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Two New and Two Poorly Known Species of *Ancistrum* (Ciliophora, Scuticociliatia, Thigmotrichida) Parasitizing Marine Molluscs from Chinese Coastal Waters of the Yellow Sea

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Abstract. The morphology and taxonomy of two new and two poorly known ciliate species of *Ancistrum*, found in the mantle cavity (mainly on gills) of marine molluscs from culture beds and pools along the Chinese coast of the Yellow Sea, were investigated using living observation and silver impregnation. *Ancistrum haliotis* n. sp. was isolated from the abalone *Haliotis discus hannai* Ino, *A. crassum* Fenchel, 1965 from the purple clam *Saxidomus purpuratus* (Sowerby), *A. acutum* n. sp. from the surf clam *Mactra veneriformis* Reeve, and *A. japonicum* Uyemura, 1937 from both the venus clam *Cyclina sinensis* (Gmelin) and from *Dosinia japonica* (Reeve). *Ancistrum haliotis* differs from its most similar relative *A. mytili* (Quennerstedt, 1867) by the body outline (anterior portion narrower vs. wider than the posterior portion), the macronuclear shape (broadly ellipsoidal vs. reniform or sausage-like), and by having fewer somatic kineties (28–32 vs. usually more than >40). *Ancistrum crassum* is characterized by the naked area at the apical end of the cell, the relatively short buccal field occupying about two thirds of the body length, and the posterior-dorsal cone-shaped prolongation. *Ancistrum acutum* n. sp. and *A. japonicum* are almost identical in morphometry, but differ distinctly in the live morphology (anterior end pointed and posterior end rounded vs. anterior end narrowly rounded and posterior-dorsal end protruded) and ciliary pattern (all right-side kineties extend to posterior body end vs. all right-side kineties excluding somatic kinety 1 distinctly shortened posteriad, forming a glabrous zone). We neotypify *Ancistrum japonicum* and discuss the taxonomic status of the four species. Based on an evaluation of all nominal species of *Ancistrum* and *Ancistrumina*, we recognize nine valid species of *Ancistrum* and provide a tabular guide to their identification. *Fenchelia* Raabe, 1970 is regarded as a junior synonym of *Ancistrum* Maupas, 1883. We synonymize *Ancistrumina nucellae* Khan, 1970 with *Ancistrum japonicum* Uyemura, 1937 and *Ancistrum edajimanum* Oishi, 1978 with *A. crassum* Fenchel, 1965.

Key words: *Ancistrum haliotis* n. sp., *Ancistrum crassum*, *Ancistrum acutum* n. sp., *Ancistrum japonicum*, marine parasitic ciliate, morphology, neotype, new species, taxonomy.

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INTRODUCTION

The ciliate subclass Scuticociliatia comprises the orders Philasterida, Pleuronematida and Thigmotrichida (Lynn 2008). The first two orders include generally free-living and facultatively parasitic species inhabiting various, mainly marine, aquatic environments, whereas Thigmotrichida contains predominantly symbiotic forms parasitizing invertebrates, primarily in the mantle cavity of marine molluscs (Corliss 1979). Recent morphological and molecular investigations have uncovered an unexpectedly high diversity of scuticociliates in marine environments (Miao *et al.* 2010, Fan *et al.* 2011, Pan *et al.* 2013). Consequently, it is reasonable to expect that there is still a large undiscovered diversity of marine scuticociliates.

Classically, the order Thigmotrichida is a heterogeneous group characterized by thigmotrich cilia and composed of a variety of orders according to modern ciliate classification schemes (Raabe 1967, 1970, Corliss 1979, Lynn 2008). Recent phylogenetic analyses indicate a close relationship between Thigmotrichida and Pleuronematida within the subclass Scuticociliatia and the monophyly of Thigmotrichida in which two representative genera, *Ancistrum* and *Boveria,* were closely related (Gao *et al.* 2010, 2012a, b).

The thigmotrichid genus *Ancistrum* Maupas, 1883 is characterized by the oral ciliature starting from the anterior body portion and making a conspicuous loop near the posterior pole, where the mouth is situated (Raabe 1959, 1970). This genus has had a complicated history since it was established. Hatzidimitriou and Berger (1977) presented a historic review of the taxonomy of *Ancistrum*. Members of *Ancistrum*, which are mainly found in marine and freshwater habitats as symbionts in the mantle cavity and on the gills of molluscs, have attracted the attention of some distinguished researchers (e.g. Chatton and Lwoff 1949, Fenchel 1965, Raabe 1970). However, few of these studies were carried out using modern methods, hence identity of many species remains ambiguous.

During surveys of the parasites of marine molluscs from the Yellow Sea coast of the northeast of China, we isolated two new and two poorly known species of *Ancistrum.* In this paper the morphology and morphometry of the four species are provided and the taxonomic status of each is discussed.

MATERIALS AND METHODS

Specimens of *Ancistrum* were isolated from the gills and mantle cavity of host molluscs from marine culture beds and pools along the Yellow Sea coast of the Shandong peninsula, northern China. *Ancistrum haliotis* n. sp. was isolated from the abalone *Haliotis discus hannai* Ino at the Qingdao coast (36°08′N, 120°43′E). *Ancistrum crassum* Fenchel, 1965 was isolated from the purple clam *Saxidomus purpuratus* (Sowerby) at the Rongcheng coast (37°15′N, 122°49′E) and was also frequently found in the short-necked clam *Ruditapes philippinarum* (Adams). *Ancistrum acutum* n. sp. was isolated from the surf clam *Mactra veneriformis* Reeve, and *A. japonicum* Uyemura, 1937 from the venus clam *Cyclina sinensis* (Gmelin), both at the Rizhao coast (35°40′N, 119°57′E). *Ancistrum japonicum* was also isolated from the Japanese Dosinia *Dosinia japonica* (Reeve) at the Rongcheng coast.

The host molluscs were maintained in the laboratory for examination. The fluid of the mantle cavity was first examined for ciliates, and then the gills were removed and washed with sterilized seawater in a Petri dish. The ciliates were isolated with a micropipette and counted under a dissecting microscope. For each mollusc examined, the intensity of *Ancistrum* infection was estimated semi-quantitatively by assigning values ranging from <20 individuals (light intensity of infestation), through 20–100 (moderate intensity), to > 100 individuals (heavy intensity) on a single host.

Live ciliates were observed by differential interference contrast microscopy. The protargol stain (Wilbert 1975) was used to reveal the infraciliature and nuclear apparatus. The wet silver nitrate method, as described in Foissner (1991), was used to reveal the silverline system. *In vivo* measurements were conducted at magnifications of 100–1,000 ×. Counts and measurements of silvered specimens were performed at a magnification of 1,000 ×. Drawings of live cells were based on free-hand sketches; those of silvered specimens were made with the help of a drawing device. Systematics follows Lynn (2008). Terminology is mainly according to Raabe (1970) and Corliss (1979).

