First Japanese Record of *Epistylis wuhanensis* (Ciliophora: Epistylididae) Attached to *Lernaea cyprinacea* (Copepoda), with a List of *Epistylis* Species Attached to Metazoans in Japan

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The epistylid ciliate *Epistylis wuhanensis* Wang, Zhou, Guo, and Gu, 2017 was found attached to the body surface of *Lernaea cyprinacea* Linnaeus, 1758 (Copepoda: Lernaeidae) parasitizing *Rhinogobius similis* Gill, 1859 (Perciformes: Gobiidae) from the Arida River in Wakayama Prefecture, Japan. This epistylid was described based on live ciliates, stained specimens, and scanning electron microscope observation with molecular information. This is the first record of *E. wuhanensis* from Japan and the second record of the epistylid attached to metazoans. A list of records of *Epistylis* Ehrenberg, 1830 species attached to metazoans in Japan is proposed.

**Key Words:** new country record, peritrich ciliates, epibionts, *Rhinogobius similis*.

**Introduction**

The epistylid ciliate genus *Epistylis* Ehrenberg, 1830 contains over 260 nominal species (Lu et al. 2020). It is considered that a certain number of species are free-living but most of them are considered as epibionts on aquatic metazoans such as crustaceans, insects, rotifers, and aquatic plants in marine and fresh waters (Sládecek 1986). Occasionally, these epibiont species cause diseases such as red sore diseases in fish hosts (Basson and van As 2006; Ksepka 2006). The epibiont species have mainly focused on fish pathology (e.g., Miyazaki and Egusa 1973; Sejima et al. 1973) and their role as indicators for quantifying various properties of activated sludge in water treatment (e.g., Morishita 1964, 1968; Morishita et al. 2006). The diversity of free-living species of *Epistylis* in activated sludge has been well-documented by Morishita (1964, 1968) and Morishita et al. (2006), with 22 species listed. However, the diversity of the other species found attached to aquatic metazoans has not been evaluated. Therefore, I have assembled the published records of *Epistylis* attached to metazoans found in Japan and provide them herein as a list.

Peritrich ciliates attached to the anchor worm, *Lernaea cyprinacea* Linnaeus, 1758 (Copepoda: Lernaeidae) parasitizing eight species of freshwater fish, and ten of the twelve records described the collection sites, and six of which are from western to southern Japan (Table 1). However, most specimens have not been morphologically described (see references for Table 1), so it is not clear how many ciliate species are present on anchor worms and those effects on the host. To date, only Fukuda (2000) has reported an un-identified *Epistylis* species and published a picture taken via a scanning electron microscope (SEM) (see references for Table 1). The taxonomic study of those peritrich ciliates is required from the perspective of fish pathology and biodiversity (Nagasawa and Nitta 2019). Ciliates previously reported as peritrich ciliates from the Arida River in Wakayama Prefecture using a hand-net and examined for parasites under a dissecting microscope on 7 October 2019. Four of the six collected fish were infected by *Lernaea cyprinacea*, and in total six specimens of *L. cyprinacea* were collected. Identification of the copepods followed Nagasawa and Nitta (2019). Each two specimens of *L. cyprinacea* were fixed in hot (60°C) Bouin’s solution or 99% ethanol along with the attached ciliates. The ciliates attached to two other anchor worms were cover-slipped for in vivo observation under a microscope.

Six specimens of *Rhinogobius similis* Gill, 1859 were collected from the Arida River (34°04′41.8″N, 135°09′34.5″E), at Takigahara, Miyahara-cho, Arida City, Wakayama Prefecture using a hand-net and examined for parasites under a dissecting microscope on 7 October 2019. Four of the six collected fish were infected by *Lernaea cyprinacea*, and in total six specimens of *L. cyprinacea* were collected. Identification of the copepods followed Nagasawa and Nitta (2019). Each two specimens of *L. cyprinacea* were fixed in hot (60°C) Bouin’s solution or 99% ethanol along with the attached ciliates. The ciliates attached to two other anchor worms were cover-slipped for in vivo observation under a microscope.

These live ciliates were observed using an Olympus BX60 light microscope (Olympus, Tokyo, Japan) at ×40–1000 magnification, with photographs and movies taken using a CANON 70D digital camera (Canon, Tokyo, Japan) fitted to
the microscope, then fixed in acetic acid-formalin-alcohol (AFA). The infraciliature was revealed using the protargol staining method (Ji and Wang 2018) using specimens fixed in AFA. The samples were subsequently freeze-dried, then observed via SEM (Hitachi S-2150) at an accelerating voltage of 20 kV.

