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# A new species of *Holosticha* (Ciliophora: Hypotrichida) from the coastal waters of Nagasaki, Japan: *Holosticha nagasakiensis* sp. nov.

Xiaozhong Hu*† and Toshikazu Suzuki*‡

*Faculty of Fisheries, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852-8521, Japan.
†Laboratory of Protozoology, Ocean University of China, Qingdao 266003, People's Republic of China.
‡Corresponding author, e-mail: huxzchen@yahoo.com.cn

The morphology and infraciliature of the marine hypotrichous ciliate *Holosticha nagasakiensisi* sp. nov. from the coastal waters of Nagasaki, Japan are described based on living and protargol-impregnated specimens. The new species is characterized by: medium-sized marine *Holosticha*, *in vivo* 100–150×30–40 μm with elongated body shape and slightly greyish to reddish cell colour; cortical granules arranged in conspicuously longitudinal rows; 24–32 adoral membranelles; ~20 midventral cirral pairs and 7–10 transverse cirri; 28–42 left and 25–38 right marginal cirri; constantly 4 frontal cirri and 3 dorsal kineties; 23–63 macronuclear nodules.

## INTRODUCTION

*Holosticha* is one of species-rich ciliate genera known. To date, up to 40 species have been acknowledged (Kahl, 1932; Borror, 1972; Foissner et al., 1991; Petz et al., 1995; Song & Wilbert, 1997a,b; Hu & Song, 1999; Berger, 2001). However, only a few of these are marine forms. During a recent survey on marine ciliated fauna along the coast of the Yellow Sea, China, two unknown forms were described (Gong et al., 2001; Hu & Song, 2001). Here, we add a further new species found in the waters of the New Fishing Port of Nagasaki, Japan.

## MATERIALS AND METHODS

Samples were isolated from New Fishing Port of Nagasaki (32°48′N 129°46′E), Japan on two occasions in December, 2002. Water salinity was about 34 psu, water temperature 8 and 17 °C, and pH 7.9. Specimens were cultured in boiled seawater to which squeezed rice grains were added.

The infraciliature was revealed with the protargol staining method according to Wilbert (1975). Counts and measurements on prepared specimens were performed at a magnification of ×1000. *In vivo* measurements were conducted at magnifications of ×100–1000. Illustrations of living organisms were based on *in vivo* observations, while those of stained cells were made with a camera lucida. Terminology is according to Borror (1972).

## RESULTS AND DISCUSSION

Order HYPOTRICHIDA Stein, 1859
Family HOLOSTICHIDAE Fauré-Fremiet, 1961
Genus *Holosticha* Wrzesniowski, 1877
*Holosticha nagasakiensis* sp. nov.
(Figures 1A–I, 2A–J; Table 1)

*Diagnosis*

Medium-sized marine *Holosticha*, *in vivo* 100–150×30–40 μm with elongated body shape and slightly greyish to reddish cell colour; cortical granules arranged in conspicuously longitudinal rows; 24–32 adoral membranelles; ~20 midventral cirral pairs and 7–10 transverse cirri; 28–42 left and 25–38 right marginal cirri; constantly 4 frontal cirri and 3 dorsal kineties; 23–63 macronuclear nodules.

*Type location*

Nagasaki New Fishing Port, Nagasaki (32°48′N 129′46′E), Japan.

*Etymology*

Named after the location (City of Nagasaki) where the species was discovered.

*Type specimens*

One holotype and one paratype as two slides of protargol-impreganted cells (registration no.: 2002: 12: 01; 2002: 12: 02) have been deposited in the Laboratory of Protozoology, College of Fisheries, Ocean University of China, People's Republic of China. Data relating to the type population are given in the upper line in Table 1.

