

Morphologically, the description of digenean under this work agreed in many respects, such as the shape of general body and suckers; location of genital pore, testes, ovary, vitellaria; the absence of an ecsoma, preoral lobe and plications, and with *Podocotyloides parupenei*. Metrical data, when incorporated in the descriptions, was given as ranges followed by the mean in parentheses.

Measurements based on 10 Whole mounted of mature specimens from one host. Body elongate, widest at level of acetabulum, rounded to tapering posteriorly, bulging around the ventral sucker, tegument unarmed, longer than wide 1046 – 1842 (1487) µm in length, 202 – 356 (281) µm in width at level of ovary; forebody 151 – 518 (403) µm in length; oral sucker subterminal, spherical to sub-spherical, smaller than acetabulum 70 – 107 (84) µm in length, 64 – 125 (91) µm in width; prepharhynx very not seen; pharynx well developed, subspherical, short 40 – 105 (66) µm in length, 34 – 77 (62) µm in width; oesophagus short 13 – 75 (41) µm in length, 12 – 39 (30) µm in width; caeca long, double, narrow, terminate blindly nearly at posterior extremity; intestinal bifurcation forebody near level of anterior edge of acetabulum, a intestinal bifurcation to ventral sucker 26 – 118 (53) µm in length; mouth subterminal; ventral subspherical, sucker pedunculate to surmounted by puckered margin of peduncle, in anterior quarter of body, larger than oral sucker, 127 – 200 (165) µm in length 112 – 228 (176) µm in width; gonads separated; testes double, ovoid, tandem, post-ovarian, separated and well separated from posterior extremity, in mid-hindbody, anterior testis 100 – 193 (153) µm in length, 114 – 200 (160) µm

in width; posterior testis 125 – 214 (165) µm in length, 112 – 216 (157) µm in width; post-testicular region 253 – 638 (424) µm in length; ovary entire, pretesticular, on the right of median line, between uterus and anterior testis 104 – 134 (93) µm in length, 73 – 160 (98) µm in width; uterus restricted to area between ovary and acetabulum in anterior hindbody; post-uterine region 634 – 1148 (875) µm in length; cirrus-sac long, narrow, not highly developed, extending backward beyond acetabulum from just within hindbody to level of posterior oesophagus 179 – 320 (218) µm in length, 40 – 90 (67) µm in width; seminal vesicle long, narrow 169 – 304 (208) µm in length, 28 – 72 (49) µm in width; pars prostatica feebly developed, narrow; ejaculatory duct long; genital atrium small; genital por on left body margin at level of posterior part of pharynx; anterior extremity to genital pore 107 – 192 (150) µm in length; receptaculum seminis "seminal receptacle canalicular" dorsal to ovary; Mehlis' gland distinct; Laurer's canal present; excretory vesicle I-shaped, extending beyond ovary; excretory pore terminal at posterior end of body; vitellaria fields extend from close to posterior extremity to level of posterior part of acetabulum 17 – 45 (30) µm in diameter; anterior extremity to vitellarium 244 – 498 (359) µm in length; posterior extremity to vitellarium 0 – 38 (14) µm in length; eggs comparatively few, tanned, operculate, medium in size 36 – 50 (41) µm in length, 19 – 25 (21) µm in width (Fig. 1, 2).

