Trichopodiella faurei n. sp. (Ciliophora, Phyllopharyngea, Cyrtophoria): Morphological Description and Phylogenetic Analyses Based on SSU rRNA and Group I Intron Sequences

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ABSTRACT. A new marine cryptophycean ciliate Trichopodiella faurei n. sp., which belongs to the order Dysteriida, family Hartmannulidae, was investigated at the morphological and molecular levels. A combination of morphological features of the organism including the oval body shape, 2–3 contractile vacuoles, 22–28 nematodesmal rods in the cytopharyngeal basket, and 31–39 somatic kineties, distinguishes it from all other known congeners. In reconstructed small subunit (SSU) rRNA phylogenies, T. faurei groups with Isochona, a representative genus of the subclass Chonotrichia. The similarity of the infraciliature between hartmannulid and several chonotrichian examples also suggests that these taxa should be closely related. A new S943 intron belonging to group IC1 was identified in the SSU rRNA gene of this species. This intron is phylogenetically related to the S891 introns previously found in the suctorians Acineta sp. and Tokophrya lennarum, and their internal guide sequences share four nucleotides, indicating that these introns were vertically inherited from a common phyllopharyngean ancestor and that reverse splicing might have been involved in the transposition.

Key Words. 18S rRNA, chonotrichia, dysterida, hartmannulidae, infraciliature, marine ciliate.

Materials and Methods

Source of organisms. Two populations of T. faurei n. sp. were collected, one from coastal waters of the Yellow Sea, Qingdao (36°08’N; 120°43’E) northern China, and the other from Daya Bay (22°42’N; 114°31’E), South China Sea, southern China.

Morphological observations. Cells were first examined at 10–63X magnification using a stereomicroscope to observe behavior and movement, then transferred onto glass slides and placed under a microscope at 100–1,250X magnification to reveal living features. Protargol staining was used in order to reveal the infraciliature (Wilbert 1975). Drawings of stained specimens were performed at 1,250-fold with the aid of a camera lucida.

DNA extraction, PCR amplification, and sequencing. Isolated cells of the Daya Bay population were repeatedly washed to remove other protists, and genomic DNA was extracted with REDExtract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO) according to the manufacturer’s protocol, with the modification suggested by Gong et al. (2007). The SSU rRNA gene was amplified by PCR with primers Euk A and B (Medlin et al. 1988). Temperature cycling was 40 cycles of denaturation for 15 s at 95 °C, primer annealing for 1 min at 60 °C, and extension for 3 min at 72 °C. The PCR products were purified with a Gel Extraction Kit (QIAgen, Chatsworth, CA), inserted into the pGEM-T Easy vector (Promega, Madison, WI), and transformed into Escherichia coli DH5α cells. Plasmid DNA containing the rRNA genes was purified with a Plasmid Miniprep Kit (Qiagen). Sequencing in both directions was carried out on an ABI 3700 sequencer.

Secondary structure of intron. An intron in the SSU rDNA sequence of T. faurei n. sp. was identified by the alignment of 50 intron-less ciliate sequences using ClustalX (Thompson et al. 1997). The conserved P, Q, R, and S elements (Burke et al. 1987; Cech 1988) in the intron sequence were located using BioEdit (Hall 1999). Our preliminary analyses found that: (1) the paired region of R and S elements has a bulged nucleotide A; (2) the 3′-end of Q element forms two base pairs GU and CG with the 5′-end of the R element; (3) there is no bulge in the paired region of the P and Q elements; (4) the nucleotides 3′–5′ of the R element were UCA; and (5) segment J3/4 contains nucleotides AUA. These features indicated that it belongs to the subdivision IC1 of group I introns (Machouart et al. 2004). The package INFERNAL V0.81 (http://infernal.janelia.org/) was used to build a covariance model (CM) of the seed alignment of IC1 introns,
which was downloaded from the Group I Intron Sequence and Structure Database (GIISSD, Zhou et al. 2008). The new intron and several known IC1 intron sequences were structurally aligned using CMaxOn within the INFERNAL package, resulting in annotations of the paired domains (P1–P10) and loops of the new IC1 intron, which served as a guide to plotting the putative secondary structure.

