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Original Article

New locality record of *Monaxinoides austrosinensis* (Mazocraeidea, Monaxinoididae) of finlet crevalle, *Atule mate* (Perciformes: Carangidae) from the Gulf of Thailand

Jittikan Intamong,[a] Smarn Keawviyuth,[a,*] Thanapon Yooyen,[b] Kittichai Chantima[c]

[a] Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[b] Department of Biology, Faculty of Science and Technology, Thaksin University Patthalung Campus, Patthalung 93110, Thailand
[c] Environmental Science Program, Faculty of Science and Technology, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

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ABSTRACT

Out of 203 *Atule mate* fish specimens examined, only 23 had parasitic monogeneans, *Monaxinoides austrosinensis* from the Gulf of Thailand. The prevalence and intensity of infection were 12.81% and 1.27%, respectively. Morphologically, the leaf-like body of *M. austrosinensis* was 5.88–8.07 mm long and 1.84–3.34 mm wide with a fan–shaped opisthohaptor at the posterior end. Numerous pores on the body tegument were observed using scanning electron microscopy. The muscular structure around the vaginal pore presented a number of sensory papillae. The opisthohaptor was one row of clamps which appeared similar in size. The presence of *M. austrosinensis* in this study is a new locality record in Thailand and is the first description based on scanning electron microscopy.

Introduction

Monogenetic trematodes are mostly ectoparasites that mainly parasitize gill fish species and are more widely distributed in tropical than in moderate regions (Ramasamy et al., 1995). About 3500 described species have been reported from marine fish (Rohde, 2005). They cause severe destruction of the gills and lead to serious problems with a marked pathogenicity (Okamoto, 1963; Morsy et al., 2012). The Polyopisthocotylea is a large group of monogeneans that have a cosmopolitan distribution in marine ecosystems, where marine opisthocotyleans feed on the blood of fish hosts and can induce anemia by heavy infestation of worms (Hayward, 2005). They have well developed attachment structures with hard structures in the form of hooks, anchors or clamps (Halton, 1974; Monteroa et al., 2004; Rohde, 2005).

Scanning electron microscopy (SEM) is a tool that provides three-dimensional images with high magnification that permits an understanding of the spatial relationships among surface structures; in monogeneans, some characteristics such as sensory structures, secretory pores, wide variation in morphological aspects on surface modifications and the appearance of microvillus in different monogenean species may be visible using SEM (Smyth and Halton, 1983). It has also been applied to validate monogenean species and separate species that present as being morphologically identical under light microscopy (Hirschmann, 1983; Gibbons, 1986).

The present study investigated the finlet cravalle, *Atule mate* (Cuvier, 1833) (synonym of *Caranx mate*) as it is an important commercial fish species in Thailand, with several species of monogenea being found in the gills of this fish species such as *Gastrocotyle* sp., *Diclybothrium* sp. and *Leuresticola* sp. caught from the Gulf of Thailand (Premkit, 1989) and *Pseudaxine kurra* from China (Jianying et al., 2003). Ding et al. (2003) reported a new species of *Monaxinoides austrosinensis* from China which had been described previously only using light microscopy. From the literature review, the occurrence of *M. austrosinensis* was the first reported on *A. mate* from the Gulf of Thailand but the SEM description was not reported (Ding et al., 2003). Therefore, this study investigated aims to study the occurrence of this monogenean and to describe it using both light and scanning electron microscopy.

Materials and methods

During 2014, 203 finlet crevalle fish samples were collected from Chonburi province (13°18′14.29″N, 100°54′7.69″E) located in the inner Gulf of Thailand and from Chanthaburi (12°28′54.89″N,

* Corresponding author.
E-mail addresses: i.jittikan@gmail.com (J. Intamong), fscismk@ku.ac.th (S. Keawviyuth).

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102°3′44.43″E) and Trat (12°10′14.86″N, 102°23′33.65″E) provinces located in the eastern Gulf of Thailand. All fresh fish were purchased from local fishers and were kept in an ice box and then transported to the laboratory at the Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand. Fish identification was carried out based on external morphology according to Yoshida et al. (2013). Fish samples were thawed and measured for body weight and standard length or total length before fish dissection. The gills were removed and placed in individual Petri dishes containing normal saline then examined for monogenea under stereomicroscope. The monogenean specimens were counted, recorded and calculated for prevalence and intensity of infection (Gudivada et al., 2012).