*Description*

Cell flexible, *in vivo* 100–150×30–40 μm, usually body shape as shown in Figures 1A & 2A, right margin almost straight, left side conspicuously convex, mostly widest at mid-body with both ends narrowly rounded. Ratio of body length to width about 3–4:1, and dorsoventrally flattened ~3:1 (Figure 1B). Buccal cavity prominent, about 25–30% of body length with cytopharyngeal fibres visible *in vivo*. Pellicle soft, cortical granules conspicuous, spherical, yellow-greenish at high magnification, 0.5–1 μm in diameter, arranged in longitudinal rows and densely packed at both ends, which render cell slightly greyish to reddish in colour at low magnification (Figures 1E & 2B–E). Endoplasm colourless, containing many globules (1–5 μm across), bacteria and diatoms. Contractile vacuole located on left about anterior 1/3 of body

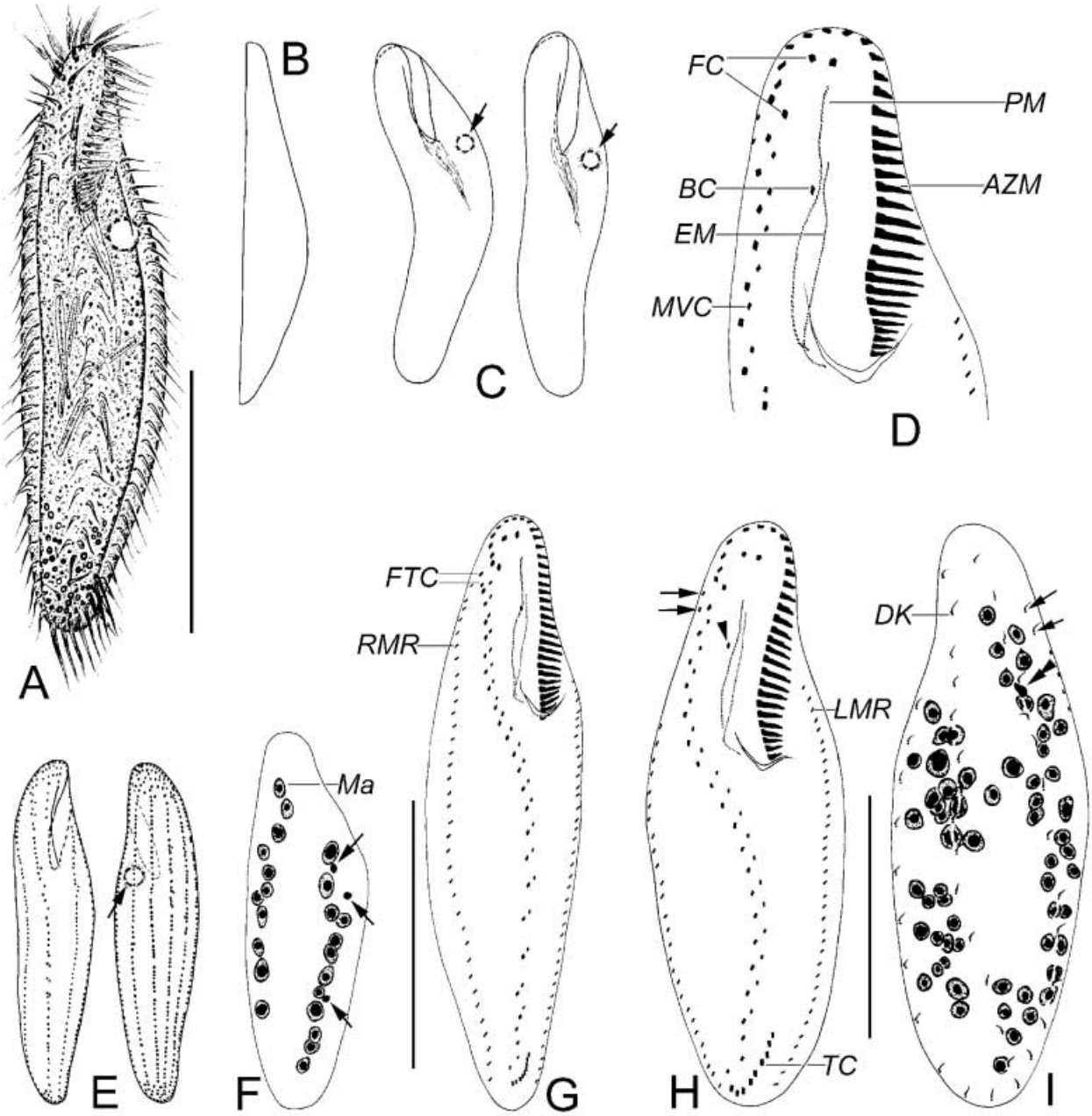


**Figure 1.** Morphology and infraciliature of *Holosticha nagasakiensis* sp. nov. from life (A–C, E) and after protargol impregnation (D, F–I). (A) Ventral view; (B) left lateral view; (C) variable body shapes, arrows to show contractile vacuole; (D) detail of structure in buccal field; (E) arrangement of cortical granules on ventral and dorsal sides, arrow to indicate contractile vacuole; (F) macronuclear nodules and micronuclei (arrows); (G–I) infraciliature on ventral (G,H) and dorsal (I) sides, arrows in (H) to show frontoterminal cirri, arrows in (I) to mark basal bodies pairs located at the anterior end of right marginal row, arrowhead in (H) to denote buccal cirrus, double arrowhead