Two species of philometrid nematodes (Philometridae) from marine fishes off Japan, including *Philometroides branchiostegi* sp. n. from *Branchiostegus japonicus* (Malacanthidae)

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Abstract: Gravid females of two species of philometrid nematodes (Philometridae) were collected from marine perciform fishes in Japanese waters, mainly from the southern Sea of Japan. Based on light microscopy and scanning electron microscopy examinations, the previously described but poorly known species *Philometra cryptocentri* Yamaguti, 1961 is redescribed from specimens recovered from the abdominal cavity of *Acanthogobius flavimanus* (Temminck et Schlegel), *Pterogobius elapoides* (Günther) and *P. zonoleucus* Jordan et Snyder (all Gobiidae) (all new host records); the number (14) and arrangement of cephalic papillae in this species are described for the first time. The new species, *Philometroides branchiostegi* sp. n. from head tissues of *Branchiostegus japonicus* (Houttuyn) (Malacanthidae), based on a single specimen, is mainly characterized by the embossment of the entire body except for the cephalic end, presence of four submedian pairs of large cephalic papillae of external circle and two small lateral single papillae of internal circle, pair of large papilla-like caudal projections, the oesophagus with a distinct anterior inflation, by a markedly small body (length about 18 mm) and the larvae 306–465 µm long.

Keywords: parasitic nematode, *Philometra*, *Philometroides*, marine fish, *Branchiostegus*, *Acanthogobius*, *Pterogobius*, Sea of Japan, Japan

Nematodes of the family Philometridae Baylis et Daubney, 1926 include a large number of species parasitic in the body cavity and various body tissues of fishes (Moravec 2006). To date, 26 species of these parasites have been reported from marine and freshwater fishes in Japanese waters, including representatives of the genera *Clavinema* Yamaguti, 1935, *Philometra* Costa, 1845 and *Philometroides* Yamaguti, 1935 (Ishii 1916a,b, 1931, Yamaguti 1935, 1941, 1961, Fujita 1940, Machida 1970, Moravec and Nagasawa 1989, Moravec et al. 1998, 2002, 2008a, Nagasawa 2008, Quiazon et al. 2008a,b). However, it can be expected that the Japanese fauna of philometrids, particularly of those parasitizing marine fishes, is still little known and most species have not yet been discovered.

During recent investigations into the parasites of some marine fishes from the Sea of Japan, off the coast of Japan, gravid female specimens of philometrid nematodes were collected from the yellow goby *Acanthogobius flavimanus* (Temminck et Schlegel), *Pterogobius elapoides* (Günther) and *Pterogobius zonoleucus* Jordan et Snyder (all Gobiidae, Perciformes), and from the horsehead tile-

fish *Branchiostegus japonicus* (Houttuyn) (Malacanthidae, Perciformes). Taxonomic evaluation of this nematode material is presented in this paper.

Acanthogobius flavimanus (maximum size 30 cm) is a commercial temperate marine, brackish and freshwater fish occurring mainly along the shore of bays and estuaries, distributed in Japan, Korea and Siberia. Two other gobiids, *P. elapoides* (maximum size 8.3 cm) and *P. zonoleucus* (maximum size 6.7 cm), are small temperate marine fishes occurring near rocky shores, distributed in the Northwest Pacific (*P. elapoides* from central Japan and Korea to Hong Kong, *P. zonoleucus* in Japan and the Korean Peninsula). *Branchiostegus japonicus* (maximum size 46 cm) is a commercial tropical marine fish, distributed in the western Pacific from central Honshu to the South China Sea, also reported as far south as the Arafura Sea (Froese and Pauly 2011).

MATERIALS AND METHODS

Four specimens of *Pterogobius elapoides* were sampled during SCUBA diving in the southern Sea of Japan, off Sekumi (35°33'N, 135°50'E), Wakasa, Fukui Prefecture on 7–9 Novem-

