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Pseudobacciger harengulae from the Atlantic herring *Clupea harengus*: a new host and locality record

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Abstract

During the summer and autumn of 1994, 1995 and 1996, 406 juvenile herring caught off the Swedish west coast were examined for parasites. Amongst those found was the digenean *Pseudobacciger harengulae*, which represents new host and locality records for this parasite. *Pseudobacciger harengulae* has been reported from several species of clupeiformes, mostly from tropical and temperate regions of the Atlantic, Pacific and Indian Oceans. The morphology of *P. harengulae* is described and compared with earlier descriptions of *P. harengulae* and *P. manteri*. The possible relationships between *P. harengulae* and *P. manteri* are discussed and the validity of the *P. manteri* is questioned. Most of the specimens (75%) of *P. harengulae* were found in the pyloric caeca and the remainder (25%) in the intestine.

Introduction

Because of its abundance and long-standing commercial importance, the Atlantic herring *Clupea harengus* has been the subject of many parasitological studies, most of which have focused on adult rather than juvenile herring. In their checklists of parasites reported from herring, Arthur & Arai (1984) and MacKenzie (1987) listed 21 and 23 named species of digenean respectively. Only one species of the family Fellodistomidae, *Pronoprymna petrowi* (Layman, 1930) Bray & Gibson 1980, reported by Layman (1930) from the Pacific herring *C. pallasii* was reported in these lists. The present paper reports the finding of another species of fellodistomid, *Pseudobacciger harengulae* Yamaguti, 1938, during a general survey of the parasites of juvenile (0+) Atlantic herring *C. harengus* caught off the west coast of Sweden.

Materials and methods

During the summer and autumn of 1994, 1995 and 1996, 406 specimens of 0-ring herring, ranging in length from 40 to 110 mm, were collected from Gullmarsfjord and Brofjorden off the Swedish west coast. Samples of fish from each haul were transferred to and kept alive in tanks supplied with filtered seawater and were killed immediately before examination. Examination of fishes took place 2–10 days after they had been transferred to the tanks.

Each fish was measured to the nearest millimetre then weighed and sexed. The digestive tract was removed and each part of it was examined separately. The intestine and stomach of 161 fish were screened for parasites. For the remaining 245 fish, the pyloric caeca, in addition to the intestine and stomach, were examined. The *t*-test ($\alpha = 0.05$) was carried out to test the significance of differences between the two methods of examination.

Fresh specimens of the fellodistomid were studied under the light microscope and measurements made with calibrated eye-pieces. Whole-mounts were either: (i) fixed in Berland's fluid, transferred to 70% ethanol and then to lactic acid and stained with 'Mexican Red', Dylon, textile stain (Berland, 1995) and mounted in glycerol-jelly; or (ii) fixed in Bouin's fluid and stained either with acetic carmine or haematoxylin.

Specimens for serial sectioning were fixed in Bouin's, sectioned at 5–8 μm thick and routinely stained with haematoxylin–eosin. Material for scanning electron

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Table 1. Measurements of *Pseudobacciger manteri*, *P. harengulae* and *P. cablei* (in μm).

	Present paper *P. harengulae*	Manter (1947) *P. harengulae*	Nahhas & Cable (1964) *P. manteri*	Yamaguti (1938) *P. harengulae*	Kim & Chun (1984) *P. harengulae*	Madhavi (1975) *P. cablei*
Host	*Clupea harengus*	*Harengula clupeola*	*H. clupeola*	*Harengula zunasi*	*Konosirus punctatus*, *H. zunasi*	*S. fimbriata*, *S. gibbosa*
Locality	West coast of Sweden	Florida, USA	Jamaica	Japan	South Korea	India
Length	370–1130	390–615	373–747	400–630	440–520	528–704
Width	280–650	277–450	266–420	200–500	310	368–512
Body surface	Spined	Spined	Spined	Spined	Spined	Smooth
Oral sucker	45–70	45–69†	45–68	60–84	40–54†	39–62 × 58–74
Ventral sucker	40–55	60–84†	57–90	58–85	40–64†	59–90
Sucker ratio	1:0.9–1.2	?	1:1–1.3	?	?	1:1.0–1.2
Pharynx	30–50	25–46†	33–45	24–47	20–38†	35–59
Oesophagus	30–65	35–54†	33–66	40–120	36–46†	73–97
Ovary	60–90	75 × 75–67 × 107†	60–90	59–70†	64 × 55†	?
Testes	40–80	100 × 92–75 × 156†	60–90 × 65–105	50–120	60 × 50–64 × 50†	78–175 × 58–137
Eggs	21–25 × 18–23	20–22 × 15–19	21–24 × 15–20	21–29 × 15–19	20–24 × 11	23–25 × 12–16