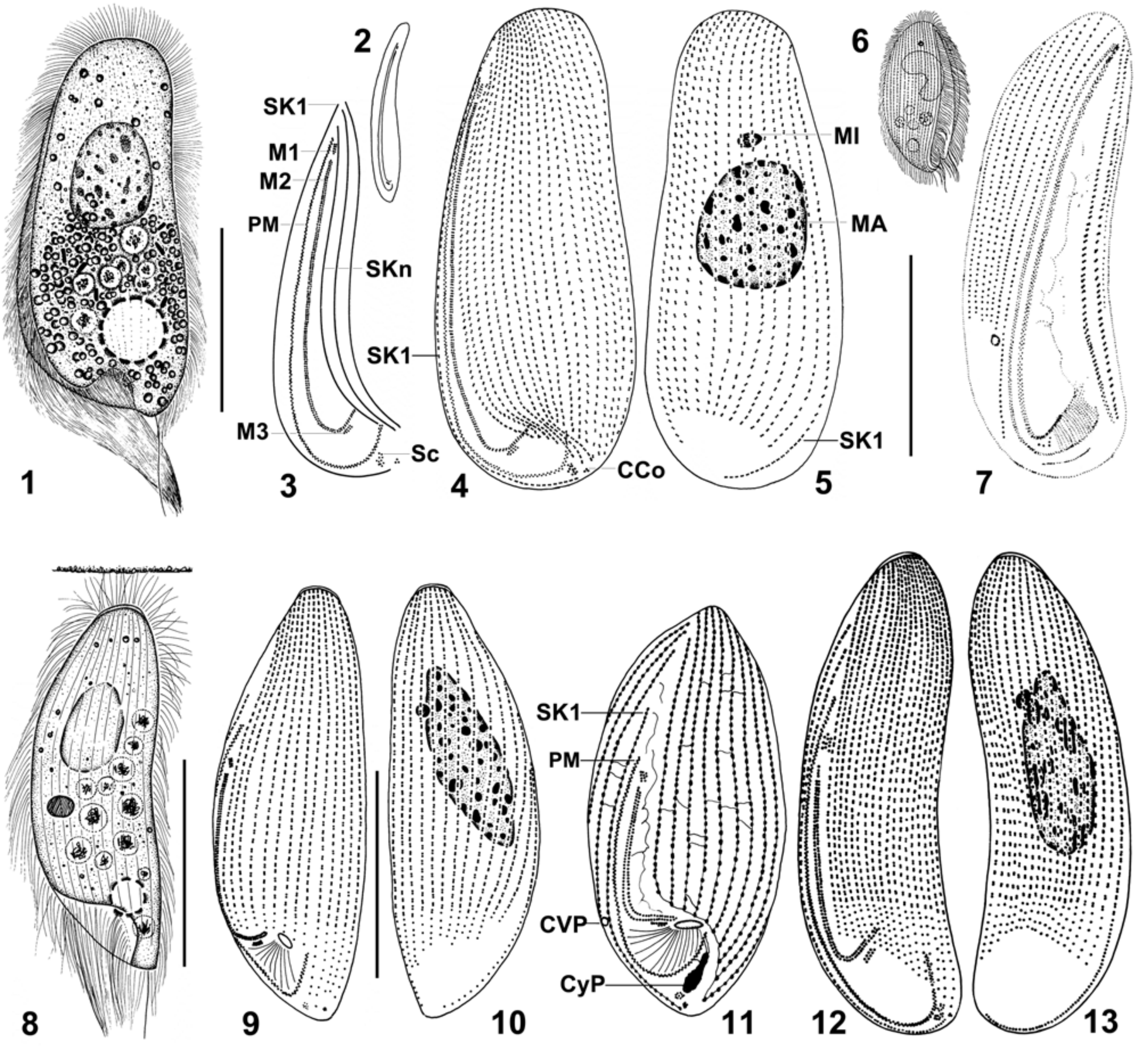
RESULTS

Order Thigmotrichida Chatton and Lwoff, 1922
Family Ancistridae Issel, 1903
Genus *Ancistrum* Maupas, 1883
Syns. *Ancistruma* Strand, 1926; *Fenchelia* Raabe, 1970

Ancistrum haliotis n. sp. (Figs 1–5; Table 1)

Nomenclature: *Ancistrum haliotis* Xu, Song and Warren, 2003 in Song *et al.* (2003, p. 129) is invalid because it was not indicated as a new species (see Article 16.1 of the ICZN 1999).

Diagnosis: Size about 70 × 25 µm *in vivo*; body more or less quadrilateral with anterior end narrower than posterior end. One ellipsoidal macronucleus and



Figs 1−13. *Ancistrum haliotis* n. sp. from the abalone *Haliotis discus hannai* Ino (1–5), *Ancistrum mytili* (Quennerstedt, 1867) from the blue mussel *Mytilus edulis* (6, after Kidder 1933) and the horse mussel *Modiolus modiolus* (7, after Hatzidimitriou and Berger 1977) and *Ancistrum crassum* Fenchel, 1965 from the purple clam *Saxidomus purpuratus* (Sowerby) (8–11) and from the short-necked clam *Ruditapes philippinarum* (12, 13, after Xu *et al.* 1997), from life (1, 2, 6, 8) and after protargol (3–5, 9, 10, 12, 13) and silver nitrate impregnation (7, 11). **1** – left lateral view of a representative specimen; **2, 3** – ventral view to show the oral structure; **4, 5** – left and right lateral view of the holotype specimen; **6, 7** – lateral and ventral view of *A. mytili*, which possesses a characteristic reniform macronucleus and a broad buccal field; **8** – left lateral view of body variants; **9–13** – lateral and ventral view of three specimens to show the ciliary pattern. CCo – caudal complex; CyP – cytoproct; M1–3 – membranelles 1–3; MA – macronucleus; MI – micronucleus; PM – paroral membrane; Sc – scutica; SK1, n – somatic kineties 1, n. Scale bars: 30 µm (4, 5 and 7, 9–13 drawn to scale).

Table 1. Morphometric data on *Ancistrum haliotis* n. sp. from *Haliotis discus hannai* (first line), *A. crassum* from *Saxidomus purpuratus* (second line), *A. japonicum* (Uyemura, 1937) n. comb. from *Cyclina sinensis* (third line), and *A. acutum* n. sp. from *Mactra veneriformis* (fourth line).

Characteristics[a]	Min	Max	Mean	SD	SE	CV	n
Body, length	65	75	70.1	2.4	0.6	3.5	18
	54	72	62.6	5.5	1.5	8.7	14
	71	95	83.4	6.3	1.8	7.6	12
	79	93	85.3	4.5	1.2	5.2	14
Body, width	18	31	25.0	3.7	0.9	14.9	18
	20	33	22.7	3.4	0.9	14.9	14
	18	30	25.2	3.8	1.1	14.9	12
	31	42	36.4	3.4	0.9	9.3	14
Buccal area, length	59	64	61.7	2.1	0.5	3.3	15
	32	45	39.2	4.0	0.3	10.2	14
	60	73	67.5	4.0	1.6	5.9	10
	60	67	64.0	3.1	0.8	4.9	14
Macronucleus, number	1	1	1	0	0	0	50
	1	1	1	0	0	0	50
	1	1	1	0	0	0	50
	1	1	1	0	0	0	50
Macronucleus, length	14	22	18.3	2.4	0.6	13.2	16
	20	30	23.2	2.7	0.7	11.6	14
	17	30	25.5	3.7	1.1	14.6	12
	20	25	22.7	1.7	0.5	7.6	12
Macronucleus, width	9	18	13.9	2.8	0.7	20.0	16
	7	16	10.4	3.5	0.9	33.8	14
	11	23	15.5	3.2	0.9	20.9	12
	17	22	18.5	1.6	0.5	8.5	12
Somatic kineties, number	28	32	30.4	1.3	0.3	4.3	20
	27	33	29.1	1.5	0.4	5.3	14
	18	21	19.9	1.3	0.4	6.5	10
	21	25	23.2	1.4	0.4	6.0	12

[a] Data based on protargol-stained specimens from field samples. Measurements in µm. CV – coefficient of variation in %; Min – minimum; Max – maximum; M – median; SD – standard deviation; n – number of individuals analyzed.

one micronucleus. On average 30 somatic kineties, with several ventral-left kineties distinctly curved near posterior end of cell, right kineties distinctly shortened in posterior region of cell forming a distinct glabrous area. Buccal area extending to posterior pole and occupying on average 88% of body length. Three membranelles each composed of two rows of kinetosomes: membranelles 1 and 3 short; membranelle 2 occupying about 70% of body length.

