Redescriptions of Two Poorly Known Marine Suctorian Ciliates, *Ephelota truncata* Fraipont, 1878 and *Ephelota mammillata* Dons, 1918 (Protozoa, Ciliophora, Suctoria), from Qingdao, China

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Summary. Two poorly known marine suctorian ciliates, *Ephelota truncata* Fraipont, 1878 and *Ephelota mammillata* Dons, 1918, collected from the coastal waters off Qingdao, China, were investigated using both live observations and protargol impregnation methods. Improved diagnoses for both species are supplied based on previous and current studies. The adult form of each species has two types of tentacle, a long stalk and a ramose macronucleus. In addition, *E. truncata* has a short column-shaped body about 75–250 × 100–200 µm *in vivo*, 12–16 suctorial tentacles, 80–100 prehensile tentacles and a stalk that is 500–1200 µm long; the swarmer is ovoid, about 40 × 30 µm *in vivo*, with 19–24 somatic kineties and a C-shaped macronucleus. *E. mammillata* has a bowl-shaped body about 55–150 × 80–150 µm *in vivo*, ca. 10 suctorial tentacles, 30–50 prehensile tentacles and a stalk that is 350–800 µm long. The SSU rRNA genes were sequenced for both species in order to compare them with those of closely related congeners.

Key words: Morphology, reproduction, SSU rRNA, taxonomy.

INTRODUCTION

Comparatively few suctorians have been investigated using modern methods, most having been described only from fixed material or from specimens observed *in vivo*. Consequently the systematics of the Suctoria is somewhat confused (Guilcher 1951, Grell & Benwith 1984, Grell & Meister 1984, Matthes 1988, Chen et al. 2008). Species of the genus *Ephelota* have been found worldwide in marine biotopes and are characterized by the absence of a lorica and by the possession of two types of tentacle, a stalk and a ramose macronucleus (Ehrenberg 1833; Claparède & Lachmann 1859; Hertwig 1876; Kent 1880–1882; Wailes 1925; Wang & Nie 1932; Kahl 1934; Guilcher 1951; Jankowski 1967). In terms of species identification, *Ephelota* is one of the most confused genera of suctorians. There are two main reasons for this: firstly many of the morphological features used in species descriptions overlap, and secondly the vast majority of species descriptions are based only on *in vivo* observations with comparatively few having been described using silver staining or other modern methods (Guilcher 1951, Chen et al. 2008).
During faunistic surveys of the ciliate fauna in marine waters of north China, several new or little-known suctorians have been isolated and reported (Chen et al. 2005, Gong et al. 2005, Chen et al. 2008). In the present study two Ephelota spp., namely E. truncata and E. mammillata, isolated from the littoral zone in coastal waters off Qingdao, were investigated both in vivo and following protargol impregnation. In addition the reproduction process in E. truncata and a description of its swarmer are documented for the first time.

MATERIALS AND METHODS

All samples were collected using artificial substrates in the form of glass slides which were immersed at a depth of ca. 1 m for one to two weeks to allow colonization by the ciliates (Li et al. 2008).

Two populations of Ephelota mammillata were collected (10 May 2004 and 8 April 2007) from scallop-culturing waters near Qingdao (Tsingtao, 36°08’N; 120°43’E) China. The salinity was approximately 31‰ and pH about 8.0. Ephelota truncata was isolated on 26 March 2007 from coastal waters off Qingdao. The salinity was approximately 30‰ and pH about 7.8.

Living cells were examined and measured at 100× to 1000× magnification using differential interference microscopy (Hu 2008). The infraciliature was revealed by the protargol impregnation method according to Wilbert (1975). Drawings of stained specimens were performed at 1250× with the aid of a camera lucida. Terminology and systematics are according to Corliss (1979) and Hausmann et al. (2003).