† Measurements not mentioned in the relevant paper and were extracted from the drawings of the corresponding papers.
? Not mentioned in the relevant paper and could not be concluded from figures.

microscopic (SEM) studies was washed several times in physiological saline, fixed for 3–4 h in 2.5% glutaraldehyde buffered with cacodylate (pH 7.4, 4°C), washed in buffer and post-fixed for 3 h in cacodylate-buffered OsO_4 (4°C), dehydrated in ethanol and acetone and finally critical point dried. Specimens were coated with a gold-palladium spatter and examined using a DSM 950, Zeiss scanning electron microscope.

In the present study, holotype and paratypes of *Pseudobacciger manteri*, deposited as number USNM 60256 in the Helminthological Collection of the US National Museum were examined and compared with specimens from the Swedish west coast.

Fellodistomids in the present study were divided into three groups depending on their stage of maturity: without eggs, with less than 10 eggs and with 10 or more eggs.

Measurements are given in μm unless otherwise stated and shown as mean (minimum–maximum).

Results

Morphology

Specimens of *Pseudobacciger* from the Atlantic herring off the Swedish west coast were similar to the holotype and paratypes of *P. manteri*. However, their morphological characters also overlapped those of *P. harengulae* (table 1).

Pseudobacciger harengulae was originally described by Yamaguti (1938) and has been redescribed by Dimitrov *et al.* (1999). Consequently, the present paper will focus only on those features which have not previously been adequately described. A total of 25 juvenile and adult specimens were examined. The measurements are from adult specimens only, fixed in Bouin's solution.

Body small, oval to pyriform, length 651 (370–1130), width 407 (280–650) (table 1), at the level of the anterior half of testes. Body-surface, including suckers, covered by minute, simple, pointed spines (fig. 1). No gland cell in parenchyma of forebody. Oral sucker terminal, 63 (45–70) in diameter, retractable inside body (fig. 1), with mouth in its central posterior (fig. 2). Ventral sucker 50 (40–55) in diameter, just within the anterior part of the body. The ventral sucker, unlike the oral sucker and unlike suckers of most other digeneans, disc-like, may transform to cup-shape, and not observed retracted within the body (fig. 3a–d). The intestinal caeca bifurcate at the level of anterior margin of the ventral sucker, terminate blindly at the pre-, mid- or post-testicular level. This level varies between individual specimens and also with their size. Excretory pore terminal, leading into a short-stemmed Y-shaped vesicle, the stem being so short that the vesicle looks V-shaped. Arms more conspicuous, relatively longer, and more closely situated in young specimens. Seminal vesicle, bi-lobed, at the level of the mid ventral sucker, anterior lobe larger and more rounded than the posterior one. Vitelline glands, paired, lobed pre-equatorial, lateral and at the level of the ventral sucker. Vitelline canals extend postero-centrally from each gland towards the single ovary, and join just anterior to the ovary. Seminal receptacle spherical to elongate, posterior to the ovary. Uterus ventral to and overlapping testes, depending on the number of eggs within the uterus. In

