Morphology and phylogeny of three karyorelictean ciliates (Protista, Ciliophora), including two novel species, *Trachelocerca chinensis* sp. n. and *Tracheloraphis dragescoi* sp. n.

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This paper investigates the morphology and infraciliature of three karyorelictean ciliates, *Trachelocerca chinensis* sp. n., *Tracheloraphis dragescoi* sp. n. and a rarely known form, *Geleia acuta* (Dragesco, 1960) Foissner, 1998, which were isolated from the intertidal zone of sandy beaches at Zhanjiang and Qingdao, China. *Trachelocerca chinensis* sp. n. is distinguished from related forms by having 26–30 somatic kineties, a narrow glabrous stripe and a single nuclear group composed of approximately four to six macronuclei and two micronuclei. *Tracheloraphis dragescoi* sp. n. can be recognized through its 14–22 somatic kineties, wide glabrous stripe and a single nuclear group composed of about four macronuclei. Phylogenetic analyses based on small-subunit (SSU) rRNA gene sequences indicated that the genera *Trachelocerca* and *Tracheloraphis* are closely related but that neither of them appears to be a clearly monophyletic group. Nonetheless, the monophyly of *Trachelocerca* is not rejected by the approximately unbiased (AU) test ($P=0.143, <0.05$), although that of *Tracheloraphis* is rejected ($P=0.011, <0.05$). *Geleia acuta*, meanwhile, branched with *Geleia fossata* and falls in the *Geleia* clade.

INTRODUCTION

The ciliate class Karyorelictea is a highly specialized group and is very common in marine interstitial environments (Gao et al., 2010; Xu et al., 2013a, c), and recent studies on trachelocercids performed in the seas around China have reiterated the wide diversity of this group (Xu et al., 2011a, c; Yan et al., 2013). As the largest group within the Karyorelictea, the Trachelocercidae currently includes more than 70 species (Alekperov et al., 2007; Al-Rasheid, 1996, 1997, 1998, 2001; Al-Rasheid & Foissner, 1999; Carey, 1992; Dragesco, 1960; Foissner, 1998). However, most of these have only been described on the basis of live observations (Carey, 1992; Dragesco, 1960). As a result, the generic classification of trachelocercids was bewildering for a long time, until a series of studies carried out in the 1990s led to the establishment of a working classification (Foissner, 1997a, b, 1998; Foissner & Al-Rasheid, 1999a, b; Foissner & Dragesco, 1996a, b). The evolution of the Trachelocercidae has been difficult to deduce by morphological characters because of the great homogeneity of the somatic infraciliature, and because only oral structures are useful for reconstructing evolution (Foissner, 1998). Since few trachelocercids have been examined by molecular techniques (Yan et al., 2014), further taxonomic and molecular sampling is needed in order to investigate the evolutionary relationships within the Trachelocercidae.

The genus *Geleia* was first established by Kahl (1933), before being declared a *nomen nudum* and then being re-established by Foissner (1998). An improved diagnosis of *Geleia* was given by Xu et al. (2011b): members of the Geleidae with cylindrical body shape; cells completely ciliated with longitudinal rows consisting of dikinetids; buccal field located subapically; preoral kinety present;
typical geleid oral structure with dominant conspicuous adoral polykineties that comprise numerous long rows of kineties. So far, 13 species have been assigned to this genus, of which information on the infraciliature is available for only six (Xu et al., 2011b). The phylogenetic position of the genus was first reported by Andreoli et al. (2009), and 11 small-subunit (SSU) rRNA gene sequences of six species are now available. Geleia acuta has never been redescribed since it was first reported by Dragesco (1960), who described it based mainