Taxonomic tools for the identification of *Allogenarchopsis bareillensis* n. sp. (Digenea: Hemiuroidea: Derogenidae) from *Channa striata* of Rohilkhand, India based on light and scanning electron microscopic studies

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Received: 2 September 2015 / Accepted: 7 October 2015 / Published online: 16 March 2016
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Abstract The stomach of the freshwater snakehead murrel, *Channa striata* (n = 250) collected from fresh water habitats of Bareilly, Uttar Pradesh, India revealed *Allogenarchopsis*, a trematode parasite (Plagiorchiida: Derogenidae). An illustrated account of light (Olympus BX-53 with Cellsens software imaging system) and scanning electron microscopy (SEM) (Neo JCM-6000) of *Allogenarchopsis bareillensis* n. sp. (prevalence 37 %, intensity 1–4 par/host) is provided. Light microscopy based studies warranting creation of a new species are the robust shape of the body with broad mid-body, blunt anterior and tapering posterior end, large ventral sucker, ratio of oral sucker: ventral sucker 1:3.5, pharynx broader than long, egg size small with relatively short egg filament, excretory vesicle U-shaped bifurcating at posterior end and terminating above intestinal shoulders. Key characteristics of the tegumental microtopography by SEM are (1) an aspinous tegument with a variety of papillae of different shapes (button-like, dome-shaped) distributed randomly on the dorsal and ventral surface (2) oral sucker and oral cavity

muscular with fringed margins and morphologically different oral papillae (3) lip of ventral sucker papillated including two unequally spaced rows of papillae with sensory receptacles interspersed in between, (4) unique differentially texturized cytoplasmic processes on the ventral sucker and (5) body folds grooved, gradually becoming narrower towards posterior end. This is the first record and study on *Allogenarchopsis* from *Channa* of Rohilkhand, India using electron optics. We recommend that the newly recognized morphological features as revealed by light microscopy and SEM be utilized as taxonomic tools in future for the identification of the species.

Keywords Trematoda · Derogenidae · *Channa* · Stomach · Papillae

Introduction

Light microscopy-based studies on the hemiuroid worm, genus *Genarchopsis*, usually parasitic in the oesophagus and stomach of fishes have a rich history from India (Gupta 1951; Kakaji 1969; Rai 1971; Pandey 1973; Madhavi 1978; Varma and Sahay 1983; Bhadauria and Dandotia 1984; Pardeshi and Hiware 2012), adjoining countries (Bashirullah and Elahi 1972; Rehana and Bilqees 1980; Khan 1991; Shaikh et al. 2011), and elsewhere (Ozaki 1925; Shimazu 1995; Urabe 2001; Gibson et al. 2002; Urabe et al. 2012) focusing on taxonomic descriptions and life cycle patterns. The genus *Allogenarchopsis*, with *A. problematica* from Japan as the type species (Urabe and Shimazu 2013), a new genus of endoparasitic digenetic worm is an offshoot of *Genarchopsis*, *G. goppo* from *Mogurnda obscura* (a synonym of *Odontobutis obscura*) in Japan being the type species (Ozaki 1925). We discovered some

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digenean trematodes from the stomach of *Channa striata* (syn. *Ophiocephalus striatus*, *O. vagus*) collected in Bareilly, India. Prior to molecular characterization, precise morphological analysis is necessary. Keeping this in view, morpho-analysis including light microscopy and scanning electron microscopy (SEM) were performed to provide a detailed taxonomic description of these specimens.

Materials and methods

Study site

This study was carried out for 12 months (January–December 2013) at Bareilly, Uttar Pradesh, India situated in the north Indian state of Uttar Pradesh at 28°10'N and 78°23'E, located on the river Ramganga with seven other rivers passing through the district. The lower Himalayas are 40 km north of the river. It is the capital of Bareilly division and the geographical region of Rohilkhand. The city is 252 km north of the state capital, Lucknow, and 250 km east of the national capital, New Delhi. It is the seventh-largest metropolis in Uttar Pradesh and the 50th-largest in India. The total area is 235 km² and is situated at an elevation of 268 m. The average annual rainfall is 1096 mm, received mostly during the monsoon season and average annual temperature is 45 °C (maximum) and 4.8 °C (minimum).