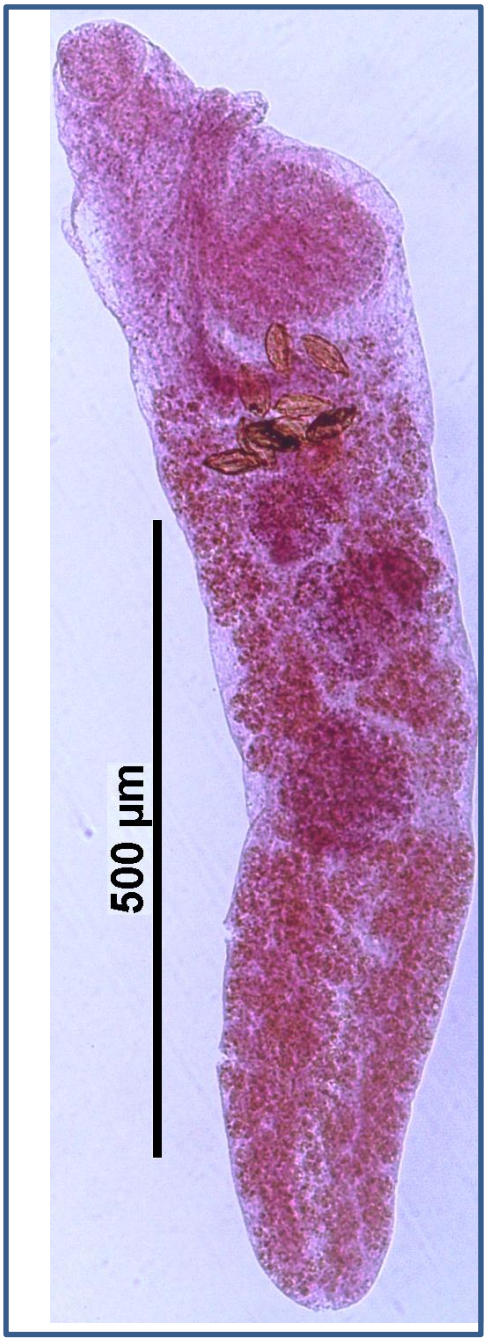

Figure 1: *Podocotyloides parupenei* (Manter, 1963; Pritchard, 1966) ex *Nemipterus furcosus*. Specimen stained with alum-carmine and photographed by advanced microscope. All scale bars are in micrometres.

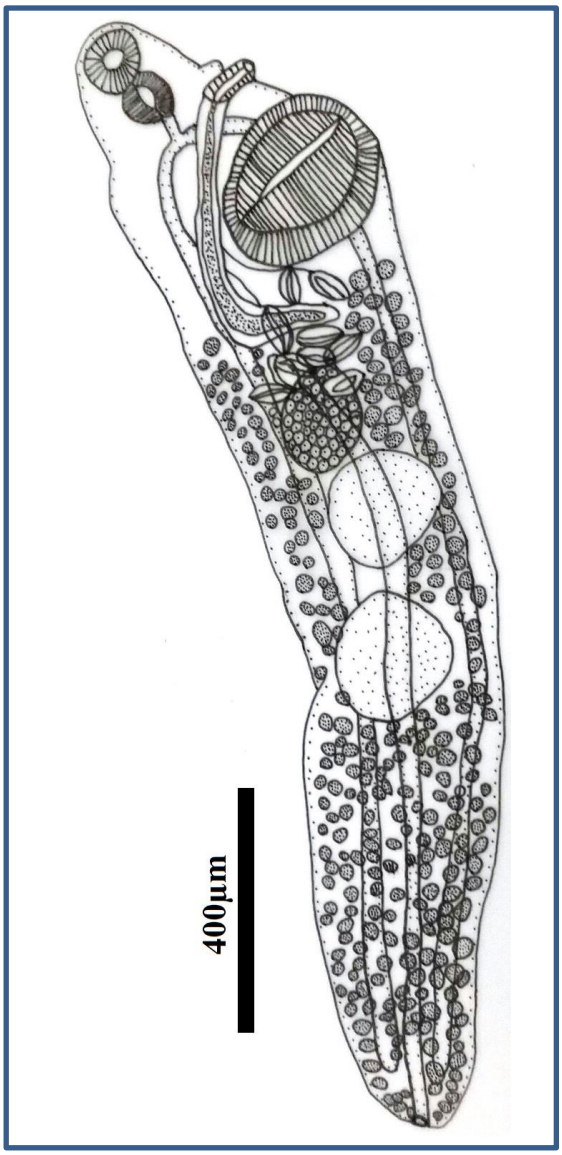

Figure 2: *Podocotyloides parupenei* (Manter, 1963; Pritchard, 1966) ex *Nemipterus furcosus*. Whole-mount ventral view drawn by camera lucida. All scale bars are in micrometres.