ber 2006 and 1 August 2007. Five specimens of *P. zonoleucus* were also collected during SCUBA diving in the southern Sea of Japan, off Tsudo (36°17'N, 133°24'E), Okinoshima, Shimane Prefecture on 14 August and 4 October 2009. Two specimens of *Branchiostegus japonicus* were commercially caught on 28 October 2009 in the southern Sea of Japan, off Hagi (35°03'N, 131°52'E), Yamaguchi Prefecture and these were bought at a fish market in Shimonoseki of the same prefecture next day. The fresh fish were brought to the laboratory, where they were examined. The philometrid from *B. japonicus* was obtained by "head washing", when the head was removed from the fish and put into water in a 500-ml bottle; the bottle was shaken and then detached ectoparasites and the philometrid were found in the water. The nematodes were fixed in hot 70% ethanol. In addition, nematode specimens from *Acanthogobius flavimanus* collected in an unknown locality off the coast of Japan were used. For light microscopical examination (LM), the nematodes were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. Specimens used for scanning electron microscopy (SEM) were transferred to 4% formaldehyde solution and then postfixed in 1% osmium tetroxide, dehydrated through a graded acetone series, critical point dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV GB low modem. All measurements are in micrometres unless otherwise stated. Fish names follow FishBase (Froese and Pauly 2011).

RESULTS

Family Philometridae

Philometra cryptocentri Yamaguti, 1961 Figs. 1, 2

Description of female (4 gravid specimens from *A. flavimanus*; measurements of 4 [1 complete and 3 incomplete] gravid specimens from *P. elapoides* in parentheses and those of 5 gravid specimens from *P. zonaleucus* in square brackets): Body of fixed specimens yellowish to brownish, with finely transversely striated cuticle (Fig. 2 D); body length 81.64–129.61 (140.00) [47.00–88.94] mm, maximum width 802–1,156 (762–1,047) [857–979], its maximum width/body length ratio 1:95–124 (1:134) [1:48–97]; posterior part of body narrower than anterior part. Width of cephalic end 204–244 (218–231) [204–326], of caudal end 231–299 (218–231) [136–231]. Cephalic end rounded, cephalic papillae very small, indistinct when viewed laterally (Fig. 1 A,B). Oral aperture rather small, circular, surrounded by four pairs of submedian cephalic papillae of external circle and six single papillae (two lateral and four submedian) of internal circle; each pair of external papillae consisting of one rounded and one more elongate papilla (Figs. 1 C, 2 A,B). Pair of lateral amphids slightly outlined. Bottom of mouth formed by flat surfaces of three oesophageal sectors. Oesophagus including only slightly outlined anterior inflation 707–1,251 (802–1,224) [680–983] long, representing 0.7–1.4 (0.9) [0.8–1.4]% of body length; anterior inflation 93–122 (82–109) [109–163] long and

99–120 (95) [95–109] wide. Oesophageal gland well developed, opening into oesophagus just posterior to nerve ring; large cell nucleus at about middle of gland (Fig. 1 A). Nerve ring 177–272 (231–272) [163–190] from anterior end of body. Small ventriculus 23–39 (–) [27] long and 51–87 (–) [54] wide (Fig. 1 A). Oesophagus opening into intestine through distinct valve. Intestine brownish, broad at anterior end, ending blindly; its posterior end narrow, attached by long ligament ventrally to body wall near caudal end. Vulva and anus absent. Ovaries long, narrow, reflected, situated near body ends (Fig. 1 E,F). Uterus occupying most space of body, filled with numerous larvae and eggs; anteriorly uterus may extend up to level of anterior oesophageal inflation. Larvae from uterus 414–426 (408–441) [435–525] long, maximum width 15–18 (15–18) [15–18]; length of their oesophagus 126–135 (114–135) [120–147], representing 30–33 (28–31) [26–32]% of body length; sharply pointed tail 114–120 (96–126) [126–156] long, representing 28 (23–29) [27–33]% of body length (Fig. 1 D). Posterior end rounded, without any caudal projections (Figs. 1 G,F, 2 C).

Male: Unknown.

Hosts: Yellowfin goby *Acanthogobius flavimanus*, *Pterogobius elapoides* and *Pterogobius zonaleucus* (all Gobiidae, Perciformes). Originally reported from the gobiid *Myersina filifer* (Valenciennes) (as *Cryptocentrus filifer*) (type host) (Yamaguti 1961).

Site of infection: Abdominal cavity.

Localities: Unknown locality off the coast of Japan for *A. flavimanus* (no data for body length) (collected 7 March 1984); southern Sea of Japan, off Sekumi (35°33'N, 135°50'E), Wakasa, Fukui Prefecture, Japan for *P. elapoides* (body length 72–95 mm) (collected 7–9 November 2006 and 1 August 2007); southern Sea of Japan, off Tsudo (36°17'N, 133°24'E), Okinoshima, Shimane Prefecture, Japan for *P. zonaleucus* (body length 44–54 mm) (collected 4 October 2009).

