Redefinitions of Two Marine Suctorion Ciliates, *Ephelota gemmipara* (Hertwig, 1876) Bütschli, 1889 and *E. crustaceorum* Haller, 1880 (Ciliophora, Suctoria), with a Brief Description of the Asexual Reproduction Process in *E. gemmipara*

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Summary. Two “well-known” marine suctorion ciliates, *Ephelota gemmipara* (Hertwig, 1876) Bütschli, 1889 and *Ephelota crustaceorum* Haller, 1880, isolated from coastal waters near Qingdao (Tsingtao), China, were reinvestigated using both live observations and protargol impregnation methods. Based on the previous and the current studies, improved diagnoses for both species are supplied. A brief description of the asexual division process in *Ephelota gemmipara* is also presented, which can be summarized as follows: initially the basal bodies that give rise to the locomotor cilia in the transient larval (swarmer) stage appear to undergo multiplication before the budding process has commenced. The mastoid buds then develop in the apical region of the body, and one elongated branch of the parental macronucleus enters each bud. The buds undergo further development for several hours before eventually becoming swarvers which depart from the parent cell, swimming freely in the water.

Key words: *Ephelota*, marine habitat, morphogenesis, morphology, reproduction, swarmer.

INTRODUCTION

Suctorians constitute a special group of ciliated protozoa many of which, especially among the freshwater forms, have been known for over two centuries. By contrast, comparatively little attention has been given to the marine forms (Ehrenberg 1833; Wright 1858, 1859; Claparède & Lachmann 1859; Hertwig 1876, 1877; Kent 1880–1882; Kahl 1934; Guilcher 1950, 1951; Jankowski 1967; Grell and Benwith 1984; Grell and Meister 1984; Curds 1985; Matthes 1988; Stankovic *et al.* 2002; Fernandez-Leborans *et al.* 2006).

The well-known *Ephelota* is commonly found in marine biotopes. Species of this genus usually have two kinds of tentacle and a ramose macronucleus. 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 (Grell 1950, 1951; Grell and Benwith 1984; Grell and Meister 1984).

In the present study, two Ephelota spp. isolated from coastal waters off Qingdao, China are described from both live and silver-impregnated specimens. The asexual division process in E. gemmipara is also documented.

MATERIALS AND METHODS

Sampling. Samples were collected from Jiaozhou Bay near Qingdao (Tsingtao, 36°08′N; 120°43′E) China using artificial substrates in the form of microscope slide, which were immersed at a depth of 0.8–1.5 m for periods of 7 to 15 days to allow colonization by the suctorians to occur. (Song and Wilbert 1995).

Ephelota gemmipara. Four populations were sampled (6 November 2000; 30 June 2005; 24 October 2005; 11 May 2007) from scallop-farming waters. The water temperature was about 17°C to 23°C, salinity about 31‰, and pH about 8.0.

Ephelota crustaceorum. Three populations were collected (24 May 2004; 11 June 2005; 05 November 2006) from an abalone-culture pond and scallop-farming waters. The water temperature was about 15 to 20°C, salinity about 31‰, and pH about 7.8.

Observations on living cells were undertaken using differential interference microscopy. Protargol impregnations were carried out in order to reveal the infraciliature and the macronucleus (Wilbert 1975).

Counts and measurements on stained specimens were performed at a magnification of 1250×. Drawings were made with the help of a camera lucida. Two voucher slides with protargol-impregnated specimens of E. gemmipara are deposited in the Natural History Museum, London, UK with registration numbers: 2008:5:10:1 and 2008:5:10:2.

Terminology. Terminology and systematics are according to Corliss (1979) and Hausmann et al. (2003). To avoid confusion, the following terms used in the present paper are here defined. Note that the posterior end of the body in the adult is at the junction with the stalk while the anterior is at the end opposite the stalk junction. The anterior end of the swarmer is conspicuously rounded and blunt whereas the posterior end is narrowed.