Genomic DNA extraction, PCR amplification, and rRNA gene cloning and sequencing of E. truncata and E. mammillata were performed according to Miao et al. (2007). The nucleotide sequences of E. gemmipara and E. sp.-QD-05, which are used in this paper for comparative purposes, are available from the GenBank database under accession numbers: EU600180 (E. gemmipara) and DQ834370 (E. sp.-QD-05). The sequences were aligned using Clustal W, ver. 180 (Thompson et al. 1994, Yi et al. 2008).

RESULTS AND DISCUSSION

Ephelota truncata Fraipont, 1878
(Figs 1–21, 45; Tables 1–3)

Ephelota truncata has never been reinvestigated using modern methods since it was described by Fraipont (1878). A redescription and improved diagnosis are here provided based on the original description and on present studies.

Improved diagnosis: Body short-column-shaped, about 75–250 × 100–200 µm in vivo; two types of tentacle in adult cell, i.e., 12–16 sectorial tentacles on apical surface surrounded by ca.100 prehensile tentacles; stalk transparent, about 500–1200 µm in length, uppermost region conspicuously expanded; macronucleus ramose, irregularly branched; swarmer ovoid, ca. 40 × 30 µm in vivo, with 19–24 somatic kineties and C-shape macronucleus.

Deposition of specimens: Voucher slides with protargol-impregnated specimens are deposited in the Natural History Museum, London, UK with the following registration numbers: 2008:5:12:1 (adult) and 2008:5:12:2 (swarmer).

Description of the Qingdao population.

Adult. Adult form comprises a body borne upon a long stalk. Body usually about 75–200 × 100–200 µm in vivo, generally short-column-shaped, circular in cross section (Figs 2, 5, 12, 13). Two types of tentacle: 10–16 sectorial tentacles about 20 µm in length, usually retracted, confined to apical region of body; 80–100 prehensile tentacles ca. 150–180 in length, thin and sharply pointed, with many granular protuberances, and concentrated in anterior half of body surrounding the sectorial tentacles (Figs 2, 11–13).

Stalk hollow and transparent, highly variable in length (500–1200 µm), uppermost 200 µm of stalk con-

Table 1. Morphometric characters of Ephelota truncata (1st line) and E. mammillata (2nd line).

<table>
<thead>
<tr>
<th>Character</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length in vivo</td>
<td>75</td>
<td>250</td>
<td>153.3</td>
<td>62.5</td>
<td>6.9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>100</td>
<td>67.9</td>
<td>19.1</td>
<td>1.4</td>
<td>14</td>
</tr>
<tr>
<td>Body width in vivo</td>
<td>100</td>
<td>200</td>
<td>149.4</td>
<td>35.4</td>
<td>3.9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>110</td>
<td>71.1</td>
<td>19.4</td>
<td>1.4</td>
<td>14</td>
</tr>
<tr>
<td>Stalk length in vivo</td>
<td>500</td>
<td>1200</td>
<td>827.8</td>
<td>217.7</td>
<td>24.2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>375</td>
<td>850</td>
<td>553.6</td>
<td>133.3</td>
<td>9.5</td>
<td>14</td>
</tr>
<tr>
<td>PT length in vivo</td>
<td>150</td>
<td>180</td>
<td>163.9</td>
<td>11.9</td>
<td>1.32</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>160</td>
<td>123.9</td>
<td>28.0</td>
<td>2.0</td>
<td>14</td>
</tr>
<tr>
<td>Swarmer length*</td>
<td>45</td>
<td>60</td>
<td>53.3</td>
<td>5.77</td>
<td>0.48</td>
<td>12</td>
</tr>
<tr>
<td>Swarmer width*</td>
<td>35</td>
<td>60</td>
<td>48.3</td>
<td>7.49</td>
<td>0.62</td>
<td>12</td>
</tr>
<tr>
<td>No. of somatic kineties*</td>
<td>19</td>
<td>24</td>
<td>21.5</td>
<td>1.51</td>
<td>0.13</td>
<td>12</td>
</tr>
</tbody>
</table>

* All data for the swarmer are based on protargol impregnated specimens. Measurements in µm. CV – coefficient of variation in %; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of specimens investigated – PT, prehensile tentacles; SD – standard deviation; – denotes missing data.
spaciously expanded to a width of about 40–60 µm, remainder of stalk gradually narrowing to distal end (Fig. 2); usually with evenly spaced transverse striations on stalk surface (Fig. 17); without specialized attachment disc at distal end.