Total genomic DNA was isolated from specimens fixed in 99% ethanol using a NucleoSpin Tissue XS (Macherey-Nagel, Düren, Germany) kit as per the manufacturer’s instructions. Partial fragments of the 18S rDNA gene were amplified via polymerase chain reaction (PCR) using the primer pairs Peri18S-F1 (5'-ACC TGG TTG ATC CTG CCA GT-3') and Peri18S-R2 (5'-GAT CCC CTA ACT TTC CCA GT-3'), while part of the ribosomalosomal incorporating the internal transcribed spacers 1 and 2 and the 5.8S rDNA (ITS1-5.8S-ITS2) gene were amplified using the primer pair ITS-F (5'-TAC TGA TAT GCT TAA GTT CAG CGG-3') and ITS-R (5'-GTA GGT GTA GGT TTC CAG CTC-3') and Peri18S-R2 (5'-GAT CCC CTA ACT TTC CCA GT-3'). Amplified PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel), and sequenced with the PCR primers using a Big Dye Terminator v3.1 (Applied Biosystems, Foster, USA) and a 3130xl Genetic Analyzer (Applied Biosystems). The sequences obtained were submitted to the DNA Data Bank of Japan Centre (DDBJ) and compared with the available se-

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### Table 1. Records of *Lernaea cyprinacea* attached by peritrich ciliates or peritrich like ciliates in Japan.

| Reported name | Host of *Lernaea cyprinacea* | Host family | Locality | Site | Prefecture | References |
|---------------|-------------------------------|-------------|----------|------|------------|------------|
| Vorticella sp. | Oryzias latipes (Temminck and Schlegel, 1846) | Adrianichthyidae | Laboratory-reared | — | — | Suzuki (1965) |
| *Epistylis wuhanensis* Wang, Zhou, Guo, and Gu, 2017 | Rhinogobius similis Gill, 1859 | Gobiodae | The Arida River, Arida City | Wakayama | Nagasawa and Nitta (2019); this study |
| *Epistylis* sp. | — | — | — | — | — | Fukuda (2000) |
| Vorticellidae gen. sp. | Rhinogobius bruneus (Temminck and Schlegel, 1845) | Gobiodae | The Genka River, Nago City | Okinawa | Nagasawa et al. (2019) |
| Vorticellidae? gen. sp. | R. similis as *R. giurinus* (Rutter, 1897) | Gobiodae | Lake Ikeda, Ibusuki City | Kagoshima | Fukushima et al. (2020) |
| *Tridentiger brevispinis* Katsuyama, Arai, and Nakamura, 1972 | Gobiodae | Lake Ikeda, Ibusuki City, Amami Oshima Island | — | — | Fukushima et al. (2020) |
| Xiphophorus helleri Heckel, 1848 | Poceliidae | Omi River, Tatsugo Town, Amami Oshima Island | — | — | Fukushima et al. (2020) |
| *Rhinogobius nagoyae* Jordan and Seale, 1906 | Gobiodae | Omi River, Tatsugo Town | Kagoshima | Fukushima et al. (2020) |
| Peritrich ciliates | Lepomis macrochirus Rafinesque, 1819 | Centrarchidae | Katata Naiko, Lake Biwa | Shiga | Grygier (2004) |
| *O. latipes* | Adrianichthyidae | Kohoku-machi | Saga | Nagasawa et al. (2012) |
| *Squalidus gracilis gracilis* (Temminck and Schlegel, 1846) | Cyprinidae | The Seno River, Okayama City | Okayama | Nagasawa et al. (2017) |
| *O. latipes* | Adrianichthyidae | Shizuoka City | Shizuoka | Nagasawa et al. (2020a) |
| *O. latipes* | Adrianichthyidae | Shima City | Me | Nagasawa et al. (2020b) |
| *O. latipes* | Adrianichthyidae | Matsu moto City | Oita | Nagasawa et al. (2020c) |
| Peritrich-like ciliates | *O. latipes* | Adrianichthyidae | The Okoe River, Saeki City | — | — | — |
| *O. latipes* | Adrianichthyidae | — | — | — | — | — |
| *O. latipes* | Adrianichthyidae | — | — | — | — | — |

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* a Nagasawa and Nitta (2019) reported the ciliate as peritrich ciliates, and newly collected specimens were identified as *E. wuhanensis* in this study.

b Beattie et al. (2008) did not mention the ciliates attached on *L. cyprinacea*, but Nagasawa et al. (2020b) pointed out that the anchor worms may have been attached by peritrich-like ciliates based on the figures shown by Beatty et al. (2008: fig. 2B, C).
quences for species of Ciliophora in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) software on 1 April 2021.