Somatic kineties. Horseshoe-shaped kineties on the ventral side of the body in the swarmer. This is a common feature in Ephelota spp., and is possibly a generic character.

Dorsal kinety. A single, bow-shaped kinety with densely arranged cilia, positioned near the posterior margin of the cell in the swarmer.

Scopuloid. Special structure comprising an irregular ring on the ventral side of the swarmer that is impossible to observe in vivo but is clearly visible after protargol impregnation. Its function is unknown.

RESULTS AND DISCUSSION

Ephelota gemmipara (Hertwig, 1876) Bütschli, 1889 (Figs 1–5; Table 1, 2)

Synonyms:
Podophrya gemmipara Hertwig, 1876
Hemiophrya gemmipara Kent, 1880–1882
Hemiophrya microsoma Maupas, 1881
Dendrophrya gemmipara Sand, 1895

We here provide an improved diagnosis based on previous and present studies.

Improved diagnosis. Body pyriform, about 100–400 long × 100–300 µm wide in vivo; two types of tentacle in adult cells, i.e. six to ten suctorial tentacles and 30–50 prehensile tentacles; stalk transparent, about 300–1600 µm in length; macronucleus ramose, irregularly branched; swarmer ellipsoidal or ear-shaped, with 22–33 somatic kineties arranged in horseshoe-shape, 10–20 fragment kineties, and a C-shaped macronucleus.

Description of the Qingdao populations. Adult form comprises a body borne upon a long stalk. Body usually about 100–300 × 100–250 µm in vivo, variable

Table 1. Morphometric characterization of swarmer in two populations of Ephelota gemmipara (first line: 2000-06-30-population; second line: 2005-11-06-population). Data based on protargol impregnated specimens (CV – coefficient of variation in %; M – arithmetic mean; Max – maximum; Min – minimum; n – sample size; SD – standard deviation). All measurements in µm.

<table>
<thead>
<tr>
<th>Character</th>
<th>Min</th>
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<td>5.3</td>
<td>1.5</td>
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<tr>
<td>No. of right kineties</td>
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<td>7.7</td>
<td>1.3</td>
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<td>field</td>
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<td>13</td>
<td>11.5</td>
<td>1.1</td>
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<tr>
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<td>12.8</td>
<td>2.0</td>
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<td>25</td>
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<tr>
<td>pore</td>
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<td>20</td>
<td>16.3</td>
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In vivo}
in shape but generally pyriform (Figs 1B; 3A, C, E). Widest at anterior end, narrowing gradually towards posterior end, circular or slightly flattened in cross-section. Two types of tentacle: six to ten suctorial tentacles about 20–30 µm in length, usually retracted, confined to apical region of body (Figs 1B; 3A, E, K); 30–50 prehensile tentacles up to 200 µm long, thin and sharply pointed, with many granular protuberances, and concentrated in anterior half of body in a crown-like pattern surrounding the suctorial tentacles (Figs 1B; 3A).

Stalk hollow and transparent, highly variable in length (300–1500 µm), widest at junction with body, gradually narrowing to distal end (Figs 1B, H; 3G); without specialized attachment disc at distal end (Fig. 3D, arrow); usually with evenly spaced transverse striations (Figs 1E; 3H).

Fig. 1. Ephelota gemmipara and its swarmer from life (A–D, E–H) and after protargol impregnation (I, J). A – lateral view of spherical resting cyst, with transparent and gelatinous coats; B – lateral view of a typical individual, showing the ramose macronucleus, the suctorial tentacles (arrow) and prehensile tentacles (double-arrowheads); C, D – ear-shaped swarmer, showing dorsal (C) and ventral (D) views, arrowhead in (C) indicates the dorsal kinety, double-arrowheads mark the contractile vacuole, arrows indicate the capitate tentacles, arrow in (D) shows the dorsal kinety; E–H – variations in stalk morphology, with transverse striations (E), longitudinal ridges (F, G) or two annuli (H); I – swarmer with newly formed stalk, arrow indicates the new stalk, arrowheads mark the shortened somatic kineties; J – ramose macronucleus in an irregular crown-shape. DK – dorsal kinety; FF – fragment field; Ma – macronucleus; Sc – scopuloid; SK – somatic kineties. Scale bars in A, B, J – 200 µm, in C, D – 100 µm, in I – 50 µm.
Cytoplasm colourless but usually dark grey or opaque after feeding. Macronucleus ramose and irregular with several elongated branches (Figs 1B, J; 4E). Micronucleus not observed. One or two contractile vacuoles, 15–30 µm in diameter, each with an excretory pore (two individuals had two excretory pores out of ten cells examined following protargol impregnation) (Figs 1B; 3A; 4G, H).

