



Two new species of *Mazocraes* Hermann (Monogenea: Mazocraeidae) from clupeoid fishes off Visakhapatnam, Bay of Bengal

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Abstract Two new species of *Mazocraes* Hermann (Monogenea: Mazocraeidae) are described infecting clupeoid fishes of Visakhapatnam coast, Bay of Bengal: *Mazocraes bengalensis* n. sp. from *Opisthopterus tardoore* Cuvier and *M. stolephorusi* n. sp. from *Stolephorus indicus* van Hasselt and *S. commersoni* Lacepede. *M. bengalensis* n.sp. is distinguished from the most closely related species (*M. gussevi*, *M. australis*, *M. alosae*, *M. mamaevi*) by the combination of following characters: Body size, extent of caeca, number and arrangement of testes, size and structure of the clamps and the armature of genital complex. *M. stolephorusi* n. sp. differs from all the other species of *Mazocraes* in the pedunculate nature and the sizes of the haptor clamps and the structure of the genital complex. A detailed analysis of the species of the genus *Mazocraes* is presented. The partial 28S rDNA sequences of *M. bengalensis* was generated and deposited in the GenBank.

Keywords Mazocraeidae · *Mazocraes bengalensis* n. sp. · *Mazocraes stolephorusi* n. sp. · Clupeoid fishes · Bay of Bengal

Introduction

Mazocraes Hermann is a fairly large genus comprising of species recorded from marine and freshwater fishes mostly belonging to Clupeoidea. Mamaev (1982) provided a review on the genus and discussed the validity of various

species reported. Timi et al. (1999) emended the diagnosis of the genus given by Mamaev (1982). The major diagnostic features of the genus are: the thin and leaf shaped haptor comprising 4 pairs of clamps of closed type, each clamp with six sclerites; the lappet with three pairs of hooks; the genital complex armed with a pair of lateral hooks and 8–18 smaller median hooks arranged in two transverse semicircular rows or in a circle; and the numerous testes fused into a whole mass behind the ovary. Several species were reported under the genus but many of them have been included under the category of ‘species inquirendae’ due to lack of detailed descriptions and non-availability of type specimens for restudy of morphological details (Mamaev 1982; Agrawal and Sharma 1989). At present the genus is left with 12 valid species of which 6 were recorded from India. A key for separation of various species in the genus was provided by Kumar and Agarwal (1981).

Details of the hosts and organization of the Indian species of the genus were given in the book ‘An encyclopaedia of Indian Monogenoidea’ authored by Pandey and Agrawal (2008). During the present survey on mazocraeid monogeneans of clupeoid fishes off Visakhapatnam, Bay of Bengal, two new species of *Mazocraes* were encountered. These are *M. bengalensis* n. sp. from *Opisthopterus tardoore* and *M. stolephorusi* n. sp. from *Stolephorus indicus* and *S. commersonii*. A detailed analysis of the genus *Mazocraes* is presented. The 28S rDNA sequence of *M. bengalensis* was analysed and deposited in GenBank.

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Materials and methods

Samples of clupeoid fishes were collected from trawler operations/line catches of fishermen at fishing Harbour, Visakhapatnam, Bay of Bengal, brought to the laboratory and examined for monogeneans when they are fresh, usually within few hours of their collection. The monogeneans found were collected in saline and some of them were fixed in AFA, stained with alum carmine, dehydrated in alcohol series, cleared in beechwood creosote and mounted in Canada balsam. Measurements were taken using micrometer and all the measurements in the text are given in micrometres and are expressed as the mean followed by range in parenthesis. Holotype and paratype specimens were deposited in Zoological Survey of India (Kolkata, India).

A few specimens were fixed in glycerine mainly for studying details of clamp structure and armature of the genital complex. For analysis of 28SrDNA sequences, live specimens of *M. bengalensis* were fixed in 100% ethyl alcohol. The DNA extraction and sequencing were performed according to the procedure followed earlier (Sailaja et al. 2016).