Results

In the reports of Epistylis species found attached to animals in Japan, 23 of them are valid species, including E. wuhanensis (Table 2). One species is invalid, and nine are unidentified records. The recorded hosts are diverse: nine species of Malacostraca, four of Actinopterygii, four of Bivalvia, three of Copepoda, two of Branchiopoda, one of geomyid turtle (Reptilia), one of brachionid rotifer (Euratarion), and two or more species of aquatic insects.

Colonies of Epistylis wuhanensis were attached to all six collected specimens of L. cyprinacea in this study. The colonies were mainly attached to the exposed neck and trunk of L. cyprinacea and not directly to the fish body.

Epistylis wuhanensis Wang, Zhou, Guo, and Gu, 2017 (Figs 1, 2; Supplemental Movies 1–3)

Epistylis wuhanensis Wang, Zhou, Guo, and Gu, 2017: 396–400, figs 1–5.

Peritrich ciliates: Nagasawa and Nitta 2019: 148, 149, fig. 1C, D.

Description. Colony up to 1 mm in height and comprised of less than 60 zooids, branched dichotomously with secondary or more stalks (Fig. 2A); span between first and secondary stalks relatively long, occupying half to two-thirds of the total length (Fig. 2A). Stalks non-contractile (Supplemental Movie 1), transversely wrinkled weakly when the stalk curved (Fig. 2A), with longitudinal striations on the surface (Fig. 1A), 11.3–25.9 (15.7, n = 11) width in branched regions. Expended zooid elongate bell-shaped to elliptical (Figs 1B, 2A–D, Supplemental Movies 2, 3), 163.1–220.3 (190.1, n = 15) length in vivo, 128.0–181.9 (150.2, n = 6) length in fixed specimens; in half of specimens widest close to the single-layered, thin peristomial lip 41.6–56.1 (47, n = 15) in width, 36.1–56.9 (46.3, n = 15) in width at the mid-point; peristomial lip in fixed specimens 36.2–54.7 (44.7, n = 6) wide, widest at the mid-point (47.1–64.0, 55.7, n = 6); average length to width ratio in vivo 3.3–5.3 (4.1): 1 (n = 15) at mid-point, 2.1–3.5 (2.7):1 (n = 6) in fixed specimens. Contracted zooid ovoid (Fig. 1C). Peristomial disk mammilla-shaped, 11.2–13.4×14.7–18.4 (12.1×16.1, n = 5), surrounded by adoral zone of membranelles (Figs 1A, 2B, D). Macronucleus lying in the middle or upper thirds of cell, commonly C-shaped or sometimes J-shaped (Fig. 2C, D). Cytopharynx lying in upper third of expended zooid (Fig. 2B). Contractile vacuole between macronucleus and peristomial lip (Fig. 2B). Aboral trochal band lying in lower third to fourth of zooid (Figs 1B, C, 2B–D). Pellicle with circular transverse (Fig. 1B, C, E); transverse striations distributed equally, 138–164 (149.2, n = 5) striations between peristome and aboral trochal band, 92–102 (95.6, n = 5) between aboral trochal band and scopula. Longitudinal fibers continually extending across the body, from peristome to scopula (Fig. 2C, D).

Infundibular polykineties lying in upper third of expended zooid (Fig. 2D). Haplokinety and polykinety parallel, both making ca. 1.6–1.8 turns around peristome, then entering infundibulum (Fig. 2F). Germinai kinety parallel with haplokinety in infundibular region, curving anteriorly to form half-circle anterior to infundibular polykinety 2 (P2), then crossing to infundibular polykinety 1 (P1) and P2 (Fig. 2E). Each infundibular polykinety consists of three rows of kinetosomes in lower third of infundibulum (Fig. 2E); P1 longest, consisting of three rows, extending laterally, then bending downward, extending shortly, and terminating at end of infundibulum. P2 almost parallel to lateral rows of P1, terminating at posterior curvature of P1. Polykinety 3 (P3) shortest, about half the length of P2, extending to the end of P2, then bending downward with P1 and terminating near the end of P1.