Resting cyst spherical to oval, about 200 µm in diameter. Cyst wall colorless, transparent and gelatinous (Figs 1A; 3F).

**Reproduction and swarmer development.** Multiple swarmers are produced synchronously during budding (Figs 2C; 3J, L, O, P; 4A, B). Initially the basal bodies that give rise to the locomotor cilia in the transient larval stage appear to undergo multiplication before the budding process has commenced. The mastoid buds then develop in the apical region of the body and one elongated branch of the parental macronucleus enters into each bud (Figs. 2C; 4J). The buds undergo further development for several hours before turning into swarmers which eventually depart from the parent cell, swimming freely in the water.

Swarmers about 70–110 × 35–50 µm in vivo, elongated egg-shaped or ear-shaped, anterior end widely rounded, tapering gradually to the posterior end. Dor sal side with four to eight capitate tentacles, each about 12 µm in length in vivo (Figs 1C; 3M, arrows). Capitate tentacles are impossible to observe after protargol impregnation whereas many prehensile tentacles and several suctorial tentacles are clearly visible (Figs 2B,
Fig. 3. *Ephelota gemmipara in vivo*. A – lateral view of a typical specimen, arrows indicate the prehensile tentacles, double-arrowheads mark the suctorial tentacle, arrowheads show the longitudinal edges; B – individuals on *Obelia* sp. which was attached to the glass slide (arrowheads); C – same specimens as (B) enlarged; D – distal end of stalk to show no special attachment disc (arrow); E – lateral view of a special individual with only two suctorial tentacles (arrow); F – lateral view of a resting cyst, with a transparent coat; G – transparent stalk with annulus (arrows); H – transversely striated stalk, arrowheads indicate the inconspicuous striations; I – transparent stalk with a cylindrical shape; J – apical view of an early reproduction stage, arrowheads indicate the buds; K – apical view of a typical specimen, arrows indicate the suctorial tentacles; L – same individual as (J) enlarged to show the buds (arrowheads); M – lateral view of swarmer, arrow indicates capitate tentacle, arrowhead marks the contractile vacuole; N – arrow indicates the dorsal cilia. O, P – lateral views of late stage of reproduction; Q – lateral view of a specimen with newly formed stalk; R – ventral view of swarmer. Scale bars in A, F, J – 300 µm, in B – 2000 µm, in C – 700 µm, in D, G, M, R – 30 µm, in E, K, O–Q – 100 µm, in F – 200 µm, in I – 10 µm.