Study system

Live specimens of the host fishes (n = 250) were procured from the local ponds, fish market, the river Ramganga and its tributary, Nakatia, Bareilly, Uttar Pradesh, India. Fishes were transported to the Centre of Excellence laboratory of M.J.P. Rohilkhand University in large containers and maintained in aquaria in the Animal House under proper aeration. They were fed on commercial pellets (Toya fish food containing fish and soyabean meal, wheat and rice flour and vitamins A, C, D3 and E) till sacrificed.

Light microscopy studies

Fishes were anesthetized and thoroughly examined for presence of parasites by dissecting under a stereoscopic microscope. The trematode, *Allogenarchopsis* Urabe and Shimazu, 2013 collected from the stomach was fixed in alcohol-formal acetic (A.F.A.) acid, routinely stained in Borax carmine and mounted in DPX. Photographs were taken under Olympus BX-53 microscope with Cellsens software imaging system. Range values of all measurements are given in mm unless otherwise stated. Prevalence

of infection and mean intensity were calculated following Bush et al. (1997).

Scanning electron microscopy studies

The parasites collected from the intestinal tract of *Channa striata* were washed in physiological saline and fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer for 2.5 h at 4 °C. They were transferred to the Sophisticated Instrumentation Laboratory of the Centre of Excellence of MJP Rohilkhand University. The specimens were further washed with sodium cacodylate buffer (pH 7.2) to remove the smell of glutaraldehyde and postfixed in 1 % osmium tetroxide for 2 h. The process was repeated (Abdou et al. 2001), fixed samples washed in buffer, re-fixed in OsO₄ for 12 h, buffer wash repeated and the specimens were dehydrated through ethanol series and dried to critical point. Specimens were mounted on metal specimen stubs, rotary-coated with gold palladium in NeoCoater 100–240 V for 30 s, examined in Neo JCM-6000 at an acceleration voltage of 15 kV and micrographs taken.

Systematic position

The genus *Allogenarchopsis* is placed in the superfamily Hemiuroidea Looss, 1899 (Synonym Didymozooidea Poche, 1907; Accacoelioidea Odhner, 1911; Isoparorchioidea Travassos, 1922), Family Derogenidae Nicoll, 1910 (synonym Halipegidae Poche, 1926; Liocercidae Ejsmont, 1931; Arnolidae Yamaguti, 1958), Subfamily Halipeginae Poche, 1926 (synonym Arnolinae Yamaguti, 1958; Monovitellinae Ataev, 1970; Ghaziatrematinae Nasir and Velasquez, 1977) according to Gibson et al. (2002). Gibson and Bray (1979) recognized fourteen families within Hemiuroidea, provided diagnoses and keys to generic levels and also discussed possible evolutionary pattern. The subfamily Derogeninae was erected by Nicoll (1910) for *Derogenes* Luhe, 1900 but *Derogenidae* was first used at full family level by Dollfus (1950). Gibson et al. (2002) provided a detailed revision of the systematic and taxonomy of Digenea with keys for the identification of these parasites at the superfamily, family, subfamily and generic levels. Olson et al. (2003) emended the membership of established higher taxa, erected and revised new taxa, based on molecular phylogenetic study, proposed a phylogenetically based classification and discussed it in the light of ontogeny, morphology and taxonomic history. During the present study, the classification of Gibson et al. (2002) is followed whereby the family *Derogenidae* is divided into three subfamilies: *Gonocercinae* Skrjabin and Guschanskaja, 1955; *Halipeginae* Poche, 1926 and *Derogeninae* Nicoll, 1910. The authors mentioned sixteen genera under the sub family *Halipeginae*. Recently, Urabe and

Shimazu (2013) separated *Genarchopsis yaritanago* Shimazu et al., 2011 from the genus *Genarchopsis* and erected another genus, *Allogenarchopsis* under the subfamily Halipeginae, based on the presence/absence of the Juel's organ, morphological difference of sinus organ, Mehlis's gland and excretory vesicle, and a molecular phylogenetic evidence.