Phylogenetic analyses. Exon rDNA sequences of phyllopharyngean species were obtained from GenBank as ingroup taxa: Acineta sp. (AY332717), Chilodonella uncinata (AF300281), Chlamydodon triquetus (AY331794), Chlamydodon excocellatus (AY331790), Discophrya collina (L24446), Dysteria derouxi (AY378112), Dysteria procera (DQ057347), Dysteria sp. (AY331801), Ephelota gemmipara (DQ834370), Heliophrya er-hardi (AY007445), Isochona sp. (AY242119), T. lemnarum (AY332720), Trihigmostoma steini (X71134), and one environmental sequence (EF527117). Two synhymeniidean species Orthodonella apohamatus (DQ232761) and Zosterodasys transversus (EU286812) were selected as outgroup taxa. Sequences were aligned using Hmmer Package version 2.1.4 (Eddy 2001) with default settings. Hmmer used a set of previously aligned sequences to model the primary structure of a sequence. The training alignment for model building consisted of all available ciliate SSU rDNA sequences, downloaded from the European Ribosomal Database (Wuyts et al. 2002) and aligned according to their secondary structure. The masked regions that could not be aligned unambiguously were removed, and the remaining alignment was

![Figure 1-9](image_url)
then manually refined by using BioEdit (Hall 1999). The alignment of 1,494 characters was used in the final analyses. The program Modeltest 3.7 (Posada and Crandall 1998) selected the GTR+I+G as the best model with AIC criterion, which was then used for maximum likelihood analysis using the PhyML V2.4.4 program (Guindon and Gascuel 2003). The reliability of internal branches was assessed using a non-parametric bootstrap method with 1,000 replicates. A neighbor-joining (NJ) tree was constructed using ML-corrected distances, with the settings of the variable-site $\gamma$ distribution shape parameter (G) at 0.6268, the proportion of invariable sites (I) at 0.3113, and base frequencies and a rate matrix for the substitution model were as suggested by Modeltest. Maximum parsimony (MP) trees were obtained via random addition and swapped using the tree-bisection-reconnection (TBR) algorithm. Gaps were treated as missing data. Both NJ and MP analyses were performed with the software package PAUP* 4.0b10 (Swofford 2002), and the support for the internal branches was estimated using the bootstrap method with 1,000 replicates.

Based on the annotated secondary structures, the IC1 intron of *T. faurei* n. sp. was manually aligned to the existing IC1 alignment from GISSD. The IE group intron alignment, which was also obtained from GISSD, was then manually aligned to the IC1 dataset. We used the unambiguously aligned catalytic core domains (78 nt) in P3–P8 for phylogenetic inferences. A total of 56 intron sequences from GISSD were selected to present the result. NJ tree (p-distance) and MP trees were constructed by MEGA V3.1 (Kumar, Tamura, and Nei 2004) with 1,000 bootstrap resamples. IE introns were taken as outgroup representatives.

**Fig. 10–21.** Photomicrographs of *Trichopodiella faurei* n. sp. from life (10–13, 16) and after protargol impregnation (14–15, 17–21). 10. Left lateral view, showing the cortical alveoli (arrow) which cover the posterior portion of cell; 11. A view of cross-section, arrow marks the collar-shaped, depressed glandular area and arrowhead refers to the relatively thin gel layer on the anterior portion of cell surface. 12. Close-up of the gel substance on posterior portion of cell (arrow); 13. Focusing on the surface of the cortical alveoli, to note its polygonal pattern formed by many grids (arrow). 14, 15, 17, 19–21. Ventral (14, 17, 19, 21), dorsal (15, 20) views of infraciliature. 16. Focusing on the gel filaments for attachment. 18. Showing the long slit-like cytostome (arrow) in the apical region of the cell. CR, caudal ring; CV, contractile vacuole; EF, equatorial fragment; LK, left kineties; Ma, macronucleus; PK, perioral kinety; RK, right kineties; TF, terminal fragment; TS, transpodial segment. Scale bars in (10, 11, 16) = 20 $\mu$m, in (12, 13) = 10 $\mu$m.
Table 1. Morphometric characteristics of Trichopodiella faurei n. sp.