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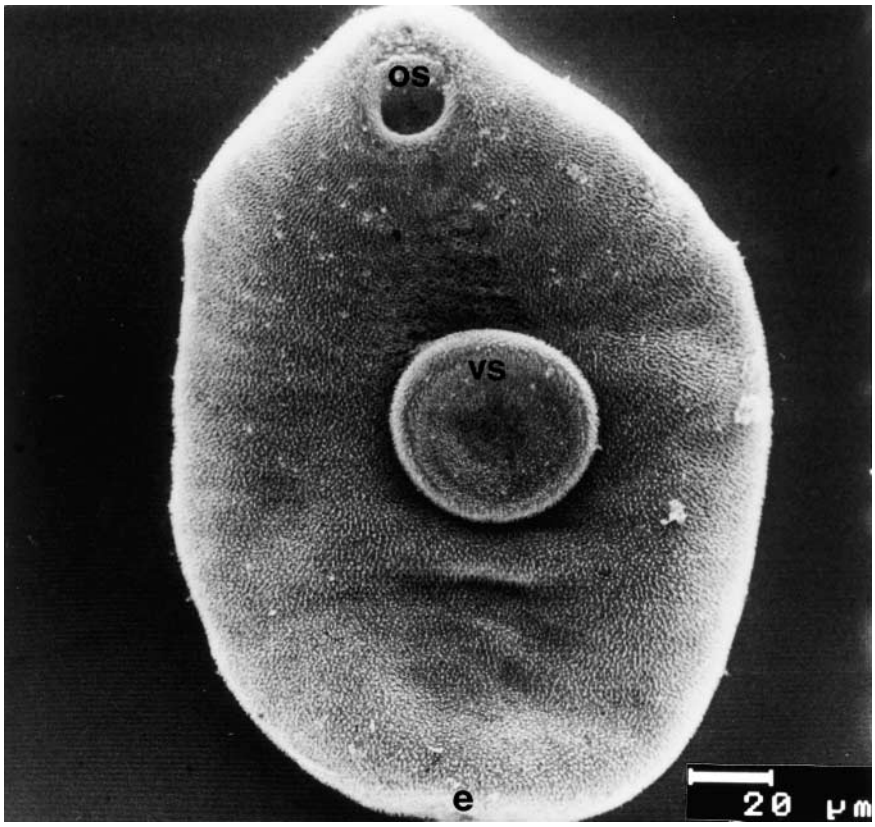

Fig. 1. Scanning electron micrograph of *Pseudobacciger harengulae* (os, oral sucker; vs, ventral sucker; e, excretory pore).

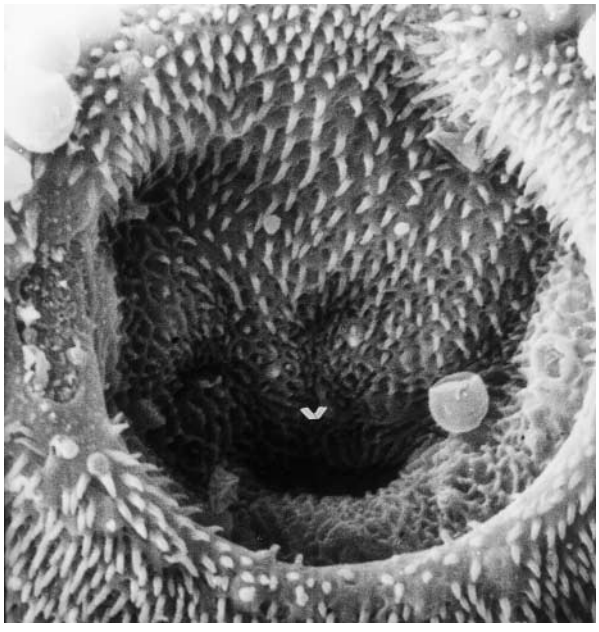

Fig. 2. Scanning electron micrograph of *Pseudobacciger harengulae* oral sucker with mouth (arrow head).

younger specimens uterus post-testicular. Eggs 22 (21–25) by 19 (18–23), oval to slightly spherical without pore or cap.