For light microscopy (LM), the monogenean samples were preserved in 4% formalin. Some of the fixed and flattened specimens were washed with distilled water to remove excess fixative and then stained with acetocarmine. Dehydration occurred by washing in an ascending alcohol series, clearing in xylene, after which samples were mounted with Canada balsam (Yooyen, 2012). The monogenean specimens were then examined under LM and identified according to the morphological characters described by Yamaguti (1958) and Ding et al. (2003).

For scanning electron microscopy (SEM), some specimens of *M. austrosinensis* were post-fixed in 1% osmium tetroxide (OsO_4) for 1 h, then dehydrated in a grade ethanol series and dried in a critical point drier (Polaron Range CPD7501; Quorum Technologies Ltd.;

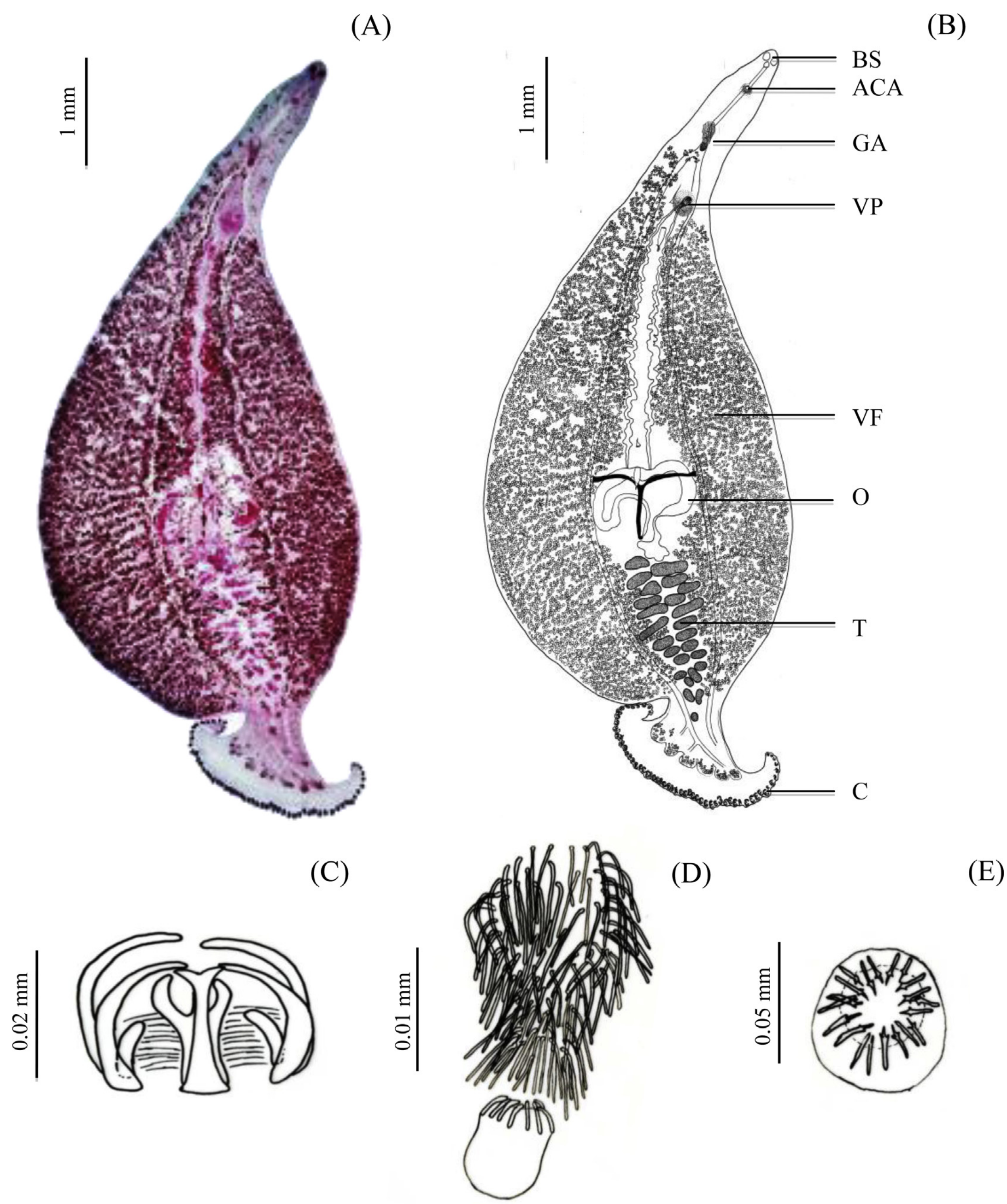

Fig. 1. Light photomicrograph of *Monaxinoides austrasiensis*: (A and B) Whole mount, ACA = accessory copulatory apparatus; BS = buccal suckers; C = clamps; GA = genital atrium; O = ovary; T = testes; VF = vitelline follicles; VP = vaginal pore; (C) clamps, microcotylid type; (D) Genital atrium showing two sets of spines—thick short spines and long spines and muscular sucker; (E) Cupped accessory copulatory apparatus.

Kent, UK), and then were coated with gold in a Sputter Coater (Polaron Range SC7620; Quorum Technologies Ltd.; Kent, UK) and examined using a scanning electron microscope (Quanta 450; FEI Co.; Eindhoven, the Netherlands) operating at 5 keV.

Results