in (I) to indicate micronucleus. AZM, adoral zone of membranelles; BC, buccal cirrus; DK, dorsal kinety; EM, endoral membrane; FC, frontal cirri; FTC, frontoterminal cirri; LMR, left marginal cirral row; Ma, macronuclear nodule; MVC, midventral cirri; PM, paroral membrane; RMR, right marginal cirral row; TC, transverse cirri. Scale bars: A & G, 60 $\mu$m; H & I, 45 $\mu$m.

(Figures 1A,C,E, arrow; 2E, arrow). Twenty-three to 63 ellipsoid macronuclear nodules, about 5 $\mu$m long after impregnation with only one large nucleolus each, distributed throughout the body (Figures 1F,I & 2F,G); 1–7 micronuclei, spherical to ellipsoid, ~1–2 $\mu$m long (Figures 1F,I, arrows and double arrowhead, respectively; 2F, arrows). Movement without specialities, crawling on debris or substrate.

Most cirri relatively fine, about 12 $\mu$m long, but transverse cirri a little stronger, ~15 $\mu$m long. Infraciliature typical for genus (Figures 1D,G,H,I & 2H,I,J). Adoral zone of membranelles evenly curved, cilia of membranelles about 15 $\mu$m long. Paroral membrane evidently longer than endoral membrane, occupying most of buccal field length. Consistently four slightly enlarged frontal cirri in normal position (Figure 1D). Single buccal cirrus situated beside mid-point of paroral membrane (Figures 1D & 2J, arrow). Two frontoterminal cirri between right end of adoral zone of membranelles and anterior end of right marginal row (Figure 1G,H, arrows). Midventral cirral row composed of oblique pairs of cirri, extending to posterior end of cell, of which the right cirri a little larger