Results

Allogenarchopsis bareilliensis n. sp

Material examined

Type host	<i>Channa striata</i> Bloch 1793 (Perciformes: Channidae)
Type locality	Bareilly (U.P.), India 28°10'N and 78°23'E
Infection site	Stomach
Etymology	The parasite is named on its type locality
Type data and depository	The holotype [AS/COE/Tr/A-01(i)] and 10 paratypes [AS/COE/Tr/A-01(ii-xi)] are deposited in the "Centre of Excellence", Department of Animal Science, MJP Rohilkhand University, Bareilly, India

Description (Figs. 1, 2)

Based on the holotype and 11 paratypes. (Range values of all measurements are given in Table 1). Body elongated, robust with mid one-third body broad, blunt anterior and pointed tapering posterior end and creamy white in color when alive. Maximum width in region of ventral sucker (Figs. 1a, 2a). Cuticle smooth, aspinous. Oral sucker sub-terminal, oval, (Figs. 1b, 2a), much smaller than ventral sucker. Ventral sucker large (Figs. 1c, 2a), muscular, spherical, occasionally overlapping intestinal caeca, located slightly post-equatorial. Sucker ratio 1:3.50 (0.128–0.285 vs 0.317–0.858 mm). Pharynx muscular, well developed. Prepharynx and oesophageal pouch absent. Drüsenmagen present, connecting to intestine on either side. Esophagus bifurcating immediately posterior to pharynx (Fig. 2a). Caeca long, thick, sinuous, corrugated, run transversely, then bend along the body margin extending posteriorly to form cyclocoel in the post gonadal region, near the posterior end of body (Fig. 2a). Testes two, entire, almost equal, post-equatorial, post-acetabular, posterolateral to ventral sucker, asymmetrical/obliquely placed, round, oval or flattened and in some, transversely elongated, rarely symmetrical (Figs. 1d, 2a). Testes, usually overlap intestinal caecum of its side, left testis lying

close or slightly away from ventral sucker. Sperm duct leads to S-shaped seminal vesicle. Prostatic cells moderate. Ejaculatory duct short. Sinus sac posterior to intestinal bifurcation, saccular. Genital pore (Fig. 2b) wide, median to sub-median, ventral to pharynx, located just below the intestinal bifurcation. Sinus organ thick walled, projecting into genital atrium and may protrude outside through genital opening. Ovary single, smooth, rounded, larger than both testes, post-acetabular, post-testicular, anterior to vitelline glands, partly overlapping intestinal caecum (Figs. 1d, 2a). Ovarian complex submedian, opposite ovary. Laurer's canal long, dilated, presence of sperms suggests its role as rudimentary seminal vesicle, opens into Juel's organ, positioned anterior to vitellaria. Seminal receptacle not observed. Ootype median or sub-median, well developed Mehlis' gland with no apparent surrounding membrane (Fig. 2c). Uterus densely folded, uterine coils occupy pre and post-acetabular space, do not stretch to back of posterior part of vitelline glands, occupy space between vitelline glands and sinus sac. With maturity of worm, eggs increase in number and size, uterus becomes more folded, uterine area increases. Eggs small, elongate, numerous (Fig. 1e), thin shelled, operculated and filamented at anopercular end (Fig. 2d), more in forebody, embryonated (young embryos) but never found with miracidium larva. Egg filament short and delicate (Fig. 2d), Vitellaria asymmetrical, post-ovarian, consisting of two compact lobed masses lying diagonally or opposite in extreme hind end of body (Fig. 1d). Excretory vesicle near posterior end, divides immediately and paired lateral limbs terminate above intestine shoulder. Excretory pore terminal, opens ventrally.

Scanning electron microscopy studies (Figs. 3, 4, 5)

To further elucidate the taxonomic features of the discovered species, SEM studies were performed. The body possesses an unarmed tegument. Oral and ventral suckers equipped with papillae. Ventral sucker protrudes ventrally. Aperture of oral sucker latitudinally wide (Fig. 3a). Inner lip ($10.4 \pm 1.6 \mu\text{m}$ wide) longitudinally segmented ($8.25 \pm 1.5 \times 2.44 \pm 0.8 \mu\text{m}$ each) (Fig. 3b) bearing papillae. Cavity of oral sucker septate (Fig. 3a). Margin thrown into 8–14 pleated rows. Oral papillae single (Fig. 3a) or paired being either equal giving a beaded appearance (Fig. 3c) or unequal (Fig. 3d).