Results

Family Mazocraeidae Price, 1936

Mazocraes Hermann

Mazocraes bengalensis n. sp.

Type host: *Opisthopterus tardoore* (Cuvier 1829)
(Pristigasteridae)

Type locality: Visakhapatnam coast, Bay of Bengal,

Prevalence: 48.2%

Mean intensity: 1.70

Type material: Zoological Survey of India, Kolkata, India, holotype W10434/1

Representative DNA sequences: GenBank Accession No. KF306255

Description (Measurements based on 6 specimens) (Fig. 1a–d)

Body long, slender, maximum width at level of ovary, gradually tapering towards anterior region, anterior end pointed. Haptor broad, well differentiated from body. Total body length 1752 (1520–2016), width 176 (112–208), haptor 458 (288–576) long, 408 (288–544) wide, approximately 26% of total body length. Tegument smooth, eye spots absent. Oral suckers two, septate almost as long as wide, 36 in length, 28 in width. Mouth opening subterminal. Pharynx oval, small, 32 (28–44) × 25 (16–40) in size. Oesophagus long with one pair of lateral diverticulae at

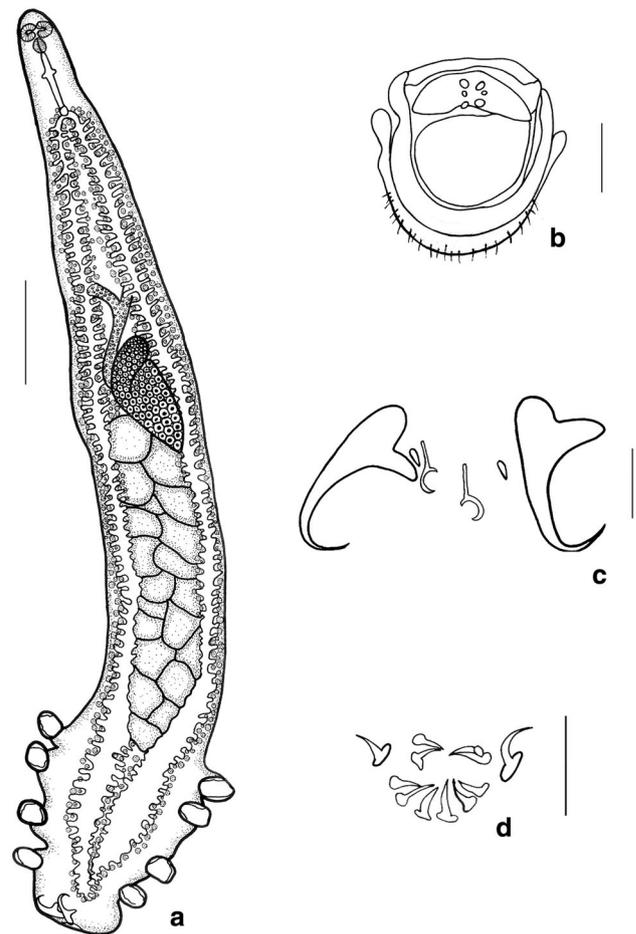


Fig. 1 *Mazocraes bengalensis* n. sp. 1. Entire worm, dorsal view; 2. Clamp; 3. Lappet showing anchors and marginal hooks; 4. Genital atrium. Scale bars: a, 200 μ m; b,c,d, 10 μ m

midlevel, bifurcates at level of genital pore. Caeca broad with lateral and median diverticula, extend into haptor, not confluent posteriorly, terminate blindly at level of 4th clamp.