Prevalence and intensity: In all 5 *P. zonaleucus* examined; 5–6 female specimens per fish. Not determined for nematodes in *A. flavimanus* and *P. elapoides*.

Deposition of specimens: All voucher specimens except one in the National Museum of Nature and Science, Tokyo, Japan (NSMT-As 3692–3696); one voucher specimen from *P. elapoides* in the Helminthological Collection of the Institute of Parasitology, Biology Centre, AS CR, České Budějovice, Czech Republic (Cat. No. N-972).

Remarks. Philometrids exhibit a relatively high degree of host specificity (Rasheed 1963, Ivashkin et al. 1971, Moravec 2006, Quiazon et al. 2008a,b, Moravec and Justine 2009) and individual species are characterized by their location in the host, particularly that of gravid females (Moravec 2004, Moravec et al. 2008b). Moravec et al. (2008b) reported a total of 17 species of *Philometra* the gravid females of which are parasitic in the body cavity of marine and brackish-water fishes, and provided a key to these species. Two additional species,

Fig. 1. *Philometra cryptocentri* Yamaguti, 1961, females from *Pterogobius zonaleucus*. **A** – anterior end of gravid specimen, lateral view; **B**, **C** – cephalic end of gravid specimen, lateral and apical views, respectively; **D** – larva from uterus, lateral view; **E**, **F** – anterior and posterior ends of subgravid specimen, lateral views; **G** – caudal end of subgravid specimen, lateral view.

Philometra gymnothoracis Moravec et de Buron, 2009 and *P. spicarae* Moravec, Gaglio, Giannetto et Marino, 2010, were described since (Moravec and de Buron 2009, Moravec et al. 2010). In contrast to the specimens of the present material, most of these species have gravid females with a distinct anterior inflation and some of them possess caudal projections.

Only four of the above-mentioned species have no or a poorly developed anterior oesophageal inflation: *Philometra cheilopogoni* Mordvinova, 1986, *P. justinei* Moravec, Ternengo et Levron, 2006, *P. pellucida* (Jäger-

skiöld, 1893) and *P. spicarae*. However, the body of their gravid females is markedly longer (196–360 mm), their hosts belong to other fish families (Centranchidae, Exocoetidae, Mullidae and Tetraodontidae) and three of them occur in the Atlantic region and one in the Java Sea.

The only species of *Philometra* so far reported from marine gobiids is *P. cryptocentri* Yamaguti, 1961 described from a single female found in the body cavity of *Myersina filifer* (Valenciennes) (Gobiidae) from the Inland Sea of Japan (Yamaguti 1961). The morphology and measurements of specimens of the present material

Fig. 2. *Philometra cryptocentri* Yamaguti, 1961, scanning electron micrographs of gravid female from *Pterogobius zonaleucus*. **A** – cephalic end, apical view (arrows indicate lateral cephalic papillae of internal circle); **B** – region of mouth, apical view; **C** – posterior end of body, subapical view; **D** – structure of cuticle near posterior end of body. *Abbreviations:* a – amphid; b – pair of submedian cephalic papillae of external circle; c – submedian cephalic papilla in internal circle; e – lateral cephalic papilla of internal circle.

are generally similar to this species, but their anterior oesophageal inflation is less distinct than that illustrated by Yamaguti (1961) and their larvae are longer than reported in the original description. However, the width of the anterior oesophageal inflation given by Yamaguti is smaller (78) than that found in the present specimens (95–120), so that this difference may be ascribed to certain inaccuracy in observations. Probably the same concerns the length of larvae, which were apparently measured by Yamaguti inside the body of the only available female specimen. Taking into account the general morphology and measurements of the present specimens, the family of their hosts, the site of infection in the host and the locality, they are considered to belong to *P. cryptocentri*. The recorded differences in the body lengths of gravid females from different host species seem to be within the limits of intraspecific variability of *P. cryptocentri*.

Unfortunately, the only available specimen presumed to be the type of *P. cryptocentri* is heavily damaged. According to recent information by Dr. T. Iwaki, a slide-

mounted specimen designated as “*Philometra* n. sp.” was found in Yamaguti’s collection, now housed at the Meguro Parasitological Museum, Tokyo. Based on the information on the host and date written on the label on the slide, this specimen (Cat. No. SY57-43) was thought by Dr. Iwaki to be identical to a specimen of *P. cryptocentri*. As Yamaguti did not designate the specimen as holotype in his paper, Dr. Iwaki discovered that slide-mounted specimen as the most possible specimen of *P. cryptocentri* among many nematode specimens.