In total, 23 of the 203 *A. mate* samples were infected with *M. austrosinensis*. The prevalence and intensity of infestation were 12.81% and 1.27% parasites per fish, respectively. On the basis of the LM study, the general morphology of *M. austrosinensis* was described ($n = 10$). The body shape was leaf-like with a pointed tapered anterior containing a mouth opening and a posterior end containing haptors. The body length was 5.88–8.07 mm and the maximum width at the level of post-ovary was 1.84–3.34 mm. The head was 1.56–1.89 mm wide with a ventroterminal mouth aperture. Two buccal suckers were elliptical in shape, aseptate and dimensioned 0.06–0.08 mm × 0.05–0.08 mm. The pharynx was oval or elliptical and the esophagus bifurcated immediately behind the genital atrium at 0.77–1.12 mm from anterior end of the body. Caeca had a lot of branching on each side, terminating separately at the posterior end of the body proper. The opisthohaptor was shaped like a fan and fringed with a single row of 53–56 clamps along its semi-circular posterior margin which were 1.01–1.95 mm (Fig. 1A and B). At one end of the opisthohaptor, there were two pairs of terminal anchors. The clamp skeleton was 0.06 mm × 0.04 mm in size, and of the microcotyle type (Fig. 1C).

The testes were obliquely long and irregular in shape, extending from behind the ovary to the near posterior end of the inter-intestinal field, approximately 1.04–1.92 mm in size. The vas deferens was raised with asymmetrical undulation and the cirrus