Haptoral clamps 4 pairs, on short peduncles, almost of equal size, measure on an average 40–64 (52) in length, 44–88 (52.6) in width. Clamp skeleton of mazocraeid type, closed type, comprises 6 sclerites: scleritumarcuat-ant-erius (SAA), scleritumantero-supplementarium (SAS), scleritummedio-basale (SMB), scleritummedio-supple-mentarium (SMS), scleritumpostero-supplementarium (SPS) and scleritumarcuat-posterius (SAP). SAA long and thin, formed by fusion of two sclerites, SAS notched, SMB broad, rectangular perforated by 6 apertures arranged in 2 rows, SMS transversely elongated, SPS not clearly demarcated, SAP thin, rod-like with clubbed tips, formed by fusion of two sclerites. Lappet with two pairs of anchors and one pair of marginal hooks. Outer pair of anchors large, 11.0 long, having broad base, a median spur and curved tip, inner pair of anchors smaller, 3.5 (3–4) long,

having a short handle and curved tip. Marginal hooks one pair, very small, rod-like, transparent each with a pointed tip, not visible in many specimens.

Testes follicular, follicles arranged in two rows in intercaecal region, occupy posterior half of body, testes area 560 (520–600) long. Seminal vesicle sinuous, in intercaecal space anterior to ovary, opens into genital complex. Genital complex 22.0 in diameter, located at level of bifurcation point, at a distance of 190 (140–268) from anterior end, armature consists of 6 pairs of genital hooks. First pair lateral, curved inwards with a broad base and pointed tip. Remaining 5 pairs simple, smaller in size arranged in two semicircular rows, anterior row with 2 pairs and posterior with 3 pairs. Ovary U-shaped, length 200 (188–204), width 54 (50–56), folded upon itself, with anterior germinal and terminal ends in contact. Oviduct proceeds from terminal end of ovary, joins genito-intestinal canal and vitelline duct to continue as ootype. Mehlis' gland postero-lateral to ovary. Uterus thin-walled. Eggs not found. Vitellarium follicular, follicles co-extensive with intestinal caeca, extend from level of genital pore, terminate in haptor at level of 4th pair of clamps. Vitelline reservoir Y-shaped.

Remarks

The genus *Mazocraes* contains 12 valid species (see Table 2). The present species closely resembles *M. gussevi* Agrawal and Sharma 1989 reported from *Hilsa ilisha* from Ganges, a freshwater locality but differs from it in the following respects: Body is much smaller, 1520–1600 × 112–208 in the present form as against 3100–3300 × 400–600 in *M. gussevi*. Testes are arranged in two rows instead of a single row. Caeca extend more posteriorly into the haptor up to level of 4th clamp and terminate blindly. In *M. gussevi* caeca extend up to the level of 3rd pair of clamps and are united posteriorly. Clamp structure is different in that the SMB bears 3 pairs of pores instead of numerous pores. The armature of genital complex is also different in the two species- the present species bears one pair of large lateral and 5 pairs of median hooks of similar size and shape. In *M. gussevi* the genital hooks are 7 pairs, the first to fourth pairs are of different sizes and shapes while the fifth, sixth and seventh pairs are tuberculate and are of similar size. The new species can be differentiated from *M. australis* Timi, Sardella & Etchegoin 1999 by the following combination of characters: Caeca and vitellaria extend into the haptor, testes more posteriorly located, clamps are of uniform size and their structure is different, the number of genital hooks is much less, 16–18 in *M. australis*. In *M. alosae* the body size is bigger and anterior pair of clamps are large in size. Clamp

structure is different. Genital hooks are large, consist of 8 thin, curved median hooks and one pair of robust lateral hooks. Testes are numerous. *M. mamaevi* reported from a cyprinid fish, *Labeo rohita* from Lucknow, differs mainly in having 6 pairs of genital hooks, of which the 5th and 6th pairs are tuberculated. Further SMB is rectangular and perforated by 12 pairs of apertures arranged in two rows. *M. bengalensis* n. sp. is morphologically very different from the remaining species described in the genus.

Mazocraes stolephorusi n. sp.