Yamaguti (1961) reports that the cephalic end of *P. cryptocentri* is without papillae. Of course, he could not observe cephalic papillae of this species in lateral view using light microscopy, because these are very small. The SEM examination shows that the number and distribution of cephalic papillae in *P. cryptocentri* are typical of most congeneric species, i.e., 4 submedian pairs of the external circle and 4 submedian and 2 lateral single papillae of the internal circle are present.

Philometra cryptocentri was not recorded since its original description by Yamaguti (1961). Based principally on biometrical features, Rasheed (1963) considered it a junior synonym of *Philometra inimici* Yamaguti, 1941, but later Moravec (2006) re-established its validity. The present findings of *P. cryptocentri* in *Acanthogobius flavimanus*, *Pterogobius elapoides* and *P. zonoleucus* represent new host records for this nematode species.

Philometra cryptocentri is the second philometrid species recorded from the yellow goby *A. flavimanus*. The other species is *Clavinema mariae* (Layman, 1930), a parasite of subcutaneous tissues mainly of pleuronectiform, scorpaeniform and gobiesociform fishes (see Moravec 2006), which was also reported from *A. flavimanus* in Japan (Sakaguchi and Fukuhara 1975, Moravec et al. 1998).

Philometroides branchiostegi sp. n. Figs. 3, 4

Description of female (1 damaged, gravid specimen, holotype): Body of fixed specimen whitish, comparatively short, somewhat tapering at both ends. Body length 17.69 mm, maximum width 476; maximum width/length ratio of body 1:37. Width of cephalic end 177, of posterior end 163. Almost whole body covered with numerous transparent, circular, irregularly scattered cuticular bosses up to 6–12 high (Figs. 3 E,F, 4 F); embossment starting anteriorly somewhat posterior to level of nerve ring and extending posteriorly to body end (Figs. 3 A,C,D, 4 C–E). Cephalic end rounded. Oral aperture almost circular. Bottom of mouth formed by flat surfaces of three oesophageal sectors (Figs. 3 B, 4 B,C). Cephalic papillae 10 in number, arranged in two circles. Papillae of external circle large, oval-shaped in apical view, arranged in four submedian pairs. Papillae of internal circle very small, represented by two single papillae situated laterally near oral aperture (Figs. 3 B, 4 A,B). Oesophagus muscular, forming distinct bulb at anterior end, with large oesophageal gland extending from level of nerve ring to end of oesophagus, possessing not well visible cell nucleus at its middle (Fig. 3 A); oesophagus including bulb 1.05 mm long, representing 6% of body length; anterior bulb 95 long and 90–122 wide. Ventriculus indistinct. Intestine light-brown, straight, displaced laterally by uterus; anterior end of intestine narrow, posterior end atrophied, forming ligament attached ventrally to body wall near posterior extremity (Fig. 3 D). Posterior end of body narrow, rounded, with two lateral papilla-like caudal projections and several irregularly scattered cuticular bosses (Figs. 3 C,H, 4 D,E). Vulva and vagina absent. Ovaries reflected, situated near anterior and posterior ends of body; anterior ovary reaching anteriorly nearly to level of nerve ring, posterior ovary posteriorly to level of intestinal ligament (Fig. 3 A,D). Uterus occupying most space in body, being filled with larvae and eggs. Larvae (n = 5) slender, 306–465 long, maximum width 15–18; oesophagus 84–147, representing 27–36% of body length; tail sharply pointed, 57–96 long, representing 19–23% of body length (Fig. 3 G).

Male: Unknown.

Type host: Horsehead tilefish *Branchiostegus japonicus* (Malacanthidae, Perciformes) (body length 340 mm).

Site of infection: Unknown (found in head washing).

Type locality: Southern Sea of Japan, off Hagi (35°03'N, 131°52'E), Yamaguchi Prefecture, Japan (collected 28 October 2009).

Prevalence and intensity: In 1 of 2 fish examined; 1 female specimen.

Deposition of type specimen: Holotype (anterior and posterior body ends mounted on SEM stub; rest of body stored in a vial in ethanol) at the Helminthological Collection of the Institute of Parasitology, Biology Centre, AS CR, České Budějovice, Czech Republic (Cat. No. N-971).