Juvenile specimens are more elongated with genitalia situated more closely together. In older specimens, filled with eggs, the uterus extends as far as the ventral sucker and covers most of the genitalia. Under the SEM, a partial loss of spines, especially on the ventral part of the body in the larger and older specimens is observed.

Prevalence and intensity of infection

From a total of 406 fish examined, 17.5% were infected with *P. harengulae* in the intestine, reaching 20.4% in fish in which both the intestine and pyloric caeca were examined, with an intensity of infection of 5.2 and a worm range of 1–52. *Pseudobacciger harengulae* first appeared in herring between mid-July and mid-August when the water temperature was about 18°C. Both prevalence and intensity of infection increased continuously, reaching a peak in September.

Of the total number of worms recovered from herring, 74.7% and 25.3% were located in the pyloric caeca and the intestine respectively, with no specimens being found in the stomach. The number of worms found in the pyloric caeca was significantly greater than in the intestine (*t*-test,

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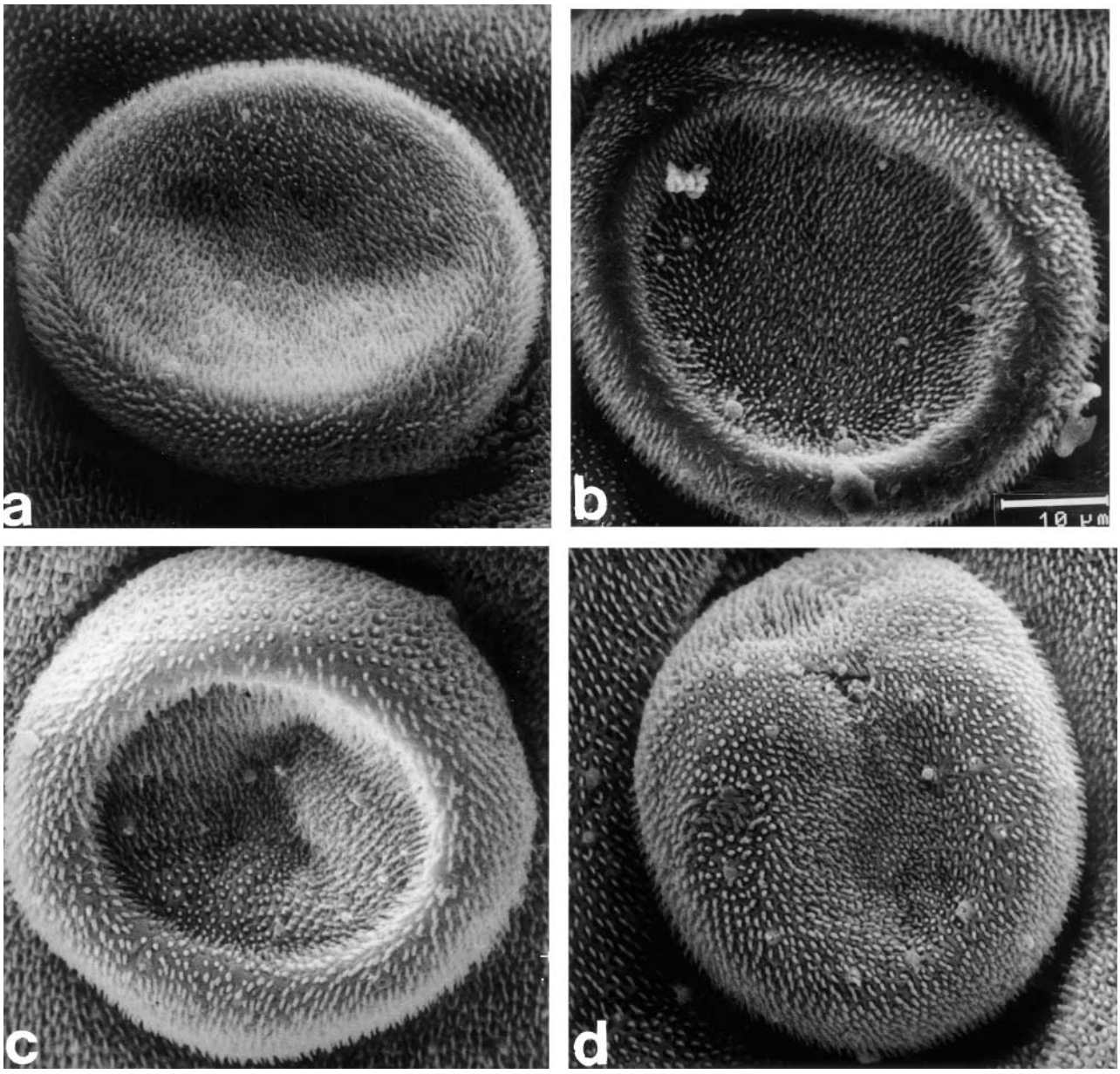