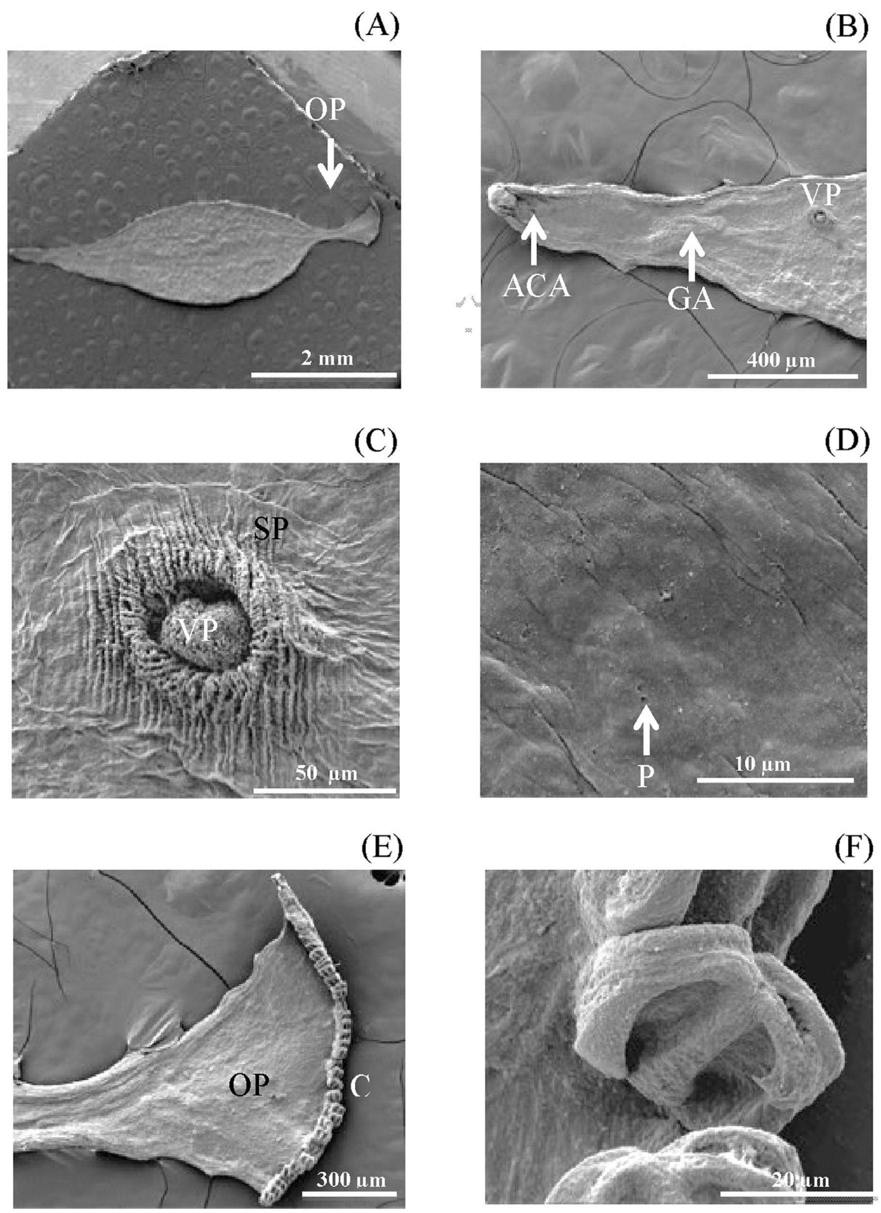

Fig. 2. Scanning electron micrograph of *Monaxinoides austrasiensis*: (A) Whole mount, 23×. PR = prohaptor, OP = opisthohaptor; (B) Anterior region of body proper showing the opening of accessory copulatory apparatus (ACA), position of genital atrium (GA) and vagina pore (VP), 120×; (C) Vaginal pore (VP), showing tegument folds around vaginal pore with numerous sensory papillae (SP), 1000×; (D) Tegument of posterior region of body showing distribution of numerous pores, 3000×; (E) Posterior region of body proper showing an opisthohaptor (OP) with a single row of clamps (C), 150×; (F) Clamps, microcotylid type, 2500×.

opened at the genital atrium at 0.52–0.82 mm from the head end. The genital atrium consisted of two groups of spines—one group consisted of 14–16 short thick spines, each 0.023–0.024 mm long, arranged in a circle and the other group consisted of numerous long spines, each 0.05–0.061 mm long, formed in a vase-shape. At the base of the genital atrium was a muscular atrial sucker, 0.060–0.071 mm in size (Fig. 1B and D). An accessory copulatory apparatus (ACA) was located between the buccal suckers and the genital atrium and 0.28–0.43 mm from the anterior end. The ACA was a muscular cup with 21–24 spanner-like spines, arranged in a complete ring (Fig. 1E). The ovary was an inverted U-shape, 0.19–0.27 × 0.29–0.49 mm in size, situated in the middle of the body proper. The vaginal pore was single, unarmed, with two vaginal ducts and 0.99–1.63 mm from the anterior end of the body. Vitelline follicles were small, co-extensive with the intestine, leaving the beginning of the intestine and posterior end of the main body but not entering the haptor region.