Type host and site: Abalone *Haliotis discus hannai* Ino, gills and mantle cavity.

Type locality: Marine culture pools on the Yellow Sea coast at Qingdao, northern China (36°08′N, 120°43′E).

Type material: A protargol slide with the holotype specimen circled in ink is deposited in the Laboratory of Protozoology, Ocean University of China with registration number MD-951022-01. A protargol slide with paratype specimens is deposited in the Marine Biological Museum, Chinese Academy of Sciences (Qingdao) with registration number MD-951022-03. A second paratype slide from the same series is deposited in the Natural History Museum, London with registration number NHMUK 2014.4.8.1.

Etymology: Named after the generic name of the type host *Haliotis discus hannai*. We have retained the species name originally designated by Xu *et al.* (2003) so as not to inflate the number of ciliate species names.

Description: Size *in vivo* 50–75 × 20–30 µm, usually about 70 × 25 µm, bilaterally flattened about 2 : 1 (Table 1). Body more or less quadrilateral in lateral view, with anterior end narrower than posterior end, widest at posterior quarter of body; ventral margin slightly convex, dorsal margin slightly indented at anterior third of body and slightly projected at caudal pole, where the caudal cilium is located (Figs 1, 2). Single macronucleus broadly ellipsoidal, about 18 × 14 µm in protargol-stained specimens, with roundish nucleoli. One ellipsoidal micronucleus positioned anterior to macronucleus (Fig. 5). Contractile vacuole positioned anterior to cytostome. Cortex inflexible and no cortical granules recognizable. Cytoplasm hyaline in anterior half of body, but often opaque in posterior half due to accumulation of numerous food vacuoles and lipid droplets about 5 µm across, which made the cytoplasm appear dark when viewed at low magnification using bright field microscopy. Movement relatively slow, by rotation about main body axis; cells usually attached at an angle of approximately 45° to substrate via the anterior thigmotactic ciliature.

On average 30 (28–32) somatic kineties, slightly more on left than on right side, composed mostly of dikinetids mixed occasionally with monokinetids; cilia about 10 µm long; anterior left cilia densely arranged and rigid, forming a thigmotactic area. Kineties terminate at anterior end, except for several ventral preoral kineties (somatic kineties 1, n and adjacent kineties) which progressively shorten to form an anterior suture. Somatic kinety 1 (SK1) closely parallels paroral membrane (PM) along its entire length diverging only at posterior end (Figs 3–5). Usually six ventral-left kineties conspicuously curved towards dorsal posterior end of cell; about 12 right kineties shortened posteriorly, forming a distinct glabrous area in posterior 1/5 of cell (Figs 4, 5).

Buccal field of typical generic pattern, occupying on average 88% of body length. Oral cilia about 10 µm long anteriorly, increasing to about 25 µm long in posterior region where they form a conspicuous brush-like structure (Figs 1–5). Membranelles 1 (M1) and 3 (M3) of similar length, each composed of about 10 kinetosomes arranged in two short rows; membranelle 2 (M2) L-shaped, with two longitudinal rows of kinetosomes, occupying about 70% of body length. Paroral membrane composed of a zigzag file of dikinetids, very prominent, occupying about 85% of body length, extends slightly beyond M1 anteriorly and terminates near posterior end of body. Scutica patch-like, comprising six pairs of kinetosomes, located close to caudal cilium complex (CCo) which bears a single stiff caudal cilium about 15 µm long (Figs 1, 3, 4).

Occurrence and prevalence: *Ancistrum haliotis* has been found only on the gills and in the mantle cavity of the host abalone *Haliotis discus hannai* Ino. It was first isolated from the abalone in marine culture pools of Qingdao, China, in October 1995, when the water temperature was 21.8°C, salinity of 31.1 and pH at 8.0. It was found in 20 out of 20 specimens of the host mollusc examined (i.e., the prevalence was 20/20) and the intensity of infestation was heavy. We have not found this ciliate parasitizing other host molluscs.

Ancistrum crassum Fenchel, 1965 (Figs 8–13; Table 1)

Host, site and locality. Purple clam *Saxidomus purpuratus* (Sowerby) from the Yellow Sea coast at Rongcheng, northern China; gills and mantle cavity.

Voucher specimens. Two voucher slides with silver stained specimens, i.e. one wet-silver (RC-980430-01) and the other protargol (RC-980430-02), are deposited in the Laboratory of Protozoology, Ocean University of China.

Redescription. Size *in vivo* 50–70 × 15–25 µm, usually about 60 × 20 µm; ratio of length to width usually about 3 : 1, bilaterally flattened about 2 : 1. Body elongate-ellipsoidal with ventral margin convex and dorsal margin concave in the middle portion; anterior end narrowly rounded with a naked area, which is recognizable both *in vivo* and in protargol-stained specimens; posterior-dorsal portion forms a cone-shaped prolongation (Fig. 8). Single macronucleus ovoid or elongate-ellipsoidal, about 23 × 10 µm in protargol-stained specimens (Table 1). One micronucleus, globular to ellipsoidal, frequently positioned anterior-dorsal to macronucleus (Fig. 10). Contractile vacuole located anterior to cytostome near ventral side, 5–6 µm across. Cortex thick, with cortical granules both in clusters and loosely arranged along ciliary rows. Cytoplasm usually hyaline in anterior half of body and opaque in posterior half due to accumulation of many food vacuoles, about 6 µm across, containing bacteria-like remnants. Movement by rotation about main body axis; cells may attach to substrate via anterior thigmotactic area.

On average 23 (20–33) somatic kineties evenly distributed on left and right sides, composed primarily of dikinetids in area above cytostome; somatic cilia about 8 µm long; thigmotactic area indistinct because anterior cilia are less densely arranged (Figs 8–10). Somatic kineties mostly extending to anterior end of cell forming an imbricate structure; several right-ventral preoral kineties which are progressively shortened anteriorly; in posterior region of cell, all kineties excluding SK1 are distinctly shortened forming a distinct glabrous zone on both left and right sides (Figs 9, 10).