Fig. 3. (a–d). Different stages of the ventral sucker of *Pseudobacciger harengulae*: a, almost flat; b, beginning of cup formation; c, cup shape; d, fist-like shape.

$\alpha = 0.05$). No sex related differences in the prevalence or intensity of infection were observed. There were also differences in the proportions of worms at different stages of maturity in the intestine and pyloric caeca (fig. 4). In the pyloric caeca the proportion of fully mature worms, with more than 10 eggs, was higher than that of worms with less than 10 eggs and worms without eggs. In the intestine, however, the proportion of worms without eggs was the highest.

Discussion

The specimens described in the present study were found to be a fellodistomid belonging to the genus *Pseudobacciger*. *Pseudobacciger harengulae*, the type species of this genus, was first reported and described by Yamaguti (1938) as *Bacciger harengulae* from *Harengula zunasi* from Japan. Later, Manter (1947) reported *P. harengulae* from the intestine and pyloric caeca of *Harengula clupeola* (= *Sardinella macrophthalmus*) (= *Harengula macrophthalma*) from Florida.

A second species, *Pseudobacciger manteri*, has been reported from the intestine and pyloric caeca of *Harengula clupeola* in Jamaica (Nahhas & Cable, 1964). The description of *P. harengulae* by Manter (1947) was based on three specimens (one of which was partly crushed)

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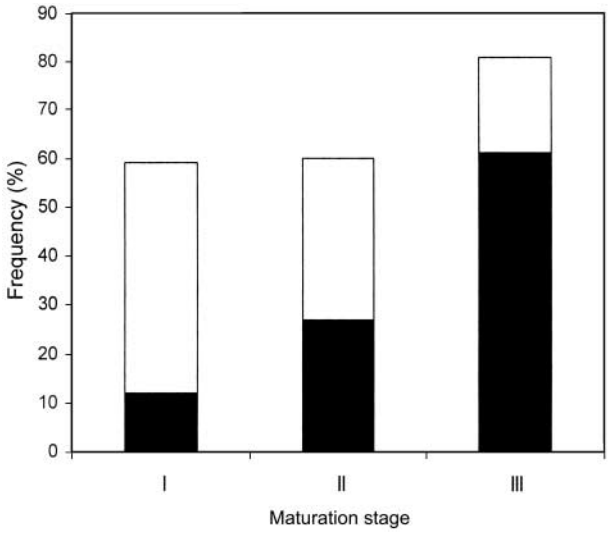

Fig. 4. Percentage of different maturation stages of *Pseudobacciger harengulae* (I, without egg; II, with up to 10 eggs; and III, 10 eggs and more) in pyloric caeca (black) and the intestine (white).