Cytoplasm colourless, but usually dark grey or opaque when full of food vacuoles. Macronucleus ramose and irregular with several elongated branches (Fig. 10). Micronucleus not observed. Several contractile vacuoles, each about 15 µm in diameter (Figs 2, 11).

**Swarmer.** Multiple swarmers produced synchronously during reproduction. Initially the basal bodies that give rise to the locomotor cilia in the transient larval stage appear to undergo multiplication before the budding process begins. The mastoid buds then develop in the apical region of the body, and one elongated branch of the parental macronucleus enters into each bud. The buds undergo further development for several hours before turning into swarmers which gather around the expanded part of stalk (Figs 5, 13, 18, 19).

Figs 1–10. *Ephelota truncata* and its swarmer from life (1, 2, 5–9) and after protargol impregnation (3, 4, 10). 1 – lateral view of swarmer; 2 – lateral view of a typical individual, showing the suctorial tentacles (arrow) and prehensile tentacles; 3, 4 – ventral and dorsal views of swarmer, showing the infraciliature (3) and macronucleus (4); 5 – an individual after reproduction, showing the variation of parental body shape and swarmers moving over the stalk; 6, 7 – ventral and dorsal views of swarmer in vivo; 8 – lateral view of a living individual (from Fraipont 1878); 9 – living individual of *Ephelota* sp. from Wailes (1943); 10 – ramose macronucleus in a crown-shape. Scale bars: Figs 1, 3, 4, 6, 7=25 µm, Fig. 2=100 µm.
Swarmer body size rather constant, about 40 × 30 µm in vivo, hemispherical-shaped, slightly dorsoventrally flattened, ventral surface flattened, dorsal side domed, width to thickness ratio approximately 3:2 (Figs 1, 16). Endoplasm colourless, usually containing two or three large food vacuoles each 10–15 µm in diameter. One contractile vacuole, ca. 5 or 6 µm in diameter, positioned on left ventral side (Fig. 6). Macronucleus curved and “C” shaped (Figs 4, 7, 21). Cilia about 3 µm long in vivo, covering ventral surface except for right and left margins (Figs 6, 14–16). Movement rather slow, creeping on substratum or on parental stalk (Figs 5, 13).
A total of 19–24 somatic kineties on ventral side (see Table 1). The outermost three or four kineties extend almost over whole body perimeter but are interrupted at one end forming an elongated horseshoe-shape; inner kineties progressively shortened, forming two conspicuous barren areas (Figs 3, 20).

**SSU rRNA gene sequence (Fig. 45; Table 3):** The SSU rRNA gene sequence of *Ephelota truncata* has been deposited in the GenBank database with accession number EU600182. The sequence length is 1691 bp. We compared the sequence with the SSU rRNA gene sequence of *E. gemmipara* and *E. sp.-QD-05*. Both *E. gemmipara* and *E. sp.-QD-05* differed in 112 nucleotides with that of *E. truncata* (Fig. 45).

**Remarks and comparison:** *Ephelota truncata* was discovered by Fraipont (1878) whose description consisted only of short references to the morphology of the adult cell *in vivo*. Our population of *E. truncata* from Qingdao is virtually identical to that described by Fraipont (1878), based on the morphology of the adult *in vivo* (e.g. size and shape of the body, two types of tentacle, conspicuously expanded stalk, ramose macronucleus) and the fact that both forms were found in marine habitats. The reproduction process and swarmer morphology, including its infraciliature, are described here for the first time.