The outer rim of oral sucker presents great variety of papillae ranging from small dome-shaped (765 nm, Fig. 3e), to button-like (Fig. 3f–bp) having pores (Fig. 3f–bpp) but never stalked or ciliated, $0.77\text{--}4.70 \mu\text{m}$ in diameter. Some embedded (Fig. 4a–ep), others protrude well above tegument giving a toothed appearance (Fig. 4a–tp,) or lie inside the oral cavity (Fig. 4a, b–ocp). Pits visible

Table 1 The present species compared with *Allogenarchopsis problematica* (all measurements are in mm unless otherwise stated)

Parasite species	<i>Allogenarchopsis problematica</i>	<i>A. bareillensis</i> n. sp.
Host	<i>Tanakia lanceolata</i>	<i>Channa striatus</i>
Author/year	Urabe and Shimazu (2013)	Present species
Locality	Japan	Bareilly, India
Habitat	Intestine	Stomach
1. Body (l × b)	1.62–3.47 × 0.52–0.97	0.955–1.175 × 0.325–0.521
2. Oral sucker (l × b)	0.20–0.37 × 0.23–0.35	0.128–0.285 × 0.128–0.186
3. Ventral sucker (l × b)	0.23–0.39 × 0.26–0.45	0.317–0.858 × 0.373–0.778
4. Ratio O/A	1:1.1–1.2	1:3.5
5. Distance between oral sucker and ventral sucker	–	0.495–0.989
6. Pharynx (l × b)	0.08–0.12 × 0.07–0.11	0.041–0.065
7. Left testis (l × b)	0.11–0.22 × 0.07–0.18	0.075–0.292 × 0.090–0.340
8. Right testis (l × b)		0.072–0.248 × 0.086–0.360
9. Ovary (l × b)	0.16–0.26 × 0.14–0.22	0.173–0.198 × 0.170–0.192
10. Right vitellaria (l × b)	0.16–0.30 × 0.10–0.20	0.063–0.129 × 0.082–0.200
11. Left vitellaria (l × b)		0.092–0.189 × 0.097–0.276
12. Egg (l × b)	56–64 × 25–32 μm	28.58–48.33 × 15.24–21.62 (discharged)
13. Egg filament	1.16	20.45–38.18 μm

only under high magnification (Fig. 4c) as minute hollow structures which maybe as less as 190 nm.

The dimensions of ventral sucker cavity $80.2 \pm 4.6 \times 70.0 \pm 4.2 \mu\text{m}$ (Fig. 4d). Floor of sucker embossed with minute, smooth, button-shaped papillae and micro-ridges (Fig. 4e). Papillae either large $4.30 \pm 0.24 \mu\text{m}$ (vsp2) or small $1.90 \pm 0.02 \mu\text{m}$ (vsp1) (Fig. 4f). Rim of sucker possess a series of finger-like ridges (Fig. 5a) $4.50\text{--}7.00 \mu\text{m}$ apart each other which probably assist the fluke in attachment to the host. The surface topography of ventral sucker characteristic in having longitudinal cytoplasmic processes (lcp) in innermost margin, followed by square-shaped (scp) and diamond-shaped (dcp) processes (Fig. 5b). Longitudinal row $6.08 \pm 0.26 \mu\text{m}$, followed by 4 rows of square ($2.45 \pm 0.25 \mu\text{m}$ each; total $10.4 \pm 1.22 \mu\text{m}$) and 7 rows of diamond shaped having total width $14.5 \pm 2.22 \mu\text{m}$. Body texture (bt) around ventral sucker comprising of 8 rows of diamond-shaped processes, $12.5 \pm 14.5 \mu\text{m}$ in length. Lip of ventral sucker clearly exhibiting two types of papillae (Fig. 5b): 10 larger inner papillae and 8 smaller outer papillae. Distance between two outer papillae range from 17.9 to $65.4 \mu\text{m}$ and of inner papillae from 14.5 to $46.4 \mu\text{m}$. Inner papillae (vsp3) corrugated, uneven, oval in shape ($7.04 \times 1.06 \mu\text{m}$) with pointed end whereas outer (vsp4) smooth and circular ($4.02 \pm 0.22 \mu\text{m}$ in diameter of which approximately $0.50 \mu\text{m}$ remains embedded). Under higher magnification, sensory receptors (sr) visible, scattered between studded papillae of ventral sucker (Fig. 5c). Both types of papillae neatly studded in lip of ventral sucker (Fig. 4d). All papillae on ventral sucker without