from the intestine of two of 33 hosts. He reported the parasite as *Bacciger harengulae* Yamaguti, 1938. Details of the structure of these specimens agreed with Yamaguti's description but some differences were observed, including the extent of the uterus, the somewhat longer caeca and more rounded eggs. From his sample, Manter (1947) was unable to observe the excretory vesicle, possibly due to the abundance of eggs. Manter (1947) and Yamaguti (1938) both assumed the presence of at least a delicate and weakly developed cirrus sac, but the latter was not described. Considering this criterion, they placed the parasite in the genus *Bacciger*. The genus *Bacciger* was named by Nicoll (1914) with *Bacciger bacciger* as the type species with a thin-walled but well developed cirrus sac. Nine species have been assigned to this genus of which only five are considered to be valid (Bray & Gibson, 1980). Although the genus *Bacciger* has usually been placed in the family Fellodistomidae, Yamaguti (1938) considered the parasite to belong to the family Heterophyidae, but Manter (1947) retained the genus as belonging to the fellodistomids. According to one of the latest reviews on the state of the family Fellodistomidae (Bray, 1988), this monophyletic family consists of seven subfamilies including Baccigerinae which has 12 genera, amongst them *Pseudobacciger*.

Nahhas & Cable (1964) described *Pseudobacciger manteri* based on 20 specimens recovered from the caeca of *H. clupeola* and considered *Bacciger harengulae* of Manter (1947) as its synonym. On the basis of the lack of a cirrus sac they also established a new genus, *Pseudobacciger*, for this species and considered *P. harengulae* of Yamaguti (1938) (syn. *B. harengulae*) as the type species. Nahhas & Cable (1964) reported that the length of caeca and excretory arms as the two most important features separating *P. manteri* and *P. harengulae*.

Pseudobacciger manteri was also referred to by Margolis & Ching (1965) who examined samples collected by Manter. Margolis & Ching (1965) furthermore considered a report of *P. harengulae* from *Harengula clupeola* from Bimini (Sogandares-Bernal, 1959) to be a misidentification of *P. manteri*.

Dimitrov *et al.* (1999) considered three species of *Pseudobacciger* to be valid; these are *P. harengulae* (Yamaguti, 1938) Nahhas & Cable, 1964 (the type species), *P. manteri* Nahhas & Cable, 1964, and *P. cablei* Madhavi, 1975. The fellodistomid specimens found in the present study overlap both in measurements and general morphology of *P. harengulae* as described in previous studies, including the latest by Dimitrov *et al.* (1999). Thus, *P. harengulae* is herewith reported from a new host, the herring, *Clupea harengus*, and from a new geographical area, the eastern North Atlantic.

When comparing morphological characters of the fellodistomid species found in the present study with those of the holotype and paratypes of *P. manteri*, these overlapping features are again evident (table 1), which explains why Rahimian & Thulin (1996) recorded this parasite as *P. manteri*. However, specimens of *P. harengulae* from the Swedish west coast differ from *P. manteri* reported from Jamaica (Nahhas & Cable, 1964) in having larger but less globular vitelline glands, which are situated more anteriorly in specimens of the present study. Furthermore, branches of the intestinal caeca in the present specimens are shorter than those of the holotype of *P. manteri*. The intestinal caeca of the present specimens, however, are slightly longer than those from *P. harengulae* reported from Japan (Yamaguti, 1938) and Korea (Kim & Chun, 1984). In the present specimens, no gland was observed in the forebody. Glands in the forebody are very pronounced in the drawings of *P. harengulae* (Yamaguti, 1938; Madhavi, 1975; Kim & Chun, 1984; Gaevskaya, 1996). However, these structures were not observed in the holotype and paratypes or in the drawings of *P. manteri*. Furthermore, the present specimens are similar to those studied by Dimitrov *et al.* (1999) in having a bipartite seminal vesicle with the anterior part larger than the posterior part. Dimitrov *et al.* (1999) concluded that the seminal vesicle is one of the most important features separating *P. harengulae* from *P. manteri* and that Fig. 79 was *P. manteri* and Fig. 78 probably *P. harengulae* in Manter's (1947) report. The most pronounced difference between these two figures, apart from the relative situation of the caeca, is the number of eggs which, in my opinion, results in differences in the relative position of the sexual organs, caeca and excretory vesicles. It is also worth noting that some of the species characteristics were observed to vary between specimens collected from different host species from the same localities, i.e. the Black Sea (Dimitrov *et al.*, 1999).