Fig. 2. *Ephelota gemmipara* from life (E, G), after protargol impregnation (A–C, I–J) and after Chatton-Lwoff silver impregnation (H). A, B – ventral (A) and dorsal (B) views of swarmer, showing the infraciliature and macronucleus; C – an individual in reproduction, to note the branched macronucleus extending towards the buds; D – to show the ramose macronucleus (from Batisse 1994); E. Lateral view of an individual (from Fraipont 1877); F – an individual in reproduction (from Hertwig 1876); G – lateral view of a typical individual (from Hertwig 1876); H – ventral view of swarmer to show infraciliature (from Guilcher 1951); I, J – ventral (I) and dorsal (J) views of swarmer of population 2005:11:06. CVP, contractile vacuole pore; DK – dorsal kinety; FF – fragment field; Ma – macronucleus; PTn – prehensile tentacles; Sc – scopuloid; SK – somatic kineties; STn – suctorial tentacles. Scale bars in A, B, I, J – 25 µm, in C – 50 µm, in D – 100 µm.
J; 5B, G). One bow-shaped kinety at anterior end, cilia about 12 µm in length (Figs 1C, arrowhead, D, arrow; 3N, arrow). Ventral side flattened and with a horseshoe-shaped ciliary field, the cilia of which are about 6 µm long (Figs 1D; 3R), and a field of densely arranged fragment kineties near the posterior end, the cilia of which are about 8 µm long. Cytoplasm colourless, often containing numerous tiny particles < 1 µm in diameter. One or two contractile vacuoles, each ca. 6–8 µm in diameter, positioned near anterior end (Figs 1C, double-arrowheads; 3M, arrowhead). Movement slow, usually swimming around the parental cell or creeping on the substrate for about one to two hours before attaching and producing a stalk.

![Images](A, B, C, D, E, F, G, H, I, J)
Marine Suctorians

Fig. 4. *Ephelota gemmipara* after protargol impregnation. A – anterior part of an individual in reproduction; B – apical view, to show multiple swarmers being produced synchronously, arrows indicate macronuclei; C, D – same swarmers as (B) enlarged to show infraciliature, arrows indicate shortened kineties; E – to show ramose macronucleus, arrows indicate suctorial tentacles; F – swarmer with a newly formed stalk, arrowheads indicate somatic kineties, arrow marks the fragment field; G – to show contractile vacuole pores, one in (G), two in (H); I – swarmer as in (B) enlarged to show band-shaped macronucleus; J – an individual in reproduction, arrows denote the branched macronucleus extending into the buds. Scale bars in B – 75 µm, in E – 100 µm, in F – 30 µm, in G – 5 µm.

Fig. 5. Swarmers of *Ephelota gemmipara* after protargol impregnation. A, B – ventral (A) and dorsal (B) views of the same specimen showing the infraciliature and macronucleus, arrows indicate the suctorial tentacles, arrowheads mark the prehensile tentacles, double-arrowheads mark the contractile vacuole pore; C, G – swarmer of population 2005:11:06; D – to show fragment field (arrows); E – dorsal view of anterior end showing the dorsal kinety (arrowheads); F – showing the irregular-shaped scopuloid (arrow). Scale bars in A – 35 µm, in C – 20 µm, in D, F – 10 µm.
The number and appearance of the somatic kineties in the swarmer varies slightly between populations (Table 1). In population 2000:06:30 there are about 22–33 somatic kineties on the ventral side arranged in an elongated horseshoe-shape, the outermost four to eight rows of which extend over almost the entire body perimeter, but are interrupted by the fragment kineties at the posterior end; the inner rows progressively shorten from outside to inside, the outermost three to eight rows usually being sparsely arranged at the posterior end of the left arm of the horseshoe-shaped structure (Figs 2A, I; 5A, C). Fragment field composed of about 10–17 densely arranged rows near the posterior end of body (Figs 1I; 2B, J; 5B, D). One bow-shaped dorsal kinety near the anterior end of the body (Figs 1I; 2B, J; 5E). The macronucleus is C-shaped, the two ends and middle part of which are expanded (Figs 2B, J; 5B, G). Usually one contractile vacuole pore is recognizable near the anterior end of the cell in protargol-impregnated specimens (Figs 2B, J; 5B, double-arrowheads). The scopuloid is visible after protargol impregnation as an irregular ring on the ventral side of the cell (Figs 1I; 2A; 5F, arrow). The function of the scopuloid is uncertain. By contrast, specimens in population 2005:11:06 have about 22–29 somatic kineties and 13–20 rows in the fragment field (Figs 2I; 5C, G).