openings. Surface tegument with transverse folds alternate with grooves. Folds just adjacent to ventral sucker broader ($2.88\text{--}3.10 \mu\text{m}$; Fig. 5d) than those at distal end ($1.50\text{--}2.15 \mu\text{m}$ Fig. 5e), former separated by grooves of variable thickness, $0.450\text{--}2.95 \mu\text{m}$. Netted structure observable in grooves at higher magnification (Fig. 5d) which can probably extend to make the body flexible for movement and attachment. Microfolds and pits not visible on tegument. Papillae randomly distributed in folds and grooves of tegument. Excretory pore at posterior extremity, opens ventrally (Fig. 5f).

Dorsal surface of body armed with papillae of various sizes (Fig. 3a inset) more abundant at anterior end, $3.27\text{--}9.02 \mu\text{m}$ in diameter. Button and domed papillae, with smooth or uneven contour and of various sizes randomly distributed on dorsal surface. Larger papillae having uneven margins unlike ventral surface papillae having a smooth contour. Papillae of different sizes visible on folds as well as in concave cavity on dorsal surface corresponding to back of ventral sucker. Cytoplasmic processes at dorsal end well marked.

Discussion

Taxonomic status of the present species

Faust (1924) named *Cercaria problematica* based on the description of larval digenean from Okayama, Japan by Kobayashi (1915). *Genarchopsis yaritanago* Shimazu et al., 2011 was reported from the intestine of *Tanakia*

Fig. 1 Light microphotographs of *Allogenarchopsis bareilliensis* n. sp. ventral surface. **a** Adult worm; **b** oral sucker; **c** ventral sucker; **d** posterior end showing cyclocoel and vitelline glands; **e** eggs in utero; **c** cyclocoel; **ep** excretory pore; **o** ovary; **os** oral sucker; **p** pharynx; **t** testis; **v** vitellaria. Scale-bars 200 μ m in **a**, **d**, 50 μ m in **c**, 100 μ m in **b**, **e**

lanceolata from Japan. Urabe and Shimazu (2013) considered *C. problematica* and *G. yaritanago* to be the same species and the new genus name *Allogenarchopsis* was erected based on the presence of Juel's organ, Laurer's canal leading into the Juel's organ and the excretory arms well separated at the intestinal shoulders. The species name was changed from *A. yaritanago* to *A. problematica* treating *G. yaritanago* as a junior synonym of *C. problematica*, according to the Law of Priority of the International Code of Zoological Nomenclature.

The present species was compared to *A. problematica* (Table 1) by light microscopy. *A. problematica* was discovered from the intestine of *T. lanceolata* and other species of Acheilognathinae from Japan, whereas the present species was from the stomach of *Channa striata* from India. Key characters differentiating the present form are

the shape of the body (broad mid body with blunt anterior and pointed posterior end), larger sucker ratio, pharynx broader than long but not barrel shaped, vitellaria smaller, egg not curved, egg size 1/3rd of *A. problematica*, relatively short egg filament, excretory vesicle U-shaped bifurcating at posterior end and terminating above intestinal shoulders.