Etymology: The specific name of this nematode relates to the genitive form of the generic name of the host.

Remarks. By the general morphology, in particular the presence of numerous irregularly scattered cuticular bosses on the body of gravid female, the specimen of the present material may be assigned to the philometrid genus *Philometroides* (see Rasheed 1963, Moravec 2006). Moravec and Bakenhaster (2010) reported 26 valid species in this genus. Most species of *Philometroides* were described only from females, whereas conspecific males are known for eight species. The majority of species (19) occur in freshwater fishes, whereas seven were described from marine fishes and one from a brackish-water fish.

The following species of *Philometroides* are known as parasites of marine and brackish-water fishes: *P. atropi* (Parukhin, 1966), *P. denticulatus* Rasheed, 1965, *P. grandipapillatus* Moravec et Bakenhaster, 2010, *P. marinus* Moravec et de Buron, 2009, *P. oveni* Parukhin, 1975, *P. paralichthydis* Moravec et de Buron, 2006 and *P. seriola* (Ishii, 1931); except for *P. paralichthydis*, they are all parasites of Perciformes (see Moravec 2006, Moravec and de Buron 2009, Moravec and Bakenhaster 2010). Of them, *P. denticulatus* and *P. seriola*, parasites of the body cavity or musculature of Haemulidae, Sciaenidae or Carangidae in the Indian or North Pacific Oceans, differ markedly from the new species in having much longer gravid females (250–320 and up to 600, respectively, vs. about 18 mm); the former species also by the presence of three oesophageal teeth protruding out of the mouth aperture, whereas the latter by the embossment of cuticle extending up to the anterior extremity (Moravec et al. 1998).

In contrast to *P. branchiostegi* sp. n., the gravid female of *P. marinus*, a parasite of the body cavity of Rachycentridae in the northern West Atlantic, is much larger (550 vs. about 18 mm long) and it is noted for the presence of four large dome-shaped projections, large caudal projections and the cuticular embossment restricted to the anterior part of body (Moravec and de Buron 2009). *Philometroides atropi* from the abdominal cavity of Carangidae in the South China Sea, Gulf of Tonkin (Vietnam) and the Red Sea differs in the absence of cuticular bosses on the anterior (oesophageal) part of body and in the shape

Fig. 3. *Philometroides branchiostegi* sp. n., gravid female. **A** – anterior end of body, lateral view; **B** – cephalic end, apical view; **C** – caudal end, lateral view; **D** – posterior end of body, lateral view; **E**, **F** – cuticular boss, lateral and apical views, respectively; **G** – larva from uterus, lateral view; **H** – caudal end, apical view.

of the cephalic region (markedly protruding out from the remaining part of body), whereas *P. oveni*, a parasite of the oculo-orbit and eye of Serranidae in the Mediterranean Sea, mainly in the absence of cuticular bosses on the caudal end (Parukhin 1966, 1975).

Both *P. grandipapillatus* and *P. paralichthydis*, parasites of the subcutaneous tissues of Carangidae and Paralichthyidae, respectively, in the region of the northern West Atlantic, are easily distinguishable from the new species by the presence of transversely elongate (oval) (vs. circular) cuticular bosses, the absence of caudal projections and by the character of external cephalic papillae (Moravec and de Buron 2006, Moravec and Bakenhaster 2010). The distinctions between *P. branchiostegi*

and congeneric species parasitizing freshwater fishes are evident from the key to *Philometroides* spp. provided by Moravec (2006) and from data on the two North American species *P. aphanonaris* Moravec, Crosby, de Buron, González-Solis et Roumillat, 2008 and *P. wellborni* Moravec, Crosby, de Buron, González-Solis et Roumillat, 2008 subsequently described from centrarchids (Moravec et al. 2008c).

Philometroides branchiostegi is the first known species of philometrid nematode parasitizing a representative of the fish family Branchiostegidae. Further studies on this nematode are necessary, including the description of the hitherto unknown conspecific male.

Fig. 4. *Philometroides branchiostegi* sp. n., scanning electron micrographs of gravid female. **A** – cephalic end, lateral view; **B** – cephalic end, apical view (arrows indicate lateral cephalic papillae of internal circle); **C** – anterior end of body, ventral view; **D** – posterior end of body, sublateral view; **E** – caudal end, apical view (arrows indicate caudal projections); **F** – cuticular boss, apical view.

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