<table>
<thead>
<tr>
<th>Character</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
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<td>50.0</td>
<td>37.0</td>
<td>6.98</td>
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<tr>
<td>SK, number</td>
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<td>39.0</td>
<td>34.8</td>
<td>2.20</td>
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<td>19.0</td>
<td>16.4</td>
<td>1.69</td>
<td>10.3</td>
<td>14</td>
</tr>
<tr>
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<td>26.2</td>
<td>2.45</td>
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<td>6</td>
</tr>
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<td>22.0</td>
<td>20.0</td>
<td>1.62</td>
<td>8.1</td>
<td>17</td>
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<tr>
<td>BB in CR, number</td>
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<td>23.0</td>
<td>20.8</td>
<td>2.17</td>
<td>10.4</td>
<td>5</td>
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<tr>
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<td>9</td>
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<td>15.0</td>
<td>9.43</td>
<td>62.9</td>
<td>5</td>
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<tr>
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<td>1.22</td>
<td>6.6</td>
<td>7</td>
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<tr>
<td>Macronuclear length</td>
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<td>18.0</td>
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<td>2.13</td>
<td>16.1</td>
<td>5</td>
</tr>
<tr>
<td>Macronuclear width</td>
<td>7.0</td>
<td>12.0</td>
<td>10.3</td>
<td>1.37</td>
<td>13.3</td>
<td>20</td>
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</tbody>
</table>

All measurements in μm.

BB, Basal bodies; CR, caudal ring; EF, equatorial fragment; NR, nematodesmal rods; PK, perioral kinety; SK, somatic kineties; TF, terminal fragment; TS, Transpodial segments.

RESULTS

Morphological description. The two populations of T. faurei n. sp. show no significant differences in morphology and infraciliature (data not shown). The size of cells is about 30–60 × 15–30 μm in vivo. The body is roughly oval, with width slightly wider than the dorsoventral thickness. The anterior portion is bluntly rounded and slightly greater than the posterior (Fig. 1, 10). The body surface is covered with a layer of transparent gel substance that is 1–2 μm in thickness, mostly relatively even, and smooth (arrowhead in Fig. 11), but it becomes distinctly foam-like in the posterior region of cell, forming cortical alveoli (Fig. 1, 10, 11). The body surface is covered with a layer of transpodial segments (TS), that are composed of ca. 10 kinetosomes situated inside the depressed chamber of the glandular area (Fig. 2, 4, 8, 12, 19, 21). The macronucleus is juxtaposed heteromerous, about 13 × 10 μm after protargol impregnation, and positioned in the body center (Fig. 3, 9, 15, 18–20); the micronucleus was not detected.

Small Subunit (SSU) rDNA. The SSU rDNA sequence of T. faurei n. sp. has been deposited in GenBank with Accession no. EU517922. This sequence has 2,105 bp, with a group I intron of 394 bp inserted at position 943 in the SSU rDNA of E. coli (J01695) (Canonne et al. 2002).

Small subunit (SSU) rRNA phylogenies. Ciliates assigned to the class Phyllopharyngea are strongly supported as a clade with suctorians on a separate branch from the cryptophorans and choanorhics (Fig. 22). Trichopodiella faurei n. sp. clusters with the environmental sequence (EF527117) and then forms a clade with the choanotrich species Isochona sp. (Fig. 22). This clade is highly or moderately supported by bootstrap values (ML/MP/NJ, 88/78/90), and shows a sister relationship with Dysteria spp. We additionally analysed two larger datasets respectively with 37 and 59 ciliate taxa from all known classes within the phylum (data not shown), resulting in identical topologies for phyllopharyngeans as shown in Fig. 22.

Structural and phylogenetic analyses of intron. The intron is named Tfa.S943 according to the nomenclature suggested by Johansen and Haugen (2001). The predicted secondary structure of Tfa.S943 supports its membership in the group IC1 intron (Fig. 23). Conserved elements P, Q, R, and S were located at positions 133–144, 244–253, 286–299, and 335–346, respectively. Five exonic nucleotides and the first intronic nucleotide at the 5′-splice site are paired with the intron internal guide sequence (IGS) at positions 33–38, forming the P1 segment. The P10 stem is composed of six base pairs, formed by six exon nucleotides at the 3′-splice site and positions 27–32. Although an open reading frame (ORF) can be detected at positions 238–330 of the intron sequence, this region includes Q and R elements and it is too short to encode a protein, thus we believe no endonuclease-encoding ORF exists in Tfa.S943.