Type host: *Stolephorus indicus* Van Hasselt (Pristigasteridae)

Other host: *Stolephorus commersonii* Lacepede (Pristigasteridae)

Type Locality: Visakhapatnam coast, Bay of Bengal,

Prevalence: 12.09% in *S. indicus*, 8.57% in *S. commersonii*

Mean intensity: 1.13 in *S. indicus*, 1.0 in *S. commersonii*

Material deposited: Zoological Survey of India, Kolkata, India: holotype W 10435/1

Description (Measurements based on 6 specimens) (Fig. 2a–d)

Body long, slender, with almost parallel sides, tapered anteriorly and posteriorly, 1552 (1056–2048) long, 218 (144–320) wide at level of ovary. Tegument smooth, without eyespots. Haptor triangular not well demarcated from body, 269 (208–320) long, 149 (128–160) wide, approximately one fifth of body length. Prohaptor represented by 2 suckers, each sucker measuring 28–32 × 28–30, partitioned into two chambers. Mouth subterminal, midventral, pharynx muscular, spherical 24–36 in diameter. Oesophagus narrow, with one pair of lateral diverticulae. Intestinal bifurcation a little posterior to genital pore at a distance of 144–192 from anterior end, intestinal caeca two, diverticulate, united and terminate in anterior part of haptor at level of 2nd pair of clamps.

Haptoral clamps 4 pairs, provided with short peduncles, clamps decreasing gradually in size distally, anterior two pairs large, more or less of equal size 60–68 × 40–44 in size, 3rd and 4th pairs of clamps of equal size, 32–40 × 28–32 in size. All clamps of closed type, each with 6 sclerites, SAA thin arch-like, SAS broad, SMS transversely elongated with broad lateral edges, narrowed in the middle, SMB rectangular, SPS triangular, small, SAP thin the two sclerites join together to form an arch. Lappet small with 2 pairs of anchors and one pair of marginal hooks. Outer pair of anchors large, 24–32 in size each with a curved tip and short handle, inner pair of anchors 6 long. Marginal hooks thin, rod-like, transparent, not visible in many specimens.

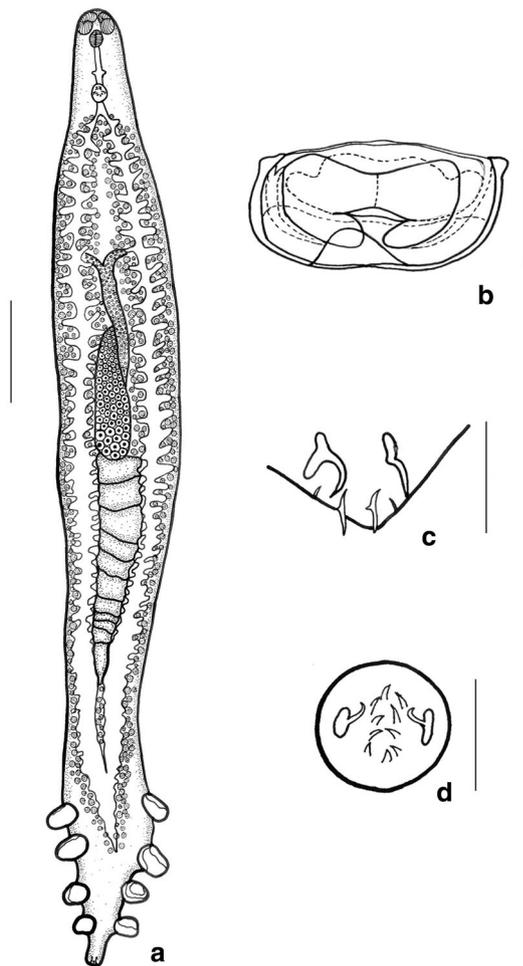


Fig. 2 *Mazocraes stolephorusi* n. sp. 1. Entire worm, ventral view; 2. Clamp; 3. Lappet showing anchors and marginal hooks; 4. Genital atrium. Scale bars: a, 200 μ m; b,c,d, 30 μ m

Testes follicular, follicles arranged linearly in posterior half of body forming a compact mass in intercaecal region. Genital complex spherical, 36–40 in diameter, located just in front of bifurcation point, at a distance of 128–134 from anterior end, surrounded by a thick zone of concentric muscles, armature consists of 1 pair of sickle shaped, large hooks situated laterally one on each side and five pairs of simple smaller hooks arranged in two vertical rows along midregion. Seminal vesicle sinuous, intercaecal.