Wailes (1943) isolated a population of *E. truncata* from the Strait of Georgia, briefly characterizing it thus: "body basin-shaped, about 100–300 µm in diameter, expanded part of stalk about 40–60 µm in diameter, stalk 100–800 µm in length, one or two contractile vacuoles sometimes visible," which is very similar with original description of *E. truncata*, notwithstanding the slight difference in body shape which in any case is somewhat variable. However, he misidentified it as *E. gemmipara* (Fig. 9; Wailes 1943).

Among the known species of *Ephelota* three, namely *E. gemmipara* (Hertwig, 1876) Bütschi, 1889, *E. corona* Kent, 1881 and *E. minima* Noble, 1929, resemble *E. truncata* in terms of body shape, tentacles and habitat. *Ephelota truncata* is most similar to the well-known form, *E. gemmipara* (Hertwig, 1876) Bütschi, 1889. The reproduction process and the swarmer morphology, including its infraciliature, are described here for the first time.

### Table 2. Morphological comparison of some closely related morphotypes in the genus *Ephelota*.

<table>
<thead>
<tr>
<th>Character</th>
<th><em>E. truncata</em></th>
<th><em>E. gemmipara</em></th>
<th><em>E. mammillata</em></th>
<th><em>E. crustaceorum</em></th>
<th><em>E. crustaceorum</em></th>
<th><em>E. plana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>ca. 75–100</td>
<td>ca. 40–100</td>
<td>ca. 150–300</td>
<td>ca. 100–200</td>
<td>ca. 150–200</td>
<td>ca. 150</td>
</tr>
<tr>
<td>Tentacle: No. of PTn</td>
<td>&gt;60</td>
<td>20–45</td>
<td>10–15</td>
<td>25–30**</td>
<td>7–15***</td>
<td>ca. 5**</td>
</tr>
<tr>
<td>Tentacle: No. of STn</td>
<td>10–15</td>
<td>10–15</td>
<td>6–8</td>
<td>4–6</td>
<td>10–20</td>
<td>7–10</td>
</tr>
<tr>
<td>Tentacle: Position of PTn</td>
<td>of body</td>
<td>of body</td>
<td>of body</td>
<td>of body</td>
<td>of body</td>
<td>of body</td>
</tr>
<tr>
<td>PTn length (µm)</td>
<td>ca. 160</td>
<td>ca. 200</td>
<td>ca. 200</td>
<td>ca. 300–400</td>
<td>ca. 300–600</td>
<td>ca. 300</td>
</tr>
<tr>
<td>Stalk length (µm)</td>
<td>500–1200</td>
<td>350–800</td>
<td>300–1600</td>
<td>200–800</td>
<td>300–600</td>
<td>300–500</td>
</tr>
<tr>
<td>Surface feature of stalk</td>
<td>smooth, always with TS and LS</td>
<td>smooth, with TS and LE</td>
<td>smooth, with TS and LE</td>
<td>smooth, with TS and LE</td>
<td>smooth, with TS and LE</td>
<td>smooth, with TS and LE</td>
</tr>
</tbody>
</table>

### Notes:
- Not including stalk.
- **Body length.** ***Data based on drawings.***
- Data unavailable. Abbreviations: LE – longitudinal edges (polygonal in cross-section); LS – longitudinal striation; PTn – prehensile tentacle; STn – suctorial tentacle; TS – transverse striation.
and size in vivo (ovoid, 40 × 30 µm vs. ellipsoidal or ear-shaped, 70–110 × 35–50 µm) and the infraciliature of the swarmer (19–24 somatic kineties, no fragment field of kineties or dorsal kinety vs. 22–33 somatic kineties, fragment field with 10–20 kineties, one dorsal kinety) (Table 2; Figs 29–30, 33–34; Chen et al. 2008). In addition, the dissimilarity of both forms is firmly supported by SSU rRNA gene sequence data with differences in 112 nucleotides between the two (structural similarity 93.4%) (Table 3; Fig. 45).