SEM revealed that the body of the presented monogenea was a leaf–like shape, 5.8 mm long and 1.6 mm broad with a tapered anterior prohaptor and a posterior opisthohaptor (Fig. 2A). The ACA was noticed on the ventral surface a short distance from the anterior end while the genital atrium was located above the vaginal pore (Fig. 2B). Numerous sensory papillae were observed on the muscular structure, which surrounded the vaginal pore (Fig. 2C). Several pores presented on the body tegument (Fig. 2D). The opsithohaptor was separated from the body proper in the posterior region with a single row of clamps which appeared similar in size. The tegument of the clamps was similar to the body tegument (Fig. 2E and F).

Discussion

Monogeneans in the genus *Monaxinoides* (Yamaguti, 1963) infesting the gills of carangid fish were reported as *Monaxinoides indica* from *Caranx kalla* (Ramalingam, 1961), *Monaxinoides laminate* from *Caranx affinis* in India (Radha, 1975) and *M. austrosinensis* from *C. mate* in China (Ding et al., 2003) as shown in Table 1. The discovered worm in the present study differed from the earlier species in the same genus by the absence of bifid accessory pieces on the median spring of clamps and short vitellaria. In addition, it resembled *M. austrosinensis* from the gills of *C. mate* in China in general appearance. It presented a single row of clamps, of the microcotyle type with the absence of a bifid accessory piece and had long and numerous spines in the genital atrium. Moreover, the presented species were reported in the same host as *A. mate* (*C. mate*) and found in the South China Sea as was also the case for the Gulf of Thailand (Ding et al., 2003). Therefore, the occurrence of *M. austrosinensis* in the present study was a new locality record from the Gulf of Thailand.

Few data are available on the ecological features of *M. austrosinensis* infecting fish in the wild. The prevalence of infection in the present study was 12.81% (23/203) compared with 25.00% (1/4) from the study of Ding et al. (2003). The heavy infestation of monogenea on fish gills feeds on large amounts of blood, causing anemia, and also marked infiltration of inflammation cells and a reduced respiration rate (Osman, 2001; Kaur and Shrivastav, 2014).

Examination of the tegument of *M. austrosinensis* using SEM in the present study showed numerous pores on the tegument body and the muscular structures around the vaginal pore clarified the morphological features. The pores are characteristic of the tegument of the majority of the species studied, including *Dilcdophora merlangi* (Halton, 1979), *Polystoma integerrimun* (Williams and McKenzie, 1995), *Paranaella luquei* (Cohen et al., 2001) and *Lammelodiscus diplodicus* (Elsayed et al., 2011). The presence of numerous pores on the tegument body is related with the exocrine discharge that occurs by means of these pores and provided evidence of a closed relationship between monogenea and cestodes (Halton, 1978, 1979). Cohen et al. (2001) suggested that the pit-like depressions and several of the pores on the tegument might increase the surface area of the region as well.

The occurrences of a vaginal pore with *M. austrosinensis* corresponded with the description of *Gotocotyle acanthura* presenting wrinkles and a folded surface or muscular structure around the vaginal pore (Pamplona-Basilio et al., 2011). These observations suggested that the well-developed muscular structure might be an accessory structure for holding the pair of worms on the surface of the gills during copulation (Hayward and Rohde, 1999). Moreover, the sensory papillae at the vaginal pore observed herein were similar to those previously recorded from other species, such as

Table 1
Morphometrics of *Monaxinoides austrasiensis* parasitic on *Atule mate* compared with others species (in mm).