Buccal field of typical generic pattern, occupying about 63% of body length. Oral cilia about 16 µm long *in vivo* in anterior region, increasing to about 25–30 µm long in posterior region where they form a conspicuous brush-like structure (Fig. 8). M1 short, composed of two or three rows of kinetosomes; M2 L-shaped, composed of two longitudinal rows often with several single-rowed kinetosomes anteriorly, occupying slightly more than one third of body length; M3 near end of M2, of similar length to M1, composed of 10 kinetosomes arranged in three oblique rows. Paroral membrane (PM) lies parallel to somatic kinety 1, composed of a zigzag file of dikinetids, extending slightly beyond M1 anteriorly and terminating posteriorly near caudal pole of body. Cytostome at base of PM and M2, ellipsoidal in outline, connected with radially arranged fibers (Figs 9, 11). Scutica patch-like, located close to caudal cilium complex which bears a single caudal cilium about 18 µm long (Figs 10, 11).

Silverline system as depicted in Fig. 11. Transverse silverlines sparsely distributed between somatic kineties, all of which are joined by silverlines at posterior end; longitudinal silverlines present in buccal area. Cytopyge (Cyp) underneath paroral membrane, connected with PM by silverlines. Single contractile vacuole pore (CVP) located near posterior end of somatic kinety 2 (Fig. 11).

Occurrence and prevalence: The population described here was found in 1998 from the purple clam *Saxidomus purpuratus* on the Rongcheng coast of the Yellow Sea, where the water temperature was about 14.8°C and salinity about 28.The prevalence was 10/10 and the infestation was moderate to heavy. It co-occurred with an ectoparasitic ciliate, *Trichodina ruditapicis*, on the same host, the prevalence of which was 8/10 with low intensity.

Ancistrum crassum was originally found by Fenchel (1965) in high numbers in the European aurora venus clam *Venerupis aurea* (Gmelin) with prevalence of 16/20, and in a specimen of *V. pullastra* (Montague) from the Øresund coast of the Baltic Sea. This species was not subsequently reported until Xu *et al.* (1997) isolated it in April 1995 from the short-necked clam *Ruditapes philippinarum* (Adams) from the Qingdao coast of the Yellow Sea, where the water temperature was about 10°C, salinity about 32 and pH 8.0. The prevalence was 50/50 and the intensity of infestation was moderate to high. Later, Song (2000) redescribed *A. crassum* based on the Qingdao population parasitizing *Ruditapes philippinarum* which he mistakenly identified as *Haliotis discus hannai* Ino. We have repeatedly found *Ancistrum crassum* parasitizing *Ruditapes philippinarum* from the China coast of the Yellow Sea (unpublished data). Xu and Song (2003) also recorded it from the venus clam *Protothaca jedonensis* (Lischke) and the purple clam *Saxidomus purpuratus* (Sowerby). However, the host record of *Ruditapes variegata* was misidentified as *Ruditapes philippinarum*, and the host record of the small sand clam *Caecella chinensis* Deshayes needs reconsideration. The population described by Oishi (1978) under the name *Ancistrum edajimanum* from *Ruditapes philippinarum* in Japan is likely also to be *A. crassum* (see Discussion below).

Ancistrum acutum n. sp. (Figs 14–17; Table 1)

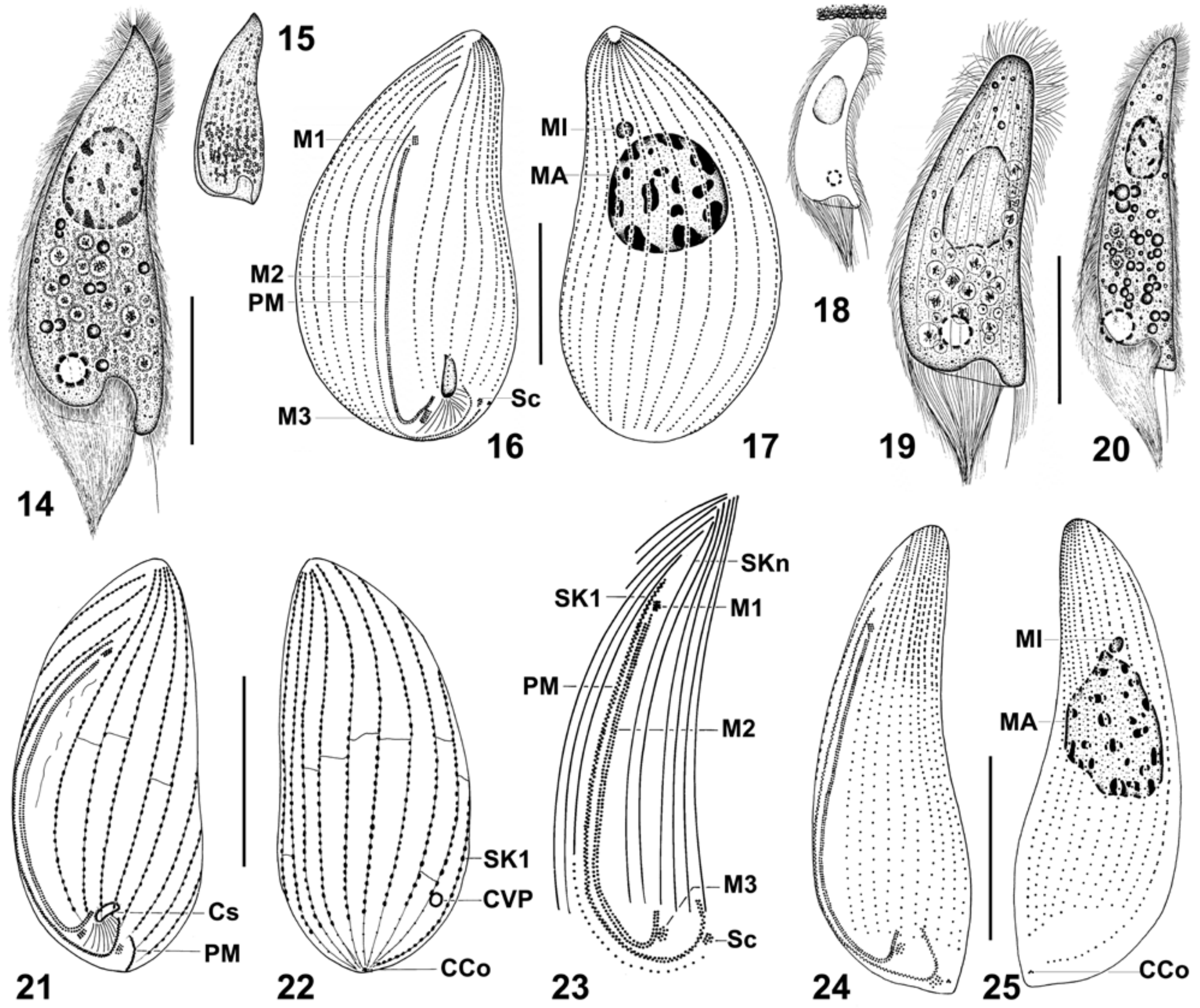
Nomenclature: *Ancistrum acutum* Xu, Songand Warren, 2002 in Song *et al.* (2003, p. 132) is invalid because it is not indicated as a new species (see Article 16.1 of the ICZN 1999).

Diagnosis: Size about 90 × 30 µm *in vivo*; body triangular with pointed anterior end. One ellipsoidal to globular macronucleus and one micronucleus. On average 23 somatic kineties, most extending to caudal pole. Buccal area extending to caudal pole and occupying about 3/4 of body length. Membranelles 1 and 3 short, each composed of three rows of kinetosomes; M2 composed of two rows and occupying about 2/3 of body length.