In terms of its large body size and long stalk, *E. truncata* resembles *E. coronata* Kent, 1881. The former differs from the latter in its body shape (usually short column-shaped vs. wedge-shaped), tentacles (ca. twelve conspicuous suckorial tentacles vs. without conspicuous suckorial tentacles) and stalk shape (conspicuously expanded in uppermost 200 µm of stalk vs. uppermost region of stalk not expanded part) (Table 2; Fig. 32; Kent 1880–1882, Kahl 1934).
Like *E. truncata*, *E. minima* also possess two types of tentacle. However, *E. truncata* differs from the latter in the body size in vivo (75–250 × 100–200 vs. 20–110 × 20–110 µm) and the length of the stalk (500–1200 vs. 15–85 µm) (Table 2; Fig. 28; Noble 1929).

**Ephelota mammillata** Dons, 1918
(Figs 22–23, 25–27, 35–45; Tables 1–3)

This organism was first reported by Dons (1918), although Kahl (1934) incorrectly cited the authority as *E. mammillata* Dons, 1915. In the original description the tentacles were almost certainly characterized incorrectly because the suctorial tentacles are extremely retractile and difficult to detect. The discovery of the population from Qingdao has enabled us to make further observations and we here provide an improved diagnosis.

**Improved diagnosis:** Body bowl-shaped, about 55–150 × 80–150 µm in vivo, with conspicuous anterior lip that is 8–10 µm thick and surrounds apical region of body; two types of tentacle in adult cell, 10–15 suctorial tentacles and 30–50 prehensile tentacles; stalk transparent, about 350–800 µm in length; macronucleus ramose with irregular branches; one or two contractile vacuoles.

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**Figs 35–44. Photomicrographs of Ephelota mammillata in vivo. 35 – living zooid under low magnification, arrowheads indicate the suctorial tentacles; 36 – lateral view, showing the extended tentacles; 37 – lateral view showing the highly retracted tentacles; 38 – lateral view of an early reproduction stage, arrowheads indicate the buds; 39 – detail of stalk annulus, also showing the transverse and longitudinal striations on stalk surface; 40 – stalk annulus, showing the conspicuous transverse striations; 41 – anterior region of cell, showing the contractile vacuole (arrow) and anterior lip (arrowheads); 42, 43 – body surface covered with mass of rod-shaped ectosymbiotic bacteria; 44 – lateral view of late stage of reproduction, showing the large swarvers. Scale bars: Figs 35, 37 = 100 µm; Figs 38, 44 = 50 µm.**
Deposition of specimens: One voucher slide with protargol-impregnated specimens is deposited in the Natural History Museum, London, UK (registration number 2008:5:12:3).

Description of the Qingdao populations: Body usually about 60–150 × 80–150 µm in vivo, variable in shape but generally bowl-shaped with conspicuous anterior lip that is 8–10 µm thick and surrounds apical region of cell (Figs 23, 27, 36, 37, 41, arrowheads). Body widest at anterior lip, narrowing gradually towards posterior end, circular in cross-section. One to several large wart-like protuberances on apical surface of body. Two types of tentacle: 10–15 suctorial tentacles ca. 20 µm in length, confined to apical region of body; 30–50 thin and sharply pointed prehensile tentacles with many granular protuberances, ca. 100 µm long when fully extended, cone-shaped when retracted, arranged in a crown-like pattern surrounding the suctorial tentacles (Figs 23, 36).

Stalk hollow and transparent, highly variable in length (350–800 µm), widest at junction with body, gradually narrowing to distal end; without specialized attachment disc at distal end; with evenly spaced transverse striations and one or two annular rings in most individuals (out of ten cells examined in vivo, ca. 3 or 4 individuals had longitudinal striations near the first ring) (Figs 22, 35, 39, 40).