Discussion

The genus was established to include those species of *Podocotyloides* which are possess a elongated body and without ecsoma, acetabulum pedunculate to surmounted by puckered margin of peduncle and vitellaria fields extend from close to posterior extremity to level of posterior part of acetabulum [11]. The genital opening was located on left body margin at level of posterior part of pharynx. Since that time the genus has generally been recognised and many additional species have been added to it, mostly by discover a new species. According to previous study *Podocotyle* Dujardin, 1845 at face value appear to be synonyms of *Podocotyloides,* and a junior synonym of *Pedunculacetabulum* [17]. Bray and Cribb (1989) recognized *Podocotyloides* Yamaguti, 1934 for marine species with vitellarium confined to the hindbody [18]. Aken'Ova widened the generic concept to cover *Pedunculatrema*, and *Podocotyloides*-like species with vitellarium extension into the forebody [19]. Andres & Overstreet described *Podocotyloides brevis* as new species from marine fish (*Conger esculentus* Poey, 1861) with vitelline follicles restricted to the hindbody [20]. This digenetic trematode can be easily differentiated from the other species of *Podocotyloides* Yamaguti,1934 by having a cirrus sac less than 10% of the body length. Of the other 13 recognized members of *Podocotyloides*, *Podocotyloides dorabus* Lokhande, 1990, also has a relatively short cirrus sac, although it is slightly longer (approximately 16% of body length), but it can also be separated from *P. brevis* and the other members of *Podocotyloides* Yamaguti,1934 by having a ratio of oral sucker length to ovarian and testicular lengths of 1: <, 1. The species *P. parupenei* (Manter, 1963) Pritchard, 1966 under this study is in close agreement with that of *P. parupenei* as provided by Manter and Pritchard [17, 21], with all of the fundamental anatomical characters being very similar, but can be clearly differentiated depending on morphometric data. Pritchard suggested that *P. parupenei* (Manter, 1963) as same species to his digenean which were described with elongated body, widest at level of acetabulum, rounded to tapering posteriorly, bulging around the ventral sucker, tegument unarmed; subterminal, spherical to sub-spherical oral sucker; prepharhynx very not seen; short pharynx and oesophagus; cirrus sac length longer than 10% of body length; and vitelline follicles interrupted at level of ovary and testes [17]. These characterization are agree in location and somewhat in the shape with the diagnostic character of *P. parupenei* under this study. It is notable that the specimens of this species are different from both digeneans above-mentioned (*P. dorabus* Lokhande, 1990 and *P. brevis* Andres & Overstreet,2013) in all measurements.

The results showed one species of family Opecoelidae Ozaki, 1925, which was reported from different marine fish of the Terengganu coastal water. The species *P. parupenei* (Manter, 1963) Pritchard, 1966 under this study was described from the intestine of *N. furcosus*. This trematodes was reported by Pritchard from a mullid fish in Fiji, while in India it was recorded from the Indian marine fish [16,21]. Approximately, one species of the genus *Podocotyloides* Yamaguti, 1934 is known from the East China Sea. This parasite (*Podocotyloides plageorchis*) was described from

Muraenesox cinereus [23]. The prevalence of digenean reported from the present study did not agreed with the previous study carried out by Miller *et al*., who described a one species of digenean from the same host fish (*N. furcosus*) in off New Caledonia. The present investigation detected the presence of digeneans *P. parupenei* with the prevalence of infection 25.2%, while Miller *et al*., described this species with the prevalence of infection 69% [24]. The parasite (*P. parupenei*) is reported here for the first time from the Malaysian coastal waters of the South China Sea.

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