Characteristic	*M. indica* (Ramalingam, 1961)	*M. laminate* (Radha, 1975)	*M. austrosinensis* (Ding et al., 2003)	Present study
Host	*Caranx kalla*	*C. affinis*	*C. mate*	*A. mate*
Locality	Manapam, India	Madras, India	Hainan, China	Chonburi and Trat, Thailand
Body length	1.970–5.640	2.220–6.700	4.053–6.690	5.880–8.071
Body width	0.985–2.820	1.100–6.700	1.293–2.885×	1.840–3.340×
Buccal sucker size	0.027 × 0.040	0.028 × 0.022	0.051–0.102 × 0.046–0.079	0.060–0.080 × 0.050–0.080
Pharynx size	0.025 × 0.020	0.025 × 0.022	0.043–0.069 × 0.036–0.064	0.034 × 0.045
ACA				
Spine number	20	30	20–28	21–24
Spine long	0.0180	0.018–0.020	0.020–0.025	0.028–0.043
Genital atrium				
Spine number of long set	Numerous	Numerous	110–130	Numerous
Spine number of short set	12	12	14–16	14–16
Spine long of long set	0.042–0.115	0.064–0.110	0.041–0.066	0.050–0.061
Spine long of short set	0.014–0.019	0.014–0.018	0.023–0.029	0.022–0.024
Testes				
Testes number	35–45	16–24	20–26	21–24
Testes size	0.014 × 0.039	0.048 × 0.084	0.052 × 0.165	0.06–0.15
Testes arrangement	Irregular	2 longitudinal rows	2 or 3 rows	2 or 3 rows
Testes region	–	–	1.096–1.519 × 0.0517–1.095	1.040–1.920 × 1.000–1.500
Clamp				
Clamp size	0.022 × 0.024	0.034 × 0.036	0.025–0.043 × 0.025–0.062	0.040 × 0.060
Clamp number	32–64	64	43–56	53–56

ACA = Accessory copulatory apparatus.

uniciliated structures on *Empleurposoma pyriforme* (Ramasamy and Brennan, 2000) and microvilli and secretory pores on *P. luquei* (Cohen et al., 2001). They suggested these structures acted as sensory structures and varied among different monogenean species. More detail on the sensory structures of related monogenean species should be obtained.

The presented monogenea in the present study belong to the genus *Monaxinoides* and were classified as *M. austrosinensis*, being the first locality record in the Gulf of Thailand. Studies on the seasonal variation and distribution of *M. austrosinensis* on its host are ongoing and will be published soon.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Cohen, S.C., Kohnand, A., Baptista-Farias, M.F.D., 2001. Scanning and transmission electron microscopy of the tegument of *Paranaella luquei* Kohn, Baptista-Farias & Cohen, 2000 (Microcotylidae, Monogenea), parasite of a Brazilian catfish, *Hypostomus regain*. Mem. Inst. Oswaldo. Cruz 96, 555–560.

Ding, X.J., Pan, J., Lin, L., 2003. Monogenea of Chinese marine fishes a new species of *Monaxinoides* from fishes of the South China Sea (Mazocraeidea, Monaxinoididae). Acta. Zootaxon. Sin. 28, 606–609.

Elsayed, M., Bayoumy, P.P., Saye, M.P., 2011. Surface topography and spermiogenesis of *Lamellodiscus diplodicus* (Monogenea, Diplectanidae), a parasite of *Gerres Oyena* [*sic*] (teleostei, Gerridae) from the Suez Gulf, Egypt. Life Sci. J. 8, 467–476.

Gibbons, L., 1986. SEM Guide to the Morphology of Nematode Parasites of Vertebrates. CAB. International, Wallingford, UK, ISBN 085198-569-6.

Gudivada, M., Vankara, A.P., Chikkam, V., 2012. Population dynamic of metazoan parasites of marine threadfin fish, *Eleutheronema tetradactylum* (Shaw, 1804) from Visakhapatnam coast, Bay of Bengal. CIBTech. J. Zoo. 1, 14–32.

Halton, D.W., 1974. Hemoglobin absorption in the gut of a monogenetic trematode, *Diclidophora merlangi*. J. Parasitol. 60, 59–66.

Halton, D.W., 1978. Trans-tegumental absorption of alfaalanine and alfa-leucine by monogenea *Diclidophora merlangi*. J. Parasitol. 76, 29–37.

Halton, D.W., 1979. The surface topography of a monogenean, *Diclidophora merlangi* revealed by scanning electron microscopy. Z. Parasitenkd 61, 1–12.

Hayward, C., 2005. Monogenean Polyopisthocotylea (ectoparasitic flukes). In: Rohde, K. (Ed.), Marine Parasitology. CABI Publishing, Egham, UK, pp. 53–66.

Hayward, C.J., Rohde, K., 1999. Revision of monogenea family Gotocotylidae (Polyopisthocotylea). Invertebr. Taxon. 13, 425–460.