Type host and site: Marine surf clam *Mactra veneriformis* Reeve, gills and mantle cavity.

Type locality: Marine culture beds at Rizhao on the Yellow Sea coast of northern China (35°40′N, 119°57′E).

Type material: A wet-silver slide with the holotype specimen encircled in ink is deposited in the Laboratory of Protozoology, Ocean University of China, with



Figs 14–25. *Ancistrum acutum* n. sp. from the surf clam *Mactra veneriformis* (14–17) and *Ancistrum japonicum* Uyemura, 1937 from the Japanese dosinia *Dosinia japonica* (18, 19, 21, 22) and the clam *Cyclina sinensis* (20, 23–25), from life (14, 15, 18–20) and after protargol (23–25) and silver nitrate impregnation (16, 17, 21, 22). **14** – left lateral view of a representative specimen; **15** – body variant and cortical granules; **16, 17** – ventral and dorsal view of the holotype specimen; **18, 19** – lateral view of living cells; **20** – lateral view of a representative specimen; **21, 22** – lateral view of same specimen; **23** – ventral ciliature; **24, 25** – lateral view of the neotype specimen. CCo – caudal complex; Cs – cytostome; CVP – contractile vacuole pore; M1–3 – membranelles 1–3; MA – macronucleus; MI – micronucleus; PM – paroral membrane; Sc – scutica; SK1, n – somatic kineties 1, n. Scale bars: 30 µm.

registration number RZ-950428-32. A protargol slide with paratype specimens is deposited in the Natural History Museum, London with registration number NHMUK 2014.4.8.2. A voucher slide with protargol-stained specimens from the host mollusc *Mactra chinensis* is deposited in the Marine Biological Museum, Chinese Academy of Sciences (Qingdao) with registration number QD-971204.

Etymology: The Latin adjective *acutus* (acute) refers to the pointed anterior end of body, a main feature of the species. We have retained the species name originally designated by Xu *et al.* (2003) so as not to inflate the number of ciliate species names.

Description: Size *in vivo* 80–110 × 25–35 µm, usually about 90 × 30 µm, while on average 85 × 36 µm in protargol-stained specimens, suggesting a slight inflation of the body during the fixation/staining process (Table 1). Body more or less triangular in lateral view, with anterior end pointed and posterior end rounded or slightly truncate, widest at posterior third of body; ventral margin slightly convex, dorsal margin sigmoid at anterior two thirds of body (Figs 14–17). Single macronucleus usually located in anterior half of body, broadly ellipsoidal, about 23 × 19 µm in protargol-stained specimens. One globular to ellipsoidal micronucleus positioned anterior to macronucleus (Fig. 17). Contractile vacuole located anterior to cytostome near the ventral side. Cortex flexible, with cortical granules loosely arranged in rows. Cytoplasm hyaline in anterior half of body but often opaque in posterior half due to accumulation of many food vacuoles, up to 5 µm across, and lipid droplets (Figs 14, 15). Locomotion relatively slow, by rotation about main body axis; cells can attach to substrate via the anterior thigmotactic area.

On average 23 (21–25) somatic kineties: 11–13 on the left and 10–12 on the right side; anterior two thirds of kineties composed mainly of dikinetids and posterior third composed mainly of monokinetids; cilia about 10 µm long, anterior-dorsal cilia very densely arranged and rigid, forming a thigmotactic area (Figs 14, 16, 17). Left-ventral and dorsal kineties terminate at apical plate of naked area, right-ventral preoral kineties progressively shortened anteriorly forming an imbricate structure; all kineties extend to posterior end of body except for several slightly shortened left-ventral kineties (Figs 16, 17).

Buccal field of typical pattern for the genus, occupying about 3/4 of body length. Oral cilia about 10 µm long anteriorly, increasing to about 25 µm long in the posterior region, where they form a conspicuous brush-like structure (Fig. 14). Membranelle 1 short, composed of 12 kinetosomes arranged in three longitudinal rows forming an acute angle with M2; M2 L-shaped, composed of two longitudinal rows, often with two kinetosomes anteriorly, occupying about two-thirds of body length; M3 positioned posterior to M2, slightly longer than M1, and composed of 18 kinetosomes arranged in three oblique rows. Paroral membrane lies parallel to somatic kinety 1, extending slightly beyond M1 anteriorly and terminating posteriorly near caudal pole; composed of a zigzag file of closely arranged dikinetids, but appearing as a single row in silver-stained specimens (Fig. 16). Cytostome at base of PM and M2, shape drop-like, connected with radially arranged fibers (Fig. 16). Scutica (Sc) patch-like, located close to caudal cilium complex which bears a single caudal cilium about 20 µm long (Figs 16, 17).

Occurrence and prevalence: We first isolated *Ancistrum acutum* from the gills and mantle cavity of the surf clam *Mactra veneriformis* Reeve in April 1995, when the water temperature was 10°C and the salinity was 22. The prevalence was 9/20 and the intensity of infestation was moderate. In December 1997 we also isolated this species from the sunray surf clam *Mactra chinensis* Philippi on the Qingdao coast (water temperature of 10°C, salinity 31), where the prevalence was 12/20 and the intensity of infestation was light.

Ancistrum japonicum Uyemura, 1937 (Figs 18–25; Table 1)

Nomenclature: The species was originally described by Uyemura (1937), based on observations both of live observations and of specimens stained with Heidenhain's haematoxylin, under the name *Ancistruma japonica*. The valid species name should be *Ancistrum japonicum* Uyemura, 1937 since *Ancistruma* Strand, 1926 is considered to be an objective synonym of *Ancistrum* Maupas, 1883 (Raabe 1970). Other synonyms include: *Ancistrumina japonica* Uyemura, 1937 and *Ancistrum japonicum* (Uyemura 1937). The identification of this species is still confused despite a taxonomic revision of the group to which it belongs (Raabe 1970). Furthermore, no type material exists. We suggest neotypification of *Ancistrum japonicum* with the specimens described in the present study which were isolated from the same host mollusc, namely *Cyclina sinensis* (Gmelin), and from the same region (East Asia) that the species was originally discovered.

Diagnosis of neotype: Size about 80 × 25 µm *in vivo*; body cuneate in lateral view, with anterior and posterior-dorsal ends both bluntly pointed. One ovoid macronucleus and one micronucleus. On average 20 somatic kineties, with both left and right kineties distinctly shortened in posterior region of cell. Buccal area extending to posterior pole and occupying on average 81% of body length. Membranelles 1 and 3 short, each composed of two or three rows of kinetosomes; membranelle 2 composed of two rows and occupying about 70% of body length.

Neotype host and site: Marine venus clam *Cyclina sinensis* (Gmelin); gills and mantle cavity.

Neotype locality: Marine culture beds at Rizhao on the Yellow Sea coast of northern China (35°40′N, 119°57′E).

Neotype material: A protargol slide with the neotype specimen circled in ink is deposited in the Laboratory of Protozoology, Ocean University of China with registration number RZ-950428-20. A paraneotype slide is deposited in the same collection with registration number RZ-950428-21.