Many *Genarchopsis* species have been described from fishes in India and adjacent countries, but in the most of them, detailed morphologies of terminal genitalia, ovary complex including Juel's organ, and excretory vesicle are not fully described. Some of them may have the characteristics of *Allogenarchopsis*, and it is obvious that they need to be re-examined both morphologically and molecularly. One of the *Genarchopsis* species in India, *G. punctati* Agrawal, 1966, shares some characteristics with

Fig. 2 *Allogenarchopsis bareilliensis* n. sp., holotype. **a** Gravid entire specimen, ventral view; **b** terminal genitalia; **c** ovarian complex; **d** freshly laid egg with egg filament (highly magnified). *c* cyclocoel, *d* drusenmagen, *e* egg, *ed* ejaculatory duct, *ep* excretory pore, *ev* excretory vesicle, *f* filament, *ga* genital atrium, *gp* genital pore, *hd* hermaphroditic duct, *jo* Juel's organ, *lc* laurer's canal, *m* metraterm, *mg* mehlis gland, *op* operculum, *os* oral sucker, *ot* ootype, *o* ovary, *pc* prostate cells, *pp* pars prostatica, *sd* sperm duct, *so* sinus organ, *ss* sinus sac, *sv* seminal vesicle, *t* testis, *u* uterus, *v* vitellaria, *vs* ventral sucker. Scale-bars 0.2 mm in **a**, free hand sketches not to scale in **b**, **c**, 20.0 μ m in **d**

Allogenarchopsis, as pointed out by Urabe and Shimazu (2013). It has the Juel's organ (Anjaneyulu 1967; Madhavi and Rao 1974) and may belong to *Allogenarchopsis*. However, *G. punctati* has an oesophageal pouch and Y-shaped excretory vesicle (Agrawal 1966), which differs from that of the present species.

Based on the above characteristics, the trematode collected from the stomach of *C. striata* is placed in the genus *Allogenarchopsis* Urabe and Shimazu, 2013 as a new species. The morphology described by the light microscopic observation and by SEM studies clearly indicate that it is a new species of the genus, and the name *Allogenarchopsis barielliensis* n. sp. is proposed to accommodate the species.

Based on the present study, the generic diagnosis of the genus *Allogenarchopsis* is revised as follows:

Generic Diagnosis: *Allogenarchopsis* Urabe and Shimazu, 2013

Type species: *Allogenarchopsis problematica* (Faust 1924) Urabe and Shimazu, 2013

Digenea, Derogenidae, Haliipeginae. Body spindle-shaped, small; tegument smooth. Oral and ventral suckers well developed; ventral sucker a little posterior to middle of body. Prepharynx absent. Pharynx well developed. Esophagus inverted Y-shaped, small, with or without ventral pouch. Drüsenmagen present. Intestines forming cyclocoel near posterior extremity of body. Testes two, entire, almost symmetrical, lateral, between ventral sucker and ovary. Seminal vesicle coiled, small. Pars prostatica vesicular; prostatic cells well developed. Ejaculatory duct short, uniting with metraterm to form short hermaphroditic duct

Fig. 3 Scanning electron micrographs of *Allogenarchopsis bareillensis* n. sp. ventral surface. **a** Oral sucker, *inset* showing anterior end of dorsal surface; **b** inner rim of oral sucker (*fine arrow* indicates dome-shaped oral cavity papilla, *thick arrow*, longitudinal segments of rim); **c** pleated margin of oral cavity with paired papillae (beaded appearance) (*inset* showing pleated margin); **d** papillae around the oral sucker opening showing unequal and single papillae; **e** outer rim of oral sucker showing dome-shaped papillae; **f** outer rim of oral sucker showing button-like papillae with and without pores. *bp* button-like papillae (without pores), *bpp* button-like papillae (with pores), *dsp* dome-shaped papillae, *pp* paired papillae, *ppu* unequal papillae, *sp* single papillae. Scale-bars 20 μ m in **a**, 5 μ m in **b**, **d–f**, 2 μ m in **c**, 5 μ m in *inset*

running in sinus organ and opening on its apex. Sinus sac membranous, enclosing seminal vesicle, prostatic complex, ejaculatory duct and distal part of metraterm. Sinus organ permanent, conical, small. Genital atrium tubular. Genital pore median, slightly posterior or ventral to esophagus. Ovary entire, lateral, post-testicular. Laurer's canal dilated, including sperm as rudimentary seminal receptacle, leading into Juel's organ. Juel's Mehlis' gland organ anterior to vitellaria, in dorsal parenchyma. Blind or canalicular seminal receptacle absent. Ootype submedian or rarely median; Mehlis' gland well developed, free in parenchyma, without any surrounding membrane. Uterus much coiled in all available field between vitellaria and sinus sac;

metraterm long, folded in forebody; uterine seminal receptacle present. Eggs numerous, operculate, fully embryonated, with long or short anopercular filament. Vitellaria two, entire, symmetrical or diagonal, close together near posterior extremity of body. Excretory vesicle Y- or U-shaped, bifurcating in hind body; arms well separated at near intestinal shoulders. Parasitic in alimentary canals of freshwater fishes.