Ovary elongate, immediately pretesticular, posterior part lying lateral to anterior testis, anterior part somewhat narrow contains compactly arranged germ cells, posterior part broader filled with mature oocytes. Oviduct originates from posterior part of ovary. Mehlis' gland small, postovarian. Vitellaria comprise two lateral bands of follicles, co-extensive with caeca, extend into haptor. Bilateral vitelline ducts converge towards midline to form common vitelline duct at level of Mehlis' gland. Vagina not visible. Egg elliptical with a blunt knob at one end.

Remarks

M. stolephorusi n. sp. differs from all the other species of *Mazocraes* in the following respects. The clamps are arranged on short peduncles. Haptor clamps are of different sizes, anterior 2 pairs large and posterior 2 pairs smaller. In all the other species of *Mazocraes* the four pairs of clamps are more or less of the same size. The structure of the genital complex is also different in that the armature consists of a pair of sickle-shaped lateral hooks situated on either side of two vertical rows of median hooks. *M. chauhani* Kumar and Agarwal 1981 reported from *Gudusia chapra*, a freshwater fish also possesses 5 pairs of median hooks as in *M. stolephorusi* n. sp. but the arrangement of the median hooks is horizontal rather than vertical.

Discussion

The genus *Mazocraes* requires a thorough revision, since the descriptions given for many of the species are inadequate, particularly lack details of the clamp structure and the genital complex which are essential for proper identification of the species. As many as 25 species were reported under the genus, but many of the species were included under the category of 'species inquirendae' or transferred to other genera. For instance Tripathi (1957) redescribed and figured *Mazocraes orientalis* Chauhan 1952 from the type host and locality *Dussumieria* sp. from Bombay. However, Price (1961) proposed *M. tripathii* for *M. orientalis* of Tripathi (1957) and transferred *M. orientalis* Chauhan to the genus *Kuhnina* as *Kuhnina orientalis* (Chauhan 1952). Mamaev (1982) transferred two species: *Kuhnina brevoortia* Hargis 1955 from *Brevoortia patronus* and *Neomazocraes sardinops* Lebedev and Parukhin 1969 from *Sardinops ocellata* to the genus *Mazocraes* as *M. brevoortia* (Hargis 1955) and *M. sardinops* (Lebedev and Parukhin 1969). Mamaev (1982) in a review of the genus *Mazocraes*, transferred *M. orientalis* (Chauhan 1952) and *M. trispina* (Unnithan 1964) to the genus *Leptomazocraes* (Mamaev 1975) on account of the presence of 5 rather than 6 sclerites in the clamps and the tuberculated and knife-like hooks in the genital atrium. He further expressed the view that *M. dussumierii* Unnithan 1964 may also belong to the genus *Leptomazocraes*. According to the data provided in WoRMS, *M. heterocotyle* (Van Beneden 1871) is accepted as *Pseudanthocotylodes heterocotyle* (Van Beneden 1871) and *M. longicauda* Mamaev 1975 is accepted as *Pseudomazocraeoides longicauda* (Mamaev 1975).