Deposition of specimens. MPM 21760 (mounted specimens) and MPM 21761a [preserved in 70% ethanol with L. cyprinacea (MPM 21761b)].

Sequence data. LC570274: partial 18S rDNA (1653 bp); LC570273: partial 18S (140 bp), complete ITS1 (140 bp), 5.8S rDNA (109 bp), ITS2 (170 bp), and partial 28S rDNA (34 bp).

Molecular data comparison. As found through a BLAST search, the newly obtained partial 18S rDNA sequence (LC570274) was identical, by original description, to E. wuhanensis with 100% coverage (KU869709: Wang et al. 2017) and E. cf. wuhanensis collected in the U.S.A. with 94% coverage (MW443028–MW443030: Ksepka and Bullard 2021). The closest hits to the newly generated sequence of ITS1-5.8S-ITS2 (LC570273) is also E. wuhanensis (KU869710, Wang et al. 2017, 99.79% similarity with 100% coverage), and the newly determined ITS1 sequence showed only one base deletion.

Remarks. Epistylis wuhanensis was originally described as attached to the fins of the yellow catfish Tachysurus fulvidraco (Richardson, 1846) (as Pelteobagrus fulvidraco) (Siluriformes: Bagridae), and the surface of the exposed neck and trunk of L. cyprinacea, parasitizing the bluegill Lepomis macrosirus Rafinesque, 1819 (Perciformes: Centrarchidae) in Hubei Province, China (Wang et al. 2017). On newly collected specimens, the maximum number of zooids was 60 (vs. 30 in Wang et al. 2017); fully expanded zooids in vivo were slightly longer than those in the aforementioned records [163.1–220.3 µm (this study) vs. 90.2–175.4 µm (Wang et al. 2017)]. Zooids in half of the living specimens were the widest at the peristomial lip, but those of the other half and fixed specimens were the widest at their mid-point. However, most measurements and morphological characteristics (the composition of the infraciliature; the position of the contractile vacuole; the form of the peristomial disk and lip; and the shape and position of the macronucleus) of the specimens collected in the present study are closely resemble to the description of Wang et al. (2017), partial 18S rDNA sequence was identical, and ITS1-5.8S-ITS2 sequence was also closely
| Species | Synonymized name | Host | Site of attachment | Location | References |
|---------|------------------|------|--------------------|----------|------------|
| *E. anodontae* | Uyemura, 1938 | *Sinanodonta lauta* (Martens, 1877) as *Anodonta lauta* | Malacostraca | Shell surface | Saitama | Uyemura (1938a) |
| *E. axile* | Chiromantes haematocheir (De Haan, 1835); *Parasesarma pictum* (De Haan, 1833) Malacostraca | Setae foreleg and pleopod | Kanagawa | | Fukui (1950a, b) |
| *E. bimarginata* | Nenninger, 1948 | *Crustacea* | Gill, body surface | Ouchiyama (1997) | |
| *E. cactistyla* | *Parasesarma pictum* (De Haan, 1833); *Macrophthalmus japonicus*; *Hemigrapsus penicillatus* (De Haan, 1835); *Upogebia major* (De Haan, 1835); *Bivalvia* | Gill, body surface | Tokyo, Saitama, Chiba or Tokyob | Uyemura (1938a) |
| *E. culexi* | *Linnaeus, 1758* (larva) | Insecta | Body surface | Kanagawa | Fukui (1950a, b) |
| *E. elongata* | *Mveneriformis* Reevere, 1854 as *Ruditapes philippinarum*; *Adams (Bivalvia* Gill | Shell surface | Chiba or Tokyob | Uyemura (1938b) |
| *E. flexistyla* | *Malacostraca* | Pleopod | Kanagawa | Fukui (1951a, b) |
| *E. lacustris* | *Imhoff, 1884* | Aquatic insects | Insecta | Body surface | — | Morishita (1981a) |
| *E. longicorpora* | *Sinanodonta lauta* Uyemura, 1938 | Bivalvia | Shell surface | Saitama | Uyemura (1938a) |
| *E. major* | *Fukui, 1950* (nomen nudum)a | *Malacostraca* | Seta of pleopod | Kanagawa | Fukui (1950a, b) |
| *E. microdiscum* | Stiller, 1963 | Aquatic organisms | — | — | Morishita (1981b) |
| *E. plicatilis* | *Ehrenberg 1838* | *Physella acuta* | Radix japonica (Jay, 1857) *Gastropoda* | Shell | Ouchiyama (1996a) |
| *E. polenici* | Banina 1984 | Mollusca | — | — | Ouchiyama (1997) |
| *E. pyriformis* | d’Udekem, 1862 | *Aquatic insects* | Insecta | Body surface | — | Morishita (1981c) |
| *E. scaralistyla* | *Fukui, 1950* | *Upogebia major* | Malacostraca | Seta of pleopod | Kanagawa | Fukui (1950a, b) |
| *E. sesarmae* | *Fukui, 1950* | *Parasesarma pictum* | Malacostraca | Oral parts, pleopod | Kanagawa | Fukui (1950a, b) |
| *E. sphaerostyla* | *Fukui, 1950* | *Gnorimosphaeroma* sp. | Malacostraca | Gill | Kanagawa | Fukui (1950a, b) |
| *E. etenuistyla* | *Fukui, 1951* | *E. tenuistyla* [lapsus] | Malacostraca | Pleopod | Kanagawa | Fukui (1951a, b) |
| *E. urceolata* | Stiller 1933 | *Trichoptera* (larva) | Insecta | — | Ouchiyama (1996b) |
| *E. wenrichi* | Uyemura, 1938 | *Bivalvia* | Shell surface | Saitama | Uyemura (1938a) |
| *E. wuhanensis* | *Wang, Zhou, Guo, and Nitta (2019)* reported the ciliate as peritrich ciliates, and newly collected specimens were identified as *E. wuhanensis* in this study. |