Description (based on neotype specimens unless stated otherwise): Size *in vivo* 60–90 × 18–30 µm, usually about 80 × 25 µm with ratio of length to width about 3.3 : 1; protargol-stained specimens on average 85 × 36 µm suggesting a slight body inflation during the fixation or staining process; strongly flattened bilaterally 3–4 : 1 (Table 1). Body more or less cuneate in lateral view with narrowly rounded anterior end and slightly protruded posterior-dorsal end; ventral margin convex, dorsal margin sigmoidal and convex in posterior quarter of cell (Figs 20, 24). Specimens from *Dosinia japonica* (Reeve) slightly smaller and stouter than those from neotype population parasitizing *Cyclina sinensis*, usually 65–70 × 20–30 µm *in vivo* with ratio of length to width about 3:1; bilaterally flattened about 2–2.5 : 1; body more or less distinctly concave in dorsal margin and prolonged dorsally in posterior region (Figs 18, 19). Single macronucleus usually located in the mid- to anterior half of body, usually ovoid, occasionally near to triangular, about 26 × 16 µm in protargol-stained specimens. One globular micronucleus positioned anterior of macronucleus (Fig. 25). Contractile vacuole located anterior of cytostome near ventral side, 5–8 µm across. Cortex flexible with cortical granules loosely arranged along ciliary rows. Cytoplasm usually hyaline in anterior half of body and opaque in posterior half due to accumulation of many food vacuoles, up to 8 µm across, and lipid droplets, about 5 µm across. Movement by rotation about main body axis with anterior and posterior ends frequently curved dorsally; cells can attach to substrate via anterior thigmotactic area (Figs 18–20).

On average 20 (18–21) somatic kineties, equidistantly spaced, on average 10 (8–11) rows on each side; anterior third of kineties composed primarily of dikinetids, posterior two thirds composed mainly of monokinetids (Figs 23–25). Somatic kineties mostly terminate at anterior end of body, except for several right-ventral preoral kineties which are progressively shortened anteriorly forming an imbricate structure; all kineties excluding SK1 distinctly shortened in posterior region of cell, forming a distinct glabrous zone on both left and right sides (Figs 24, 25). Somatic cilia 10–15 µm long, anterior cilia very densely arranged and rigid forming a thigmotactic area.

Buccal field of typical pattern for genus, occupying about 81% of body length. Oral cilia about 15 µm long anteriorly, increasing to about 25 µm long posteriorly where they form a conspicuous brush-like structure (Figs 20, 23). Membranelle 1 short, composed of two or three rows of kinetosomes; M2 hook-shaped, composed of two longitudinal rows often with several single-rowed kinetosomes anteriorly, occupying about two thirds of body length; M3 near end of M2, of similar length to M1, composed of 10 kinetosomes arranged in three oblique rows. Paroral membrane lies parallel to somatic kinety 1, composed of a zigzag file of dikinetids, extending beyond M1 anteriorly and terminating posteriorly near caudal pole. Cytostome at base of PM and M2, ellipsoidal in outline, connected with radially arranged fibers (Fig. 21). Scutica (Sc) patch-like, close to caudal cilium complex (CCo) which bears a single caudal cilium about 20 µm long (Figs 23–25).

Transverse silverlines very sparsely distributed between somatic kineties, in particular in mid-body; silverlines from right somatic kineties converge at posterior end; several longitudinal silverlines recognizable in buccal area. Single contractile vacuole pore (CVP) located near end of somatic kinety 2 (Figs 21, 22).

Occurrence and prevalence: *Ancistrum japonicum* was first reported by Uyemura (1937) in large numbers in the mantle cavity of marine bivalves including *Meretrix meretrix* (L.), *Paphia philippinarum* Adams and Reeve, *Cyclina sinensis* (Gmelin), *Mactra veneriformis* Reeve, *Mactra sulcataria* Reeve and *Dosinia bilnulata* Gray, in seas adjacent to Japan. This species was not subsequently reported until we recovered it from the venus clam *Cyclina sinensis* (with prevalence of 20/20 and light intensity of infestation) and likely also from the hard clam *Meretrix meretrix* (with prevalence of 20/22 and light intensity of infestation) at Rizhao on the Yellow Sea coast of northern China in April 1995, although this population was observed only *in vivo* and its identity awaits confirmation based on observations of silver-stained specimens (Xu and Song 1999). In April 1998 we isolated it from the Japanese dosinia *Dosinia japonica* (Reeve) at Rongcheng on the Yellow Sea coast of northern China, where the prevalence was 11/20 and the intensity of infestation was light (Xu and Song 2003).

DISCUSSION

Taxonomic review on the genus *Ancistrum*

The genus *Ancistrum* Maupus, 1883 and its type species *A. mytili* (Quennerstedt 1867) have had a complicated systematic history since they were established. The situation was well reviewed by Hatzidimitriou and Berger (1977). Briefly, Quennerstedt (1867) first described a ciliate parasitizing *Mytilus edulis* from the west coast of Sweden under the name *Opalina mytili*, which was subsequently transferred to the astome genus *Anoplophyra* by Kent (1882) due to the fact that its cytostome had been overlooked. Maupus (1883) redescribed the species and established the genus *Ancistrum* to include this species and *A. venerisgallinae* Maupas, 1883. Since then, numerous new species were described and assigned to the genus *Ancistrum* (e.g. Issel 1903, Chatton and Lwoff 1926, 1949, Fenchel 1965, Raabe 1970). Raabe (1959) split *Ancistrum* into two genera, viz., *Ancistrum sensu stricto* which has 40–60 somatic kineties and two adoral rows (= PM + adoral membranelles) running from the apical suture and extending to the posterior end where they form a large loop, and *Ancistrumina* Raabe, 1959 which has 12–30 somatic kineties and adoral rows that start a small distance from the apical suture, extend backwards down the cell and make a large loop at a distance of about 1/4 from the posterior body pole. *Ancistrumina* embraces most species formerly assigned to *Ancistrum sensu* Maupas, 1883 and also those formerly assigned to the genus *Ancistrina* Cheissin, 1930–1931, which is a homonym of *Ancistrina* Goede, 1927 (Lepidoptera). However, the split was not recognized by Corliss (1961) nor by Fenchel (1964, 1965). In his monographic series on thigmotrich ciliates, Raabe (1970) further confirmed his suggested arrangement, which was validated by Corliss (1979) and Lynn (2008). Fenchel (1964, 1965) noted that some of the characters (in particular the number of somatic kineties) that are used to separate the two genera overlap in some species, as admitted by Raabe (1959). This is clearly shown by *Ancistrum mytili*, the type species, the somatic kineties of which vary from 31 to 68 (Fenchel 1965; Hatzidimitriou and Berger 1977; Berger and Hatzidimitriou 1978). In *Ancistrum caudatum* and *A. bartatum* both the mouth and the adoral rows are posteriorly positioned but each has only about 20 somatic kineties (Issel 1903; Fenchel 1964). Likewise, the four species described here possess: (1) no more than 40 somatic kineties; and (2) a conspicuous hook-like paroral membrane that extend to the caudal pole along with somatic kinety 1.