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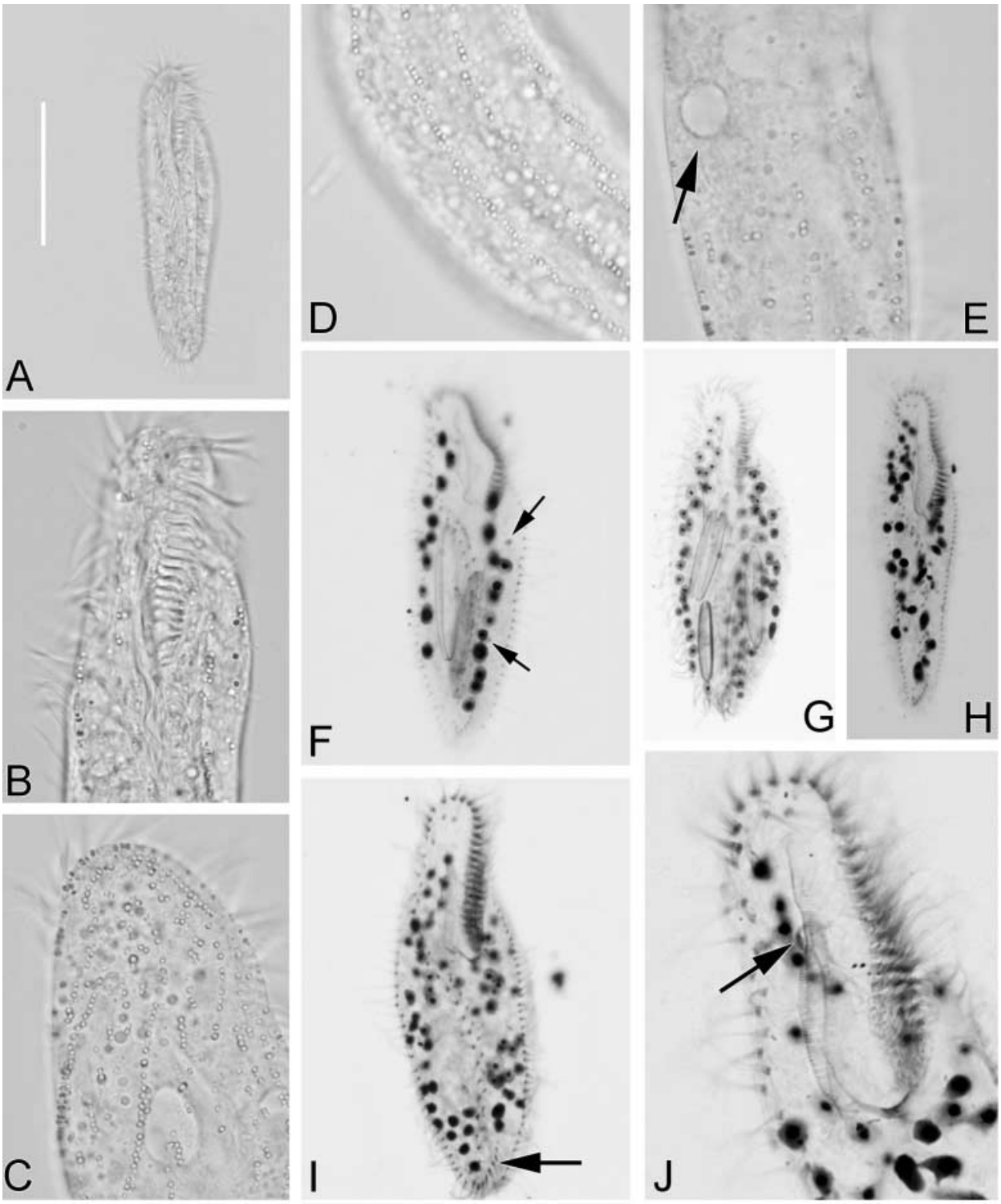


**Figure 2.** Photomicrographs of *Holosticha nagasakiensis* sp. nov. from live (A–E) and stained specimens (F–J). (A) Ventral view; (B–D) arrangement of cortical granules on ventral (B) and dorsal (C,D) sides; (E) dorsal view, note cortical granules and contractile vacuole (arrow); (F,G) macronuclear nodules and micronuclei (arrows); (H,I) infaciliature on ventral sides, arrow to show transverse cirri; (J) detailed buccal structure, arrow to indicate buccal cirrus. Scale bar: A, 50 $\mu$m.

than the left ones. Transverse cirri located caudally, arranged in J-shape (Figures 1G,H & 2I, arrow). Posterior ends of marginal rows not confluent, mostly base of each marginal cirri composed of two basal body rows, and always 2–3 basal body pairs exist at the anterior end of right marginal row on dorsal side (Figure 1I, arrows). Three dorsal kineties, middle and right one nearly extending over the whole length of the body, left one distinctly shortened anteriorly, cilia about 3 $\mu$m long *in vivo*, no caudal cirri present at the posterior end (Figure 1I).

*Comparison with other holostichs*

With reference to the body shape and size, 12 species should be compared with this new species (Table 2).