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*a* Fukui (1950a, b) only listed the name, *E. major* and would describe it at a later date (not published). Therefore, *E. major* is regarded as nomen nudum.  
*b* See, and Fukui (1950b) mentioned that he did not have enough specimens to describe *E. major*  
*c* Nagasawa and Nitta (2019) reported the ciliate as peritrich ciliates, and newly collected specimens were identified as *E. wuhanensis* in this study.  
*d* Wada (2012) reported it from ornamental fish in aquaria.
First Record of *Epistylis wuhanensis* from Japan

Fig. 1. Scanning electron micrographs of *Epistylis wuhanensis* Wang, Zhou, Guo, and Gu, 2017 from Japan. A. *E. wuhanensis* attached to exposed cephalothorax of *Lernaea cyprinacea*; B, extended zooid, lateral view; C, contracted zooid, lateral view; D, extended zooid, apical view; E, scopula of extended zooid. Abbreviations: AMZ, adoral zone of membranelles; ATB, aboral trochal band; PD, peristomial disc; PL, peristomial lip; SC, scopula; ST, stalk; ZO, zooid.
Fig. 2. *In vivo* and protargol stained specimens of *Epistylis wuhanensis* Wang, Zhou, Guo, and Gu, 2017 from Japan (MPM 21760). A, Colony of zooids; B–D, extended zooid, lateral view; E, aboral region of infraciliature polykineties; F, extended zooid, apical view. Abbreviations: ATB, aboral trochal band; AZM, adoral zone of membranelles; CV: contractile vacuole; CY, cytopharynx; G, germinal kinety; H, haplokinety; MN, macronucleus; P1–3, infundibular polykineties 1–3; PD, peristomial disc; PL, peristomial lip; Po, polykinety; SC, scopula; ST, stalk; ZO, zooid.
similar to the latter. The minor morphological differences of the specimens and the one base deletion of the newly determined ITS1 sequence are considered to be intraspecific variations, thus, the newly collected specimens were identified as a new country-specific record for *E. wuhanensis*.