We suggest the position of the oral ciliature as well as the mouth (extending to the posterior pole in *Ancistrum* vs. about 1/4 of body length distant from the posterior pole in *Ancistrumina*) is a decisive feature for separating *Ancistrum* from *Ancistrumina*, whereas the number of somatic kineties is only species- or even population-dependent. Similar to the posteriorly positioned mouth, the somatic kinety 1 closely paralleling the paroral membrane along its entire length and extending to the posterior end is another striking feature of the genus *Ancistrum*. Raabe (1959, 1970) also included the argyronemes (= silverlines) in the generic characters (scarce in *Ancistrumina* vs. a dense net of argyronemes in the naked peristomial field and numerous transversal argyronemes connecting the somatic kineties in *Ancistrum*). However, this feature also overlaps in both genera because *Ancistrum caudatum*, *A. crassum* and *A. japonicum* have only sparse argyronemes (Fenchel 1964; Figs 11, 21, 22). We do not attempt to redefine both genera because no species of *Ancistrumina* has been re-investigated using modern silver staining techniques. Thus, detailed structure of the oral and somatic ciliature and silverline system of *Ancistrumina* is still not clear and awaits investigation.

Raabe (1970) recognized only three species in *Ancistrum*: *A. mytili* (Quennerstedt 1867), *A. venerisgallinae* Maupas, 1883, and *A. modioli* Raabe, 1970. Based on demic and morphological analyses, Berger and Hatzidimitriou (1978) synonymized *A. modioli* with *A. mytili*. *Anicistrum venerisgallinae* has a mouth that is distant from the posterior pole and so should belong to the genus *Ancistrumina*, despite of its large number of somatic kineties. Likewise, some species that were transferred to *Ancistrumina* by Raabe (1970) due to their small number of somatic kineties possess a typical *Ancistrum*-like oral pattern and so should be assigned to the genus *Ancistrum*. These include *Ancistrum abrarum* Fenchel, 1965, *A. barbatum* Issel, 1903, *A. caudatum* Fenchel, 1964, and *A. japonica* Uyemura, 1937. *Ancistrum isseli* Kahl, 1931 is another “difficult” species, which was originally described by Issel (1903) from *Modiolus barbatus* in Naples under the name *Ancistrum mytili* (Quennerstedt). Kahl (1931) recognized the misidentification and named it as *A. isseli*. Fenchel (1965) indicated that *A. caudatum* is most closely related to *A. isseli*, but distinguishes it from the latter in possessing the long UM (= paroral membrane) tuft. However, *A. isseli* was not recognized by Raabe

(1970), who misinterpreted Fenchel's (1965) analysis and considered *A. isseli* and *A. caudatum* to be different forms of the same species. Further evidence of the species-level separation of *A. caudatum* and *A. isseli* is that the cytostome of the former is covered by a characteristic tongue-like prolongation, which is absent in the latter. With the exception of *A. mytili* and the four species described here, details of the oral ciliature of other *Ancistrum* spp. are not clear and await description using modern silver staining techniques.

Khan (1970) described a new species, namely *Ancistrumina nucellae,* from the gastropod dog whelk *Nucella lapillus* in South Wales, and clearly stated that the species is close to *Ancistrum* in the arrangement of adoral kineties, but assigned to *Ancistrumina* mainly due to its small number of kineties. This nominal species matches well with *Ancistrum japonicum* in almost all important features. Khan (1970) emphasized the peculiarity of the shape (oval, spherical or triangular) and variability of the macronucleus in *Ancistrumina nucellae*. However, the same macronuclear variations occur also in the Chinese specimens of *Ancistrum japonicum*. Thus, we synonymize *Ancistrumina nucellae* Khan, 1970 with *Ancistrum japonicum* Uyemura, 1937.

Raabe (1970) established the monotypic genus *Fenchelia* for *Ancistrum crassum* Fenchel, 1965, which he considered sufficiently distinct for it to be separated from the genera *Ancistrum* and *Ancistrumina*. This species is characterized by having relatively few somatic kineties, adoral kineties starting about 1/3 of the body length from the anterior pole, a posteriorly positioned mouth, and a dorso-posterior cone-shaped prolongation of the body. In our opinion, these features are not sufficient for the establishment of a separate genus. Thus, we retain *A. crassum* in the genus *Ancistrum* and submerge the genus *Fenchelia* which we consider to be a junior synonym of *Ancistrum*.

Oishi (1978) described a new ciliate, namely *Ancistrum edajimanum,* from the bivalve *Tapes philippinarum* (synonym of *Ruditapes philippinarum*) in Japan. This nominal species has a characteristic cone-shaped prolongation in the posterior-dorsal portion of cell, as observed in *A. crassum*. Furthermore, it coincides well with *A. crassum* in body size and shape, and in the number and pattern of somatic kineties and ciliary pattern, particularly with the Chinese specimens which were isolated from the same host species as the Japan population (Table 2). Thus, *Ancistrum edajimanum* Oishi, 1978 is synonymized with *A. crassum* Fenchel, 1965. Based on evaluation of all nominal species of *Ancistrum* and *Ancistrumina*, we recognize nine species of *Ancistrum* as valid and provide a tabular guide to their identification (Table 2).

Comparison with related species and populations

Compared with all known species of *Ancistrum* and *Ancistrumina*, *Ancistrum haliotis* n. sp. is most similar to *Ancistrum mytili* (Quennerstedt 1867) Maupas, 1883, the type species of the genus, both in oral structure and morphometry. However, *Ancistrum haliotis* differs distinctly from *A. mytili* in the body outline (anterior portion narrower vs. wider than the posterior portion), the macronuclear shape (broadly ellipsoidal vs. reniform or sausage-like; Fig. 6), and in having fewer somatic kineties (28–32 vs. 31–68, usually > 40) (Kidder 1933, Fenchel 1965, Raabe 1970, Hatzidimitriou and Berger 1977, Berger and Hatzidimitriou 1978). In addition, *A. haliotis* possesses a rather narrow buccal field and a glabrous area on the right side that occupies no more than 1/5 of the body length, whereas in *A. mytili* the mouth is much broader and the glabrous area on the right side extends posteriorly to about 1/3 of the body length (Figs 4, 5, 7). To date, *A. mytili* has been reported only from the host mussels *Mytilus edulis* and *Modiolus modiolus* from the Atlantic Ocean, whereas *A. haliotis* was found in the abalone *Haliotis discus hannai* from coastal waters of northern China. *Ancistrum haliotis* also resembles *A. crassum* in body size, the number of somatic kineties and macronuclear features, but differs in its body shape (nearly quadrilateral vs. elongate-ellipsoidal with posterior-dorsal prolongation), the distance between the oral ciliature and the anterior end of the cell (*ca.* 12% vs. 37% of body length), and the ratio of the length of M2 to the body length (*ca.* 70% vs. 33%).

Ancistrum crassum was originally described by Fenchel (1965) from the host clam *Venerupis aurea* and *V. pullastra* in Scandinavia, although details of the oral ciliature were not provided. Nonetheless, the population described here from the purple clam *Saxidomus purpuratus* matches the original description of *A. crassum* in most important features. This population also matches well with specimens from the short-necked clam *Ruditapes philippinarum* in body size and shape and somatic and oral ciliature (Xu *et al*. 1997, Song 2000). Fenchel (1965) reported only about 26 kineties, whereas the number of somatic kineties is slightly higher in the present populations from *Saxidomus purpuratus* (27–33, average 29) and *Ruditapes philippinarum* (26–38, average 31). In our opinion, these are only popu-

Table 2. Tabular guide to the identification of the nine species of Ancistrum recognized as valid.