Fine structure of body surface

The tegument is known to be a dynamic living cellular structure consisting of proteins, lipids, carbohydrates and

Fig. 4 Scanning electron micrographs of *Allogenarchopsis bareillensis* n. sp. ventral surface. **a** Surface view of oral sucker showing different kinds of papillae; **b** magnified view of oral sucker rim; **c** pits; **d** ventral sucker; **e** floor of ventral sucker showing microridges and papillae; **f** higher magnification of floor of ventral sucker showing microridges and large papilla (*inset* showing small papilla). *bp* button-like papillae (without pores), *ep* embedded papilla, *mr* microridges, *ocp* oral cavity papilla, *p* pits, *tp* toothed papilla, *vsp1* large sized papilla, *vsp2* small sized papilla. Scale-bars 10 μ m in **a**, **b**, **f**, 2 μ m in **c**, 50 μ m in **d**, 20 μ m in **e**

RNA. Ornamentations such as spines, sensory papillae and receptors, folds, grooves, pits and ridges of various configurations (Fried 1997; Schmidt and Roberts 2000) may be present on the trematode tegument. However, there are no spines on the tegument of *Allogenarchopsis*. Surface folds, grooves, sensory receptors and papillae, pits are the main observed features of the present worm.

The surface of *Allogenarchopsis* is encircled by tegumental folds or ridges as reported in Gorgoderioidea (e.g. *Gorgoderina attenuata*: Nadakavukaren and Nollen 1975; *Concinnum epomopis*: Otubanjo 1985; *Phyllodistomum umblae*: Bakke and Bailey 1987), Brachylaimoidea (*Leucochloridium* sp.: Bakke 1976) and Paramphistomoidea

(*Zygocotylelunata*: Sotillo et al. 2012). In *Pseudoplagiorus interruptus* (Family Opecoelidae), folds on the tegument overlying the suckers may result from body flexure and movements of the oral sucker itself (Abdou et al. 2001). Tegumentary ridges are caused by contraction waves of the internal musculature of the fluke (Otubanjo 1985) and may increase the surface area for absorptive functions assisting in micro-molecular nutrient exchange, ionic and osmotic regulations, immune responses and excretion (Balasubramanyam and Ramasamy 2010).

Tegumental sensory receptors help the parasite to establish and maintain itself in the body of the host at a site of its choice through perception of information from the

Fig. 5 Scanning electron micrographs of *Allogenarchopsis bareilliensis* n. sp. **a** Rim of ventral sucker showing finger-like ridges; **b** lip of ventral sucker showing texture and papillae; **c** higher magnification of lip showing papillae and sensory receptors; **d** body folds adjacent to ventral sucker with netted grooves; **e** body folds at distal end of body, *inset* showing dorsal surface folds; **f** posterior end showing excretory pore. *bt* body texture, *dcp* diamond-shaped cytoplasmic processes, *fl* body folds, *fr* finger-like ridges, *gr* grooves, *lcp* longitudinal cytoplasmic process, *scp* square-shaped cytoplasmic process, *sr* sensory receptors, *vsp3* corrugated large sized papilla, *vsp4* smooth, circular small sized papilla. Scale-bars 10 μ m in **a**, **b**, 5 μ m in **c–e**; 20 μ m in **f**

surrounding environment (Ip and Desser 1984; Chai et al. 1992). The sensory receptors in *Allogenarchopsis* are scattered between the two rows of papillae on the ventral sucker and the flexibility of the ventral sucker armed with sensory receptors may contribute in sensing a suitable site for attachment in the host body.