Hirschmann, H., 1983. Scanning electron microscopy as a tool in nematode taxonomy. In: Stone, A., Platt, H., Khalil, L. (Eds.), Concepts in Nematode Systematics. Academic Press, New York, NY, USA, pp. 95–111.

Jianying, Z., Tingbao, Y., Lin, L., Xuejuan, D., 2003. A list of monogeneans from Chinese marine fishes. Syst. Parasitol. 54, 111–130.

Kaur, P., Shrivastav, R., 2014. Histological effect of monogenean parasites on gills of freshwater carps. Eur. J. Biotech. Biosci. 2, 50–53.

Monteroa, E.F., Crespob, S., Padro, F., Gandarad, F., Garcad, A., Ragaa, A.T., 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae). Aquaculture 232, 153–163.

Morsy, K.S., Saady, H., Ghaffar, A.F., Bashter, A.R., Mehlhorn, H., Quraishy, S.A., Adel, A., 2012. A new species of the genus *Heterobothrium* (Monogenea: Diclidophoridae) parasitizing the gills of tiger puffer fish *Tetraodon lineatus* (Tetraodontidae). A light and scanning electron microscopic study. Parasitol. Res. 110, 1119–1124.

Okamoto, T., 1963. On the problems of monogenetic trematode infection of puffer from inland sea of Japan. Suisan. Zoshoku. (Special issue) 3, 17–29.

Osman, H.A.M., 2001. Studies on Parasitic Gill Affections in Some Cultures Freshwater Fishes (M.Sc. Thesis). Faculty of Veterinary Medicine, Suez Canal University, Ismaileya, Egypt.

Pamplona-Basilio, M.C., Barbosa, S.H., Cohen, C.S., 2011. Scanning electron microscopy on *Gotocotyla acanthura* (Monogenea, Gotocotylidae) from *Pomatomus saltatrix* (Ostheichthyes, Pomatomidae) in Brazil. Rev. Bras. Parasitol. Vet. 20, 342–346.

Premkit, W., 1989. The Abundance of Parasites of *Caranx mate* in the Gulf of Thailand. National Research Council of Thailand, Bangkok, Thailand.

Radha, E., 1975. Studies on the monogenean fauna of Madras coast. Riv. Di. Parassitol. 36, 7–27.

Ramalingam, K., 1961. On a new species of the genus *Heteraxine* (Monogenea: Axinidae) from the gills of *Caranx kalla* Cuv. &Val. Ann. Mag. Nat. Hist. 13, 1–5.

Ramasamy, P., Brennan, G.P., 2000. Ultrastructure of the surface structures and haptor of *Empleurosoma pyriforme* (Ancyrocephalinae; Monopisthocotylea: Monogenea) from the gills of the teleost fish *Therapon jarbua*. Parasitol. Res. 86, 129–139.

Ramasamy, P., Brennan, G.P., Halton, D.W., 1995. Ultrastructure of the surface structures of *Allodiscocotyla diacanthi* (Polyopoisthocotyla: Monogenea) from the gills of the marine teleost fish, *Scomberodies tol*. Int. J. Parasitol. 25, 43–54.

Rohde, K., 2005. Marine Parasitology. Desktop Concepts Pty Ltd. Australia, Melbourne.

Smyth, J.D., Halton, D.W., 1983. The Physiology of Trematodes. Cambridge University Press, UK.

Williams, J.B., McKenzie, J., 1995. Scanning electron microscopy of *Polystoma integerrimum* (Monogenea, Polystomatidae). Int. J. Parasitol. 25, 335–342.

Yamaguti, S., 1958. Systema Helminthum. Vol. I. The Digenic Trematode of Vertebrates. Part I, II. Interscience Publisher, Inc, New York, NY, USA.

Yooyen, T., 2012. Diversity of the Helminths in Some Marine Fishes from Hua-hin and Pranburi Districts, Prachaup Khiri Khan Province (Ph.D. Thesis). Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Yoshida, T., Motomura, H., Musikasinthorn, P., Matsuura, K., 2013. Fishes of Northern Gulf of Thailand. National Museum of Nature and Science, Tsukuba. Research Institute for Humanity and Nature, Kyoto, and Kagoshima University, Museum. Kagoshima, Japan.