Therefore, the above-mentioned differences in the morphological characters of the two species, *P. harengulae* and *P. manteri* are highly variable and some of these characters may be altered by differences in fixation methods and by the maturation stage of the parasite. In conclusion, specimens found in the present study are identified as *P. harengulae*, the type species of the genus. In addition, based on the high variability of the morphological characters in question, the validity of *P. manteri* should be questioned until further studies have been undertaken.

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The present study provides details of body spination as well as the special disc-like form of the ventral sucker of *P. harengulae*. The more common occurrence of *P. harengulae* in the pyloric caeca of the herring compared with the intestine suggests that the pyloric caeca is the main site of infection. The low proportion of fully mature worms in the intestine supports this. The distinct form of the ventral sucker in *P. harengulae* may produce less 'gripping' power and, as the intestine is a more hazardous environment than the pyloric caeca, worms may be washed out from the intestine. Unlike other digeneans of herring, *P. harengulae* shows undulatory movements in physiological saline (personal observation). These movements may further contribute to making the intestine a less preferred site for this digenean. Alternatively, immature worms may migrate from the intestine to the pyloric caeca as they develop.

The three species of the genus *Pseudobacciger* have been reported from different geographical areas. *Pseudobacciger harengulae* has been reported from: *Harengula zunasi* from Japan and Korea (Yamaguti, 1938; Kim & Chun, 1984); *Harengula clupeola* from Florida, USA and Bimini (Manter 1947; Sogandares-Bernal, 1959); *Konosirus punctatus* from Korea (Kim & Chun, 1984); *Sardinella fimbriata* and *S. gibbosa* from India (Madhavi, 1975); *Sardina pilchardus* and *Sardinella aurita* from the Namibian coast of the eastern South Atlantic (Gaevskaya, 1996); *Sprattus sprattus* and *Engraulis encrasicholus* from the Black Sea (Dimitrov *et al.* 1999); and *Clupea harengus* (the present study). *Pseudobacciger manteri*, on the other hand, has been reported from *Harengula clupeola* from the Caribbean Sea (Nahhas & Cable 1964), i.e. the same species which according to Manter (1947) and Sogandares-Bernal (1959) also harbour *P. harengulae*. The only report of *P. cablei* is also from *Sardinella fimbriata* and *S. gibbosa* from India (Madhavi, 1975) and these are also hosts for *P. harengulae*. These reports indicate a cosmopolitan distribution for *P. harengulae* in clupeoids, especially in tropical and subtropical regions of the Pacific and Indian oceans. The distribution of *P. cablei* is also from the tropical Indian Ocean, Bay of Bengal. The distribution of *P. manteri* seems to be limited to the tropical Caribbean Sea. The distribution range of this genus indicates a low host diversity (in one family) and a high temperature tolerance for *P. harengulae* and a low host diversity and low temperature tolerance for *P. manteri* and *P. cablei*.

Pseudobacciger harengulae usually appears in mid-July to mid-August, when the water temperature in the study area is about 18°C at a time when infective metacercariae are likely to first become available to 0-ring herring.

Pseudobacciger harengulae has not previously been reported from herring and it is unlikely that it would have escaped the attention of other investigators working on herring or on other species in the area. Therefore, it is possible that *P. harengulae* has only recently been introduced to this area. One possible way of introduction is via ballast water from oil-tankers which frequently visit the area and this speculation is reinforced by the finding of *P. harengulae* in the Black Sea (Dimitrov *et al.*, 1999). MacKenzie (1987) divided the parasites of herring into four categories ranging from 'accidental' parasites of herring to those for which herring is the most important host. Considering his categories, *P. harengulae* falls into the rank of 'parasites for which the herring appears to be one of several equally important hosts'. So it is highly likely that the parasite occurs in other clupeoids off the west coast of Sweden.

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