**Discussion**

From comparing of *E. wuhanensis* with five morphologically similar species (*E. chlorelligerum* Shen 1980, *E. chrysemidis* Bishop and Jahn, 1941, *E. picatilis* Ehrenberg, 1838, *E. hentscheli* Kahl, 1935, and *E. galea* Ehrenberg, 1831), it was revealed that *E. wuhanensis* was characterized by the shape of the mammilla-shaped peristomial disk, the C- or J-shaped macronucleus lying in the middle or upper thirds of the cell, and the contractile zood without conspicuous folds (Wang et al. 2017). While *E. wuhanensis* has not been compared to following three Epistylis species recorded attached to *L. cyprinacea*: *E. branchiophila* Perty, 1852, *E. magna* van As and Viljoen, 1984, and *E. cyprinaceae* van As and Viljoen, 1984 (see van As and Viljoen 1984). However, descriptions of *E. magna* and Viljoen (1984) are short and limited and can only be compared with a few characteristics. The infundibulum of *E. branchiophila* and *E. magna* extends to the upper thirds of the body. Furthermore, *E. wuhanensis* differs from *E. magna* by the zood width [24–46 μm (Wang et al. 2017) and 47.1–64.0 (this study)] vs. 82–145 μm (van As and Viljoen 1984)]. The colony of *E. wuhanensis* consists of uniform zooids [90.2–175.4 μm (Wang et al. 2008); 128.0–220.3 μm (this study in length)], but that of *E. wuhanensis* extends to the upper thirds of the body. Furthermore, *E. wuhanensis* differs from *E. magna* by the zooid width [24–46 μm (Wang et al. 2017) and 47.1–64.0 (this study)] vs. 82–145 μm (van As and Viljoen 1984)]. The colony of *E. wuhanensis* consists of uniform zooids [90.2–175.4 μm (Wang et al. 2008); 128.0–220.3 μm (this study in length)], but that of *E. cyprinaceae* consists of smaller dimorphic zooids: microzooids (19–33 μm) and macrozooids (45–48 μm) (van As and Viljoen 1984).

Some peritrich ciliates have been reported to be attached to *L. cyprinacea* in Japan thus far are probably *E. wuhanensis*. However, several species of *Epistylis* and the other peritrich ciliates, *Vorticella* Linnaeus, 1767 (*Vorticellidae*), have been recorded from other parasitic and free-living copepods including *L. cyprinacea* as well (e.g., van As and Viljoen 1984; Tsukii 2010). Each of these cases, ciliate specimens did not been examined nor described morphology and needs to be reexamined for certain identification.

Wang et al. (2017) reported that *E. wuhanensis* adhered to the fins of the yellow catfish, but all other records of peritrich ciliates attached to *L. cyprinacea* in Japan did not indicate that they infected the host fishes (see references for Table 1). In Japan, hosts of *L. cyprinacea* with attached peritrich ciliates are fishes of families Cyprinidae, Gobiidae, and Poeciliidae (Table 1). *E. wuhanensis* may be able to infect only bagrid catfish as the direct infection of fish.

Twenty-two *Epistylis* species attached to metazoans have been identified as valid species in Japan (Table 2), with the total number of species at 37, including species recorded as free-living and in activated sludge. However, many symbiotic *Epistylis* have not been rediscovered and identified (Table 2), with the effects of epistylidids on the host remaining un-known.

Based on molecular phylogeny, *Epistylis* was considered a polyphyletic group (Utz and Eizirik 2007; Utz et al. 2010; Wang et al. 2017). This study provided the first molecular information on the *Epistylis* in Japan, and future molecular analysis of Japanese species is expected as basic information for a detailed understanding of the phylogenetic relationships and reconstruction of the genus.

Eleven species of *Epistylis* attached to metazoans which originally described in Japan have not been reported since their original description (Table 2). For example, taxonomic studies demonstrated by Fukui (1950a, b, 1951a, b) have never been surveyed nor cited the others (e.g., Fernandez-Leborans 2009). Furthermore, Fukui (1950a, b, 1951a, b) described the following symbiotic species with inadequate descriptions by current standards: *Cothurnia anajakoe* Fukui, 1950 (*Vorticellidae*) and *Lagenophrys rotunda* Fukui, 1950 (*Lagenophryidae*) from *Upogebia major* (De Haan 1841); *Vorticella tuberculata* Fukui, 1951 and *Coturniophysis gaeticis* Fukui, 1951 (*Vorticellidae*) from *Gaetice depressus* (De Haan, 1833); and *Opercularia sarasame* Fukui, 1951 (*Operculariidae*) from *Chiromantes haematocheir* (De Haan, 1833). *Epistylis* and its related genera were considered polyphyletic groups by molecular phylogenetic analysis (Utz and Eizirik 2007; Utz et al. 2010; Wang et al. 2017), and redescriptions of the above species and determining their systematic position are important for the stability of *Epistylis* and peritrich ciliate taxonomy and understanding their biodiversity in Japan.

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Appendix

Supplemental Movies 1–3. Observation of live specimens of Epistyris wuhanensis Wang, Zhou, Guo, and Gu, 2017 (MPM 21760). https://doi.org/10.6084/m9.figshare.14754510