The intron of T. faurei n. sp. is the first one which is found at position 943 of SSU rDNA in ciliates. In the phylogenetic trees, Tfa.S943 does not cluster with the S943 introns found in other eukaryotes, such as Fungi, Chlorophyta, and Acanthamoeba (Fig. 24). Instead, it is closely related to two S891 introns from the suctorian ciliates Acinet a sp. and T. lemnarum with low to moderate bootstrap support (Fig. 24). The S1506 intron in T. lemnarum and L1925 introns in Tetrahymena spp. respectively show affinities with a S1506 from Rhodophyta and a S943 from Chlorophyta, rather than with the other ciliate introns.

DISCUSSION

Comparison with congeners. Claparède and Lachmann (1859) erected the genus Trichopus for a single species T. dysteria, about one century later, Fauré-Fremiet (1957) added the second species Trichopodiella lachmanni to the taxon. Because the generic name Trichopus was pre-occupied, Corliss (1960) suggested Trichopodiella to replace the invalid generic name. Since then, another two species of the genus Trichopodiella have been recognized (Deroux 1976).

Trichopodiella faurei n. sp. is most similar to T. lachmanni (Fauré-Fremiet 1957) in terms of living morphology and behavior.
Trichopodiella faurei n. sp.

Diagnosis. Marine Trichopodiella, size 30–60 μm in vivo, body oval, covered with a layer of transparent gel which is foam-like in the posterior region; glandular region collar-like, conspicuously depressed, secreting filiform substance for anchoring the cell to substratum; single-rowed PK longitudinally oriented, separating left and right somatic kineties; 31–39 somatic kineties with 13–19 kineties left of the PK; two to three contractile vacuoles.

Type locality. Intertidal water of Qingdao (36°08′N; 120°43′E), China.

Type specimens. One protargol-impregnated holotype slide (registration numbers 2008:06:02:1) is deposited at the NHM, London, and two paratypes are respectively deposited at laboratory of Protozoology, OUC, and the Laboratory of Protozoology, SCNU, respectively.
Dedication and etymology. Named in honor of the French ciliatologist, Prof. E. Fauré-Fremiet, who first described the morphology of the species *T. lachmanni* in detail.

Systematic position and relationships. The placement of *T. faurei* n. sp. in the order Dysteriida is apparently supported by the SSU rRNA tree, and can be justified by its *Hartmannula*-like infraciliature with its somatic kineties continuous from right to left. *Trichopodiella faurei* and its congeners have no podites, which are commonly found in dysteriids. We suppose *T. faurei* n. sp. has a mucous-secreting glandule as in its congener *T. lachmanni* (see Deroux and Dragesco 1968; Fauré-Fremiet 1957), and these glandular organelles are probably homologues to the podite, which might have become reduced or lost with diversification of *Trichopodiella* species because there were no glandular organelles reported for *T. elongata* and *T. pulex* (see Deroux 1976).

The hartmannulid *T. faurei* is closely related to the chonotrichian *Isochona* sp. in the SSU rRNA trees, which refines the relationship previously found between the order Dysteriidae and this sole representative of the subclass Chonotrichia (Snoeyenbos-West et al. 2004). The pattern on the infraciliature of swarmers of *Isochona* sp. is not known, but there are morphological similarities (e.g. the somatic kineties are continuous from right to left) between *T. faurei* and the swarmers or bud of some other chonotrichian species, such as *Cavichona laticollis* and *Cavichona brevistyla* (see Jankowski 1973), *Spirochona gemmipara* (see Fahrni 1984), and *Vasichona opiliophiла* (see Taylor, Lynn, and Gransden 1995). In addition, TS are present in both *T. faurei* and the swimmer of *S. gemmipara* (Fahrni 1984). These provide more evidence for the notion that the current classification of the chonotrichians as a subclass is inconsistent with the SSU rRNA phylogenies (Snoeyenbos-West et al. 2004; Gong et al. unpubl. data).

Although we demonstrate an affinity between *Isochona* and the family Hartmannulidae, possible relationships of some chonotrichians with the family Dysteridae cannot be ruled out. For example, at both the bud and adult stages, the chonotrichian species *Chilodochona quennerstedti* and *Heliochona scheutenii* have a dysteriid-like ciliature (Grain and Batisse 1974; Guilcher 1951), and Jankowski (1973) postulated a *Dysteria* origin of these organisms. At present, it is difficult to figure out whether the chonotrichians are evolved independently from Dysteriidae and Hartmannulidae, or the chonotrichians form a monophyletic group. We therefore conclude that it is still premature to re-designate a systematic position for chonotrichians. More sequences from both dysteriids and chonotrichians are needed to clarify the phylogenetic relationships between chonotrichians and cyrtophorians.