Remarks and comparison: Ephelota gemmipara was originally described by Hertwig (1876) under the name Podophrya gemmipara and subsequently redescribed several times from in vivo observations (Kent 1881–1882; Maupas 1881, 1883; Sand 1899; Wailes 1925; Wang 1932; Kahl 1934). Guilcher (1951) described a population using the Chatton-Lwoff silver method although he did not give detailed data about the swarmer. As a result of these previous and current investigations, E. gemmipara can be recognized by the following combination of characters: (1) adult cell 100 × 100 μm to 400 × 300 μm; (2) six to ten suctorial tentacles and 30–50 prehensile tentacles; (3) swarmer ear-shaped with horseshoe-shaped ciliary field.

The stalk surface of E. gemmipara is highly variable in appearance and has been variously described in the literature as follows: entire stalk striated transversely and additionally striated longitudinally near the junction with the body, distal end expanded and thickened (Fig. 7B) (Hertwig 1876); stalk surface transversely striated (Wang 1932); stalk surface smooth or with longitudinal ridges (Kahl 1934). In the present work at least four types of stalk appearance were observed: (1) stalk surface smooth with no special features (population 2000:11:06) (Fig. 3I); (2) stalk polygonal in cross-section with ca. eight longitudinal ridges, stalk surface with transverse striations (population 2005:06:30) (Figs 1F, G; 3A, D); (3) entire length of stalk surface striated transversely, without longitudinal ridges (population 2005:10:24) (Figs 1E; 3H); (4) stalk surface smooth but occasionally with one or two annuli (2007:05:11) (Figs 1H; 3G). All these characters appear to be conservative at the population level, therefore the value of the stalk appearance as species-level character remains uncertain.

The Qingdao populations resemble the original description reasonably well (Hertwig 1876). All key characters, i.e. body shape and size, two types of tentacle, the length and appearance of the stalk, the type of budding and marine habitat, are consistent with the original description and subsequent redescriptions of E. gemmipara, so the identification of the Qingdao populations is not in doubt.

Among its congeners, three morphotypes should be compared with Ephelota gemmipara, namely E. coronata Wright, 1858, E. plana Wailes, 1925 and E. minima Noble, 1929. Of these, E. coronata is most similar to E. gemmipara in terms of the body shape and size. However E. coronata can be clearly separated from the latter by the absence of suctorial tentacles (vs. present in E. gemmipara) and the distribution of prehensile tentacles over the whole body (vs. concentrated in the anterior half of body in E. gemmipara) (Fig. 7D; Kent 1881).

E. plana differs from E. gemmipara in having a fan-shaped stalk (vs. cylindrical in E. gemmipara) and a compressed body (vs. not compressed in E. gemmipara) (Fig. 7A; Wailes 1925).

E. minima can be distinguished from E. gemmipara by its smaller body size in vivo (22–110 μm long vs. 100–300 μm long in E. gemmipara), its body shape (oval or spherical vs. pyriform or cup-shaped in E. gemmipara) and its much shorter stalk (16–84 μm long vs. 300–1600 μm long in E. gemmipara) (Fig. 7E; Noble 1929).

Ephelota crustaceorum Haller, 1880 (Fig. 6; Table 2)

This organism was first described by Haller (1880) since when there has been no redescription. Consequently it has never been observed following silver impregnation and no morphometric data are available. An improved diagnosis is here suggested based on the original description and present investigation.
Improved diagnosis. Body pyriform or spherical, about 30–115 × 25–115 µm in vivo; two types of tentacle in the adult cell, several suctorial tentacles on the apical surface and about 10–15 prehensile tentacles covering the main body; stalk about 200–800 µm in length; one or two contractile vacuoles.

Description of the Qingdao population. Body variable in shape but generally pyriform or globular, usually about 30–80 × 30–75 µm in vivo, length: width ratio about 1:1 (Fig. 6A–D, G–I, K–M). Two kinds of tentacle, one or two suctorial tentacles about 20 µm in length on apical surface of body; about 10–15 prehensile tentacles up to 150 µm in length, radiating out from all over body surface (Fig. 6A, I, L). Stalk hollow and transparent, about 200–800 µm in length, usually eight to ten times body length, distinctly wider near junction with body than at distal end, usually with fine transverse striations and two distinct annuli (Fig. 6B, J), but sometimes with only one annulus situated near body (Fig. 6D, H, M, arrows); without specialized attachment disc.