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**Table 1.** *Morphometric characteristics of two populations of* Holosticha nagasakiensis *sp. nov. from Nagasaki. Data are based on protargol-impregnated specimens. Measurements in μm.*

| Character | Min | Max | Mean | SD | SE | CV | N |
|---|---|---|---|---|---|---|---|
| Body length | 80 | 140 | 119.4 | 16.86 | 4.22 | 14.1 | 16 |
| | 94 | 158 | 128.1 | 14.42 | 3.61 | 11.3 | 16 |
| Body width | 29 | 55 | 41.3 | 8.53 | 2.46 | 20.7 | 12 |
| | 26 | 52 | 35.9 | 5.85 | 1.46 | 16.3 | 16 |
| Adoral zone of membranelles, length | 30 | 48 | 41.1 | 4.27 | 1.07 | 10.4 | 16 |
| | 38 | 49 | 42.5 | 3.35 | 0.84 | 7.9 | 16 |
| Adoral membranelles, number | 24 | 30 | 27.4 | 1.71 | 0.43 | 6.2 | 16 |
| | 25 | 32 | 28.4 | 1.86 | 0.47 | 6.5 | 16 |
| Frontal cirri, number | 4 | 4 | 4 | 0 | 0 | 0 | 16 |
| | 4 | 4 | 4 | 0 | 0 | 0 | 15 |
| Buccal cirrus, number | 1 | 1 | 1 | 0 | 0 | 0 | 16 |
| | 1 | 1 | 1 | 0 | 0 | 0 | 16 |
| Frontoterminal cirri, number | 2 | 2 | 2 | 0 | 0 | 0 | 16 |
| | 2 | 2 | 2 | 0 | 0 | 0 | 16 |
| Midventral cirri, number | 34 | 48 | 39.6 | 4.05 | 1.05 | 10.2 | 15 |
| | 36 | 44 | 42.0 | 2.51 | 0.65 | 5.9 | 15 |
| Left marginal cirri, number | 28 | 41 | 32.8 | 4.23 | 1.06 | 12.9 | 16 |
| | 33 | 42 | 37.6 | 2.58 | 0.65 | 6.9 | 16 |
| Right marginal cirri, number | 25 | 35 | 30.4 | 3.16 | 0.79 | 10.4 | 16 |
| | 28 | 38 | 32.6 | 2.22 | 0.55 | 6.8 | 16 |
| Transverse cirri, number | 7 | 10 | 8.4 | 0.99 | 0.25 | 11.8 | 15 |
| | 8 | 10 | 8.9 | 0.77 | 0.19 | 8.7 | 16 |
| Dorsal kineties, number | 3 | 3 | 3 | 0 | 0 | 0 | 12 |
| | 3 | 3 | 3 | 0 | 0 | 0 | 15 |
| Macronuclear nodules, number | 23 | 62 | 41.5 | 11.65 | 3.01 | 28.1 | 15 |
| | 26 | 63 | 48.2 | 11.52 | 2.97 | 23.9 | 15 |
| Macronuclear nodule, length | 2 | 8 | 5.1 | 1.92 | 0.50 | 37.6 | 15 |
| | 3 | 7 | 4.6 | 1.18 | 0.31 | 25.7 | 15 |
| Macronuclear nodule, width | 2 | 6 | 3.7 | 1.23 | 0.32 | 33.2 | 15 |
| | 2 | 4 | 3.0 | 0.65 | 0.17 | 21.7 | 15 |
| Micronuclei, number | 1 | 7 | 3.4 | 1.65 | 0.44 | 48.5 | 14 |
| | 2 | 6 | 3.9 | 1.60 | 0.50 | 41.0 | 10 |

CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; N, number of specimens examined; SD, standard deviation; SE, standard error of the mean.