Evolutionary relationships among introns. Although S943 introns are commonly found in fungi (Cannone et al. 2002), its presence in *T. faurei* represents the first report of this intron in ciliates. Due to the high mobility of group I introns (Bhattacharya, Friedl, and Damberger 1996), the pattern of intron distribution we recovered here basically corresponds with neither the phylogenies of their hosts nor of the insert sites. Nevertheless, the NJ and MP analyses identified, with low to moderate bootstrap supports, a clade of introns from three phyllopharyngean ciliates, *T. faurei*, *Acineteta* sp. and *T. lemarna*. It is interesting to look into how these introns had moved and evolved.
Two mechanisms have been suggested to explain group I introns spread. The first is homing and is initiated by an intronic ORF-encoded endonuclease that recognizes and cleaves an intronless allele at or near the intron insertion site (Chevalier and Stoddard 2001). Following endonuclease cleavage at a specific 15–20 nt target sequence, the intron-containing allele is used as the template in a double-strand break repair pathway resulting in insertion of the intron and co-conversion of flanking exon sequences (Dujon 1989). Long-distance (>50 nt) intron movement can in principle be achieved by reverse splicing, in which the introns recognize their target sequence through complementary base pairing with a short (4–6 nt) IGS followed by integration into the transcript (Roman and Woodson 1995) and then putatively reverse transcription, and general recombination achieve spread. Phylogenetic analyses have suggested many sites can be targets in the spread of group I introns through putative reverse splicing (e.g. S1046/S1516, S1216/S1389 in green algae—Bhattacharya et al. 1996; S934/L1025, S1046/S1052, and S1506/S1516 in fungi—Bhattacharya et al. 2005).

In our analyses, the S943 and S891 introns in the closely related phyllopharyngean ciliates are also found to be evolutionarily related, suggesting these introns likely existed in the common ancestor of these phyllopharyngeans and were vertically inherited. Since all group I introns of ciliates known so far lack an ORF-
Table 2. Comparison of Trichopodiella faurei n. sp. with the congener for which infraciliature is known.

<table>
<thead>
<tr>
<th>Character</th>
<th>Trichopodiella faurei</th>
<th>Trichopodiella lachmanni</th>
<th>Trichopodiella lachmanni</th>
<th>Trichopodiella elongata</th>
<th>Trichopodiella pulex</th>
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<tr>
<td>Body shape</td>
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<td>Oval</td>
<td>Oval</td>
<td>Cylindrical</td>
<td>Oval</td>
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<td>45–50</td>
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<td>Left kineties, number</td>
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<td>~ 20</td>
<td>25–28</td>
<td>~ 13</td>
<td>~ 11</td>
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<tr>
<td>Right kineties, number</td>
<td>18–20</td>
<td>~ 20</td>
<td>20–22</td>
<td>~ 7</td>
<td>~ 5</td>
</tr>
<tr>
<td>Appearance of right kineties</td>
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<td>Continuous</td>
<td>Continuous</td>
<td>Continuous</td>
<td>Anteriorly shortened</td>
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<td>Nematodesmal rods, number</td>
<td>22–28</td>
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<td>~ 50</td>
<td>?</td>
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<tr>
<td>Transpodial segments, number</td>
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<td>?</td>
<td>~ 23</td>
<td>~ 7</td>
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<tr>
<td>Dikinetics in pericentral kinety</td>
<td>16–25</td>
<td>?</td>
<td>30–50</td>
<td>~ 11</td>
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<td>Deroux and Dragesco 1968</td>
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?, Data not available.

encoding region for putative endonuclease, their different locations at positions 943 and 891 in the rDNAs might have to be explained by reverse splicing in which the introns have been laterally transferred from one site in the rDNA to another. Support for this scenario comes from the identical four nucleotides in IGS (S2′-GCUC-3′) of the S943 in T. faurei and S891 in Acineta sp. and T. lemnarum.

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LITERATURE CITED


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