Following the above analysis as many as 7 species became species inquirendae (Table 1). We are of the

Table 1 List of species of *Mazocraes* regarded as species inquirendae

Species	Host	Locality
<i>M. harengi</i> (Van Beneden and Hesse 1863) Nicoll, 1915	<i>Clupea harengus</i>	Belgium
<i>M. pilchardai</i> (Van Beneden and Hesse 1863) Sproston, 1946	<i>Clupea pilchardus</i>	Belgium
<i>M. cepedianum</i> Kimpel et al. 1938	No description or figure provided	Belgium
<i>M. heterocotyle</i> (Van Beneden 1870) Sproston, 1946	<i>Clupea sprattus</i>	Belgium
<i>M. chauhani</i> Kumar and Agarwal, 1981	<i>Gudusia chapra</i>	River Ganges
<i>M. elongatus</i> Khan and Karyakarte, 1982	<i>Clupea ilisha</i>	India
<i>M. singhi</i> Gupta and Krishna, 1988	<i>Cirrhinus mrigata</i>	Lucknow

Table 2 List of valid species of *Mazocraes*

Species	Host	Locality
<i>M. alosae</i> (Hermann, 1782)	<i>Clupea alosae</i>	Europe
<i>M. villelai</i> Tandeira and Valdez 1955	<i>Alosa alosa</i>	Lisboa
<i>M. gonialosae</i> Tripathi, 1959	<i>Gonialosa manmina</i>	India
<i>M. chauhani</i> Kumar and Agarwal, 1981	<i>Gudusia chapra</i>	River Ganges, India
<i>M. brevoortia</i> (Hargis, 1955) Mamaev, 1982	<i>Bravoortia patronus</i>	Gulf of Mexico
<i>M. sardinops</i> (Lebedev and Parukhin 1969) Mamaev, 1982	<i>Sardinops ocellata</i>	South China Sea
<i>M. mamaevi</i> Agrawal, 1988	<i>Labeo rohita</i>	India
<i>M. mehrai</i> Gupta and Krishna, 1988	<i>Dussumieria acuta</i>	Puri, Bay of Bengal
<i>M. sprostonai</i> Gupta and Krishna, 1988	<i>Clupea ilisha</i>	Puri, Bay of Bengal
<i>M. gussevi</i> Agrawal and Sharma, 1989	<i>Hilsa ilisha</i>	India
<i>M. multispinalis</i> Agrawal and Sharma, 1989	<i>Hilsa ilisha</i>	India
<i>M. australis</i> Timi et al. 1999	<i>Engraulis anchoata Anchoa marina</i>	Argentina Sea

opinion that two more species *M. mehrai* (Gupta and Krishna 1988) and *M. sprostonai* Gupta and Krishna 1988 whose descriptions are incomplete and the diagrams are of poor quality, may also belong to this category. After these revisions the genus is left with 12 valid species (Table 2).

A key for differentiation of the species in the genus was provided by Kumar and Agarwal (1981). The present study added two more species *M. bengalensis* n. sp. and *M. stolephorusi* n. sp. There is every possibility that few more species may eventually be invalidated. It is evident that utmost care is needed while erecting new species in this genus. Molecular analysis of different species in the genus may help to clear the confusion prevailing in the species differentiation. Only limited information is available on this aspect. Here we have analysed the 28srDNA sequences for *M. bengalensis*. This data is also available for *Paramazocraes thrissocles* (Tripathi 1959 (Sailaja et al. 2016), *Heteromazocraes thryssaensis* (Sailaja, Shameem & Madhavi, 2017) and *H. delicatus* Sailaja, Shameem & Madhavi, 2017 (Sailaja et al. (2017) and *Kuhnia scombri* (Kuhn 1829) (Olson and Littlewood 2002). Neeraja et al. (2016) subjected 8 species of the order Mazocraeidea from

carangid fish to molecular characterization and phylogenetic study, concluded that the family Mazocraeidae formed a basal group to polyopisthocotylean parasites of teleosts. Further studies are needed with more samples to get better insight into the relationships of these mazocraeid monogeneans and their phylogenetic status.

Authors' contributions BS Collection of parasites and preparation of slides, preparation of drawings, writing the manuscript. US Assisted in the identification of the parasites, literature review and writing of manuscript. RM expert opinion and critical review of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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