A mid body depression with ventral sucker was reported in *Pseudoamphistomum truncatum* (Heckmann and Halajian 2012) but in the present trematode, the ventral sucker area was extended (Fig. 4d). This characteristic is not described in *A. problematica*, but in some other genera such as *Genarchopsis* (Shimazu 1995).

Sensory papillae present on the rim of the oral and ventral suckers show variable morphology and distribution. These characteristics are similar to other Hemiuroidea

(*Derogenes varicus*: Koie 1979, *Hemiurus luehei*: Koie 1990). Button-like papillae are distributed in singles or doubles in Gorgoderioidea (*Gorgoderina attenuata*: Nadakavukaren and Nollen 1975; *G. attenuata*, *G. bilobata*, *G. diaster*, *G. megalorchis*, *G. parvicava*, *G. megac-etabulasis*: Mata-Lopez and Leon-Regagnon 2006) as is the case in the present parasite. However, pores on spongy oral sucker of Plagiorchiida (*Haematoloechus complexus*: Whitehouse 2002) appear to be absent in Hemiuroidea. Finger-like ridges are characteristic in the rim of ventral sucker of the present fluke may serve for attachment as reported by Heckmann and Halajian (2012) in *P. truncatum*.

Cobblestone-like cytoplasmic processes of the ventral sucker were described earlier (Lee et al. 1984, 1987; Chai

et al. 1992) but the characteristic multi-texturized cytoplasmic processes (Fig. 5b) of *Allogenarchopsis* appear to be unique. Functionally, these processes may serve to increase the surface area for absorption or by its rough surface, the ventral sucker of the parasite could attach itself to the intestinal villi of the host (Nadakavukaren and Nollen 1975).

The pattern of distribution of papillae may differ in different trematodes and their possible taxonomic value has often been emphasized. This character has often been used for their classification (Eduardo 1982, 1984; Mata-Lopez and Leon-Regagnon 2006). Up to 4 types of papillae (button, ciliated, rosette, and domed papillae) were described by Hoole and Mitchell (1981), Bakke and Hoole (1988) and Mata-Lopez and Leon-Regagnon (2006) in various trematodes based on their shape. Eduardo (1982) emphasized the role of papillae in taxonomy and described up to 6 different types of papillae in paramphistomids (dome to conical non-ciliated papillae, dome to conical ciliated papillae, short and stumpy papillae covered by hair-like processes, long non-branching and non-ciliated papillae, long papillae with simple unciliated branches, and long papillae with bulb-like multiciliated branches). Domed aciliate papillae on the surface appear to be common for Brachylaimoida (*Leucochloridium* sp.) and Gorgoderioidea (*Phyllodistomum conostomum*) (Bakke 1976). However, the papillae of the oral sucker of *Schistosoma mansoni* were wart-like cuticular bosses (Miller et al. 1972), and spherical surface elevations in *Gorgoderina attenuata* (Nadakavukaren and Nollen 1975). Papillae on the trematode surface may have a regular pattern of distribution: arranged in circles; lines; or in symmetrical groups: *P. conostomum*; *Fibricola seoulensis* (Seo et al. 1984); *Phyllodistomum umblae* (Bakke and Bailey 1987); *Handerophyes nocens* (Chai et al. 1992) and *Echinostoma cinandorchis* (Lee et al. 1992). However, it may also be random: *G. attenuata* (Nadakavukaren and Nollen 1975); and *Leucochloridium* sp. (Bakke 1976).

The present study is the first report on the tegumental surface of *Allogenarchopsis*. Absence of spines, presence of variegated, randomly dispersed papillae and annular plications as observed through SEM represent diagnostic characters of *Allogenarchopsis* as present in other hemiurids. However unstalked and non-ciliated papillae, presence of single or paired papillae, minute pits, characteristic topography of cytoplasmic processes of ventral sucker and the ultra measurements provided herein maybe regarded as species specific taxonomic criteria for differentiation of the new species of *Allogenarchopsis*.

Acknowledgments This work was supported by the Government of Uttar Pradesh, Lucknow, India under the “Centre of Excellence” (sanction orders no 138/70-4-2011-46(32)/2009 and 150/70-4-2012-

46(43)/2010) who provided financial assistance to the first author for conducting research on parasite-fauna of fishes of Rohilkhand.

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