Cytoplasm colourless or slightly gray, often appearing opaque due to thickness of cell and presence of granular inclusions. One or two contractile vacuoles located in middle part of body (Fig. 6A, D, K). Macronucleus not observed.

Remarks and comparisons: Kahl (1934) included this species in his monograph on the Suctoria, briefly characterizing it thus: “body ball-shaped, 70–115 µm in length, residual characters just as E. gemmipara.” No information was given about the number of tentacles or the length of the stalk. In the absence of detailed data, we identified our organism mainly on the basis of its size, body shape, distribution of prehensile tentacles and habitat, all of which are consistent with those given by Haller (1880) for E. crustaceorum. The one possible discrepancy is the number of suctorial tentacles; in the Qingdao populations, just one suctorial tentacle is usually present (out of 15 individuals observed only one individual had two suctorial tentacles), whereas Haller’s (1880) illustration appears to show four suctorial tentacles. It is possible that Haller did not correctly illustrate this feature because the prehensile tentacles are highly retracted and could easily be misidentified as suctorial tentacles. Thus, we conclude that Haller’s form and those isolated in the present study are conspecific.

With reference to its general morphology, E. crustaceorum is most similar to E. gemmipara although it differs from latter in its smaller body size (30–115 µm vs. 100–400 µm in E. gemmipara), the presence of only one or two suctorii tentacles (vs. ca. six to ten in E. gemmipara) and the distribution of prehensile tentacles over the whole body (vs. confined to the apical body surface in E. gemmipara) (Kahl 1934).
Fig. 6. *Ephelota crustaceorum* (A–E, G–M) and *E. coronata* (F) in vivo. A – lateral view of a typical specimen, arrow marks the suctorial tentacle, arrowhead indicates the contractile vacuole; B – to show the long, transversely striated stalk with two annuli (arrowheads); C, D – to show lateral views of two individuals, arrow indicates the annulus; E – lateral view of spherical body (from Haller 1880, called *Podophrya crustaceorum*); F – to show globular body with few suctorial tentacles (from Calkins 1902); G – to show pyriform body with long stalk; H – showing transparent stalk with an annulus (arrow); I – lateral view of a typical individual, arrow indicates the suctorial tentacle, arrowheads mark the prehensile tentacles; J – to show transversely striated stalk with an annulus (arrow); K – showing the pyriform cell with two contractile vacuoles (arrowheads); L – lateral view of specimen with two suctorial tentacles (arrowheads); M – lateral view of a typical specimen, arrow indicates an annulus. Scale bars in A–D, K – 100 µm, in E, F, G, I, L – 200 µm.
Two other species to which Ephelota crustaceorum should be compared are E. minima Noble, 1929 and Corynophrya conipes (Mereschkowsky, 1877) Kahl, 1934. E. crustaceorum is similar to E. minima in terms of body shape, body size and types of tentacle. However, the former can be separated from the latter by having fewer suctorial tentacles (one or two vs. ca. six in E. minima), fewer prehensile tentacles (< 15 vs. ca. 35 in E. minima), the distribution of prehensile tentacles over the whole body (vs. confined to apical body surface in E. minima) and a longer stalk (200–800 µm vs. ca. 16–84 µm in E. minima) (Fig. 7E; Noble 1929).

Corynophrya conipes also closely resembles Ephelota crustaceorum in terms of its general morphology. Nevertheless, the latter has two types of tentacle (vs. only one type, i.e. capitate, in C. conipes) (Fig. 7C; Kahl 1934, Curds 1987). Calkins (1902) described a form under the name of Ephelota coronata which has a spherical body shape and a longitudinally striated stalk, a few suctorial tentacles, and numerous prehensile tentacles covering the main body (Fig. 6F). Hence this morphotype closely resembles E. crustaceorum (Kent 1881–1882) so the two are very likely conspecific.

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