Cytoplasm colourless but generally dark grey or opaque after feeding. Body surface covered with a mass of rod-shaped ectosymbiotic bacteria, each ca. 3 µm long (Figs 42, 43). Macronucleus ramose and irregular with several elongated branches (Fig. 26). Micronucleus not observed. One or two contractile vacuoles near region of anterior lip, ca. 15–20 µm in diameter (Figs 23, 27, 41, arrow).

SSU rRNA gene sequence (Fig. 45; Table 3): The SSU rRNA gene sequence of Ephelota mammillata has been deposited in the GenBank database with accession number EU600181. The sequence length is 1696 bp. We compared the sequence with the SSU rRNA gene sequence of E. gemmipara and E. sp.-QD-05. The sequence of E. mammillata differed in 122 and 66 nucleotides from that of E. gemmipara and E. sp.-QD-05, respectively (Fig. 45).

Remarks and comparison: In terms of the general appearance of the adult form in vivo, body size, ramose macronucleus, the distribution of tentacles on the apical body surface, habitat and especially the prominent anterior lip, the isolate from Qingdao corresponds very well with the original description by Dons (1918). The only apparent difference is the tentacles which Dons (1918) described as short and thorn-like (vs. ca. twelve suctorial tentacles about 20 µm in length and 30–50 thin, pointed prehensile tentacles, about 100 µm in length in the Qingdao population). This difference can be explained by the highly retractile nature of the tentacles, especially the prehensile tentacles, which are thin and sharply pointed when fully expanded but short and thornlike when retracted. It is likely that Dons (1918) only observed the prehensile tentacles in their contracted state, and possibly overlooked the suctorial tentacles.

Table 3. Sequence similarity of Ephelota spp. Data based on SSU rRNA genes. All measurements in %.

<table>
<thead>
<tr>
<th></th>
<th>E. truncata</th>
<th>E. mammillata</th>
<th>E. sp.-QD-05</th>
<th>E. gemmipara</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. truncata</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. mammillata</td>
<td>0.933</td>
<td>0.961</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. sp.-QD-05</td>
<td>0.934</td>
<td>0.929</td>
<td>0.926</td>
<td></td>
</tr>
<tr>
<td>E. gemmipara</td>
<td>0.934</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 45. SSU rRNA sequences of four species of Ephelota. Numbers at the end of lines indicate the number of nucleotides. The differences in sequence length were compensated for by introducing alignment gaps (–) in the sequences. Matched sites are shown by dots (.).
With reference to its general morphology and marine habitat, *E. mammillata* resembles three other species, namely *E. gemmipara* Hertwig, 1876, *E. plana* Wailes, 1943 and *E. coronata* Kent, 1881.

In terms of its tentacles, ramose macronucleus and very long stalk *E. mammillata* is most similar to *E. gemmipara* Hertwig, 1876. The former can be separated from the latter by its smaller size in vivo (55–150 × 80–150 μm vs. 100–400 × 100–300 μm), the number of suctorial tentacles (ca. 10–15 vs. ca. 6–10) and the bowl-shaped body with conspicuous anterior lip (vs. pyriform body without an anterior lip) (Table 2 and Figs 30, 32) (Chen et al. 2008). Furthermore, the dissimilarity of the two forms is firmly supported by SSU rRNA gene sequence data, the sequence of *E. mammillata* differing in 122 nucleotides from that of *E. gemmipara* (structural similarity 92.9%) (Table 3; Fig. 45).

*Ephelota plana*, which was originally isolated from the Strait of Georgia near Nanaimo, differs from *E. mammillata* in having a fan-like stalk (vs. cylindrical in *E. mammillata*) and a different body shape (compressed vs. not compressed in *E. mammillata*) (Fig. 24; Wailes 1943).

*Ephelota coronata* differs from *E. mammillata* by the absence of the suctorial tentacles (vs. 10–15 suctorial tentacles in *E. mammillata*) and the distribution of prehensile tentacles over the whole body (vs. concentrated on the apical body surface in *E. mammillata*) (Fig. 32; Kent 1880–1882).

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**REFERENCES**


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