**Table 2.** *Comparison of some closely-related* Holosticha-*species.*

| | Body length | Position of CV | EAM | Ma | AM | MVC | TC | DK | Habitat | Data source |
|---|---|---|---|---|---|---|---|---|---|---|
| *H. nagasakiensis* | 100–150μm | anterior 1/3 | absent | 23–63 | 24–32 | 35–49 | 7–10 | 3 | marine | original |
| *H. foissneri* | 130–170μm | subequatorial | absent | 5–11 | 26–36 | 32–40 | 9–17 | 4 | marine | Petz et al., 1995 |
| *H. spindleri* | 100–115μm | subequatorial | present | 1–4 | 22–43 | 18–34 | 7–14 | 4–5 | marine | Petz et al., 1995 |
| *H. heterofoissneri* | 110–150μm | subequatorial | absent | 14–16 | 33–49 | 24–30 | 11–18 | 5 | marine | Hu & Song, 2001 |
| *H. manca* | 100–120μm | above mid-body | absent | ~50–70 | 21–27 | 18–32 | 4–6 | 3 | marine | Song & Wilbert, 1977b; Kahl, 1932 |
| *H. diademata* | 50–90 μm | subequatorial | absent | 2 | 10–30 | 16–22 | 6–10 | 4–5 | marine | Hu & Song, 1999 |
| *H. pullaster* | 60–90μm | subequatorial | absent | 2 | 20–28 | 18–30 | 7–12 | 4 | freshwater or marine | Foissner et al., 1991 Petz et al., 1995 |
| *H. bradburyae* | 150–320μm | absent | present | 28–33 | 50–56 | 27–32 | 20–26 | 9–11 | marine | Gong et al., 2001 |
| *H. warreni* | 80–120μm | absent | absent | ~50 | 26–31 | 14–18 | 10–12 | 3 | marine | Song & Wilbert, 1997a |
| *H. kessleri* | 120–170μm | mid-body | absent | 2 | – | 20–25 | 10–15 | – | marine | Kahl, 1932; Foissner et al., 1991 |
| *H. gibba* | 170μm | anterior 1/3 | – | 2 | – | – | – | – | marine | Kahl, 1932 |
| *H. vernalis* | 180μm | mid-body | – | – | – | – | 5–8 | – | freshwater | Kahl, 1932 |
| *H. setifera* | 120–150μm | – | – | 2 | – | – | – | – | saltwater | Kahl, 1932 |

AM, adoral membranelles; CV, contractile vacuole; DK, dorsal kinety; EAM, elongate adoral membrane; Ma, macronuclear nodules; MVC, midventral cirri; TC, transverse cirri. –, Data not available.

Among them, *Holosticha diademata*, *H. pullaster* and *H. kessleri* possess a bipartite adoral zone of membranelles and consistently have only two macronuclear nodules (vs 23–63 in *H. nagasakiensis*), hence they can be clearly separated from this new form (Kahl, 1932; Foissner et al., 1991; Petz et al., 1995; Hu & Song, 1999). Additionally, both *H. pullaster* and *H. kessleri* lack cortical granules.

As the species mentioned above, *Holosticha foissneri* and *H. heterofoissneri* also differ from *H. nagasakiensis* in having a bipartite adoral zone of membranelles. Additional differences include the position of contractile vacuole (subequatorial vs in anterior 1/3 of body), the numbers of macronuclear nodules (5–11 and 14–16 respectively vs 23–63), transverse cirri (9–17 and 11–18 respectively vs 7–10) and dorsal kineties (4–5 vs 3) (Petz et al., 1995; Hu & Song, 2001).

*Holosticha spindleri* and *H. bradburyae* are characterized by having extremely elongate adoral membranelles at the posteriormost end of the adoral zone of membranelles (vs absent in *H. nagasakiensis*). In addition, *H. spindleri* has a maximum of only four macronuclear nodules (Petz et al., 1995). Compared with *H. nagasakiensis*, *H. bradburyae* is larger (150–320 $\mu$m long vs 100–150 $\mu$m long) and has higher numbers of adoral membranelles, cirri, and dorsal kineties (Gong et al., 2001).

*Holosticha manca* and *H. warreni* are evidently related to *H. nagasakiensis*. However, these three species differ in several respects including: the shape, size and arrangement of the cortical granules, and the number and arrangement of midventral and transverse cirri (Kahl, 1932; Song & Wilbert, 1997a,b).

Although the infraciliatures of *Holosticha gibba* and *H. setifera* remain unknown, these two species can be easily separated from *H. nagasakiensis* by possessing two macronuclear nodules (Kahl, 1932). The infraciliature of *H. vernalis* is likewise unknown. However, this species can be distinguished from *H. nagasakiensis* in terms of body length (180 $\mu$m vs 100–150 $\mu$m), position of contactile vacuole (mid-body vs in anterior 1/3 of body) and habitat (freshwater vs marine) (Kahl, 1932).

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