Ancistrum species	Size in µm	Somatic kineties, number	Buccal field/ body length	Macronuclear shape	Host species	Locality	References
A. abrarum Fenchel, 1965 Syn. *Ancistrumina abrarum* (Fenchel, 1965) Raabe, 1970	25–36 × 14	20–21	–	ovoid	Abra nitida	Gullmar Fjord, Sweden	Fenchel 1965
***A. acutum* n. sp.**[a]	80–110 × 25–35	21–25	3/4	ellipsoidal	*Mactra veneriformis*; *Meretrix meretrix*	Rizhao, China	This paper
A. barbatum Issel, 1903 Syn. *Ancistrumina barbata* (Issel, 1903) Raabe, 1970	37–51 × 14–22	16–22	–	round	*Fusus syracusanus*; *Murex trunculus*	Naples, Italy	Issel 1903; Fenchel 1964; Raabe 1970
A. caudatum Fenchel, 1964 Syn. *Ancistrumina caudata* (Fenchel, 1964) Raabe, 1970	46–55 × 20	22–23	–	oblong	*Musculus niger*; *Modiolus modiolus*	Øresund, Baltic Sea	Fenchel 1964, 1965; Raabe 1970
***A. crassum* Fenchel, 1965** Syn. *Fenchelia crassa* (Fenchel, 1965) Raabe, 1970; *Ancistrum edajimanum* Oishi, 1978	63–82 × 21–41	26	–	ovoid/oblong	*Venerupis aurea*; *V. pullastra*	Kristineberg, Sweden	Fenchel 1965; Raabe 1970;
	43–78 × 13–34	22–33	–	ellipsoidal	*Tapes* (=*Ruditapes*) *philippinarum*	Japan	Oishi 1978;
	63–77 × 20–28	26–38	0.62	ovoid	Ruditapes philippinarum	Qingdao, China	Xu *et al.* 1997;
	60–80 × 20–35	27–33	0.63	ovoid	Ruditapes philippinarum	Qingdao, China	Song 2000
	50–70 × 15–25	20–33	0.63	ovoid	Saxidomus purpuratus	Rongcheng, China	This paper
***A. haliotis* n. sp.**	50–75 × 20–30	28–32	0.88	ellipsoidal	Haliotis discus hannai	Qingdao, China	This paper
A. isseli Kahl, 1931 pro. *A. mytili sensu* Issel, 1903	66–73 × 25–40	21–23	–	spherical	Modiolus modiolus	Naples, Italy	Issel 1903; Kahl 1931
***A. japonicum* Uyemura, 1937** Syn. *Ancistruma japonica* Uyemura, 1937; *Ancistrumina japonica* Uyemura, 1937; *Ancistrum japonicum* (Uyemura, 1937); *Ancistrumina nucellae* Khan, 1970	60–90 × 18–30	18–21	0.81	ovoid	*Cyclina sinensis*; *Meretrix meretrix*; *Dosinia japonica*	Rizhao, China	This paper;
	55–76 × 14–29	18–20	–	ovoid	*Meretrix meretrix*; *Paphia philippinarum*; *Cyclina sinensis*; *Mactra veneriformis*; *M. sulcataria*; *Dosinia bilnulata*	Japan	Uyemura 1937; Raabe 1970
	45–65 × 15–25	18–20	7/8	oval, spherical or triangular	Nucella lapillus	South Wales, Britain	Khan 1970
***A. mytili* (Quennerstedt, 1867) Maupas, 1883** Syn. *Opalina mytili* Quenn.; *Anoplophyra mytili* (Quenn.) in Kent (1882); *A. musculi* Delphy, 1938; *A. modioli* Raabe, 1970 (pro. *A. isseli* Kahl, 1931 *sensu* Kidder, 1933)	52–74 × 20–38	→53	–	reniform	Mytilus edulis	Woods Hole, USA	Kidder 1933
	70–88 × 31–54	?	–	spherical?	Modiolus modiolus	Woods Hole, USA	Kidder 1933; Raabe 1970
	42–55 × 25–32	48–54	–	reniform	Mytilus edulis	Baltic Sea	Raabe 1936
	57–82 × 17–45	43–46	–	reniform	Mytilus galloprovincialis	Black Sea	Raabe 1970
	69–88 × 22–41	38–44	–	reniform	*Mytilus edulis*; likely also *Modiolus modiolus*	Baltic Sea	Fenchel 1965
	47–78 × 6–32	31–68	0.99	reniform	*Mytilus edulis*; *Modiolus modiolus*	New Brunswick, Canada	Hatzidimitriou and Berger 1977; Berger and Hatzidimitriou 1978

[a] Bolds indicate well established species; others awaiting redescription because oral ciliature is not clear.

lation-dependent differences thus the identification of *A. crissum* is assured. Present and previous investigations indicate that *Ancistrum crassum* prefers parasitizing the mollusc family Veneridae, and is a common parasite of *Ruditapes philippinarum* in the Yellow Sea.

Ancistrum japonicum was originally described by Uyemura (1937) under the name *Ancistruma japonica* from various marine bivalves in Japan. The valid name should be *Ancistrum japonicum* Uyemura, 1937 since *Ancistruma* Strand, 1926 is an objective synonym of *Ancistrum* (Raabe 1959). Raabe (1970) erroneously placed this species in the genus *Ancistrumina* based mainly on its small number of somatic kineties (18–22). However, this species has a mouth located at the posterior extremity of the body (Uyemura 1937), suggesting it belongs to the genus *Ancistrum* rather than *Ancistrumina*, as discussed above. Our specimens from the host bivalve *Cyclina sinensis* match with the original description in almost all important features, in particular the body shape, the position of mouth and oral groove, and the number of somatic kineties. Uyemura (1937) indicated that specimens of *A. japonicum* vary considerably in size depending on their hosts and have two types of body shape, i.e., oval or elongate-oval. Raabe (1970) stated that "The diversity of hosts, the separation of two types by the author, a high spread of dimensions (even among the individuals from one host) seem to indicate that we have to do with a collective species, or with a confusion of several species". Unfortunately, Uyemura (1937) did not give more details about these two morphotypes.

Our observations of specimens both *in vivo* and following silver staining indicate that those parasitizing *Cyclina sinensis* and *Dosinia japonica* are *Ancistrum japonicum*, whereas those from *Mactra veneriformis* and *M. chinensis* represent the new species, *Ancistrum acutum* n. sp. The two species are almost identical in morphometry but differ distinctly in live morphology and ciliary structure. *Ancistrum acutum* is slightly stouter and has a pointed anterior end and rounded posterior end, whereas *A. japonicum* is more or less cuneate with a narrowly rounded anterior end and slightly protruded posterior-dorsal end. Moreover, in *A. japonicum* all right-side kineties excluding SK1 are distinctly shortened posteriad, forming a glabrous zone, which is not present in *Ancistrum acutum* because all right-side kineties extend to the posterior end of the body. These differences are sufficiently distinct to justify the validity of the new species. Uyemura (1937) did not provide details of the oral structure of *Ancistrum japonicum*, nor did he deposit type material in a reference collection. Thus, we provide an improved diagnosis, a redescription based on modern methods, and neotypify *Ancistrum japonicum* with the specimens isolated from the same host *Cyclina sinensis* from the same region (East Asia), where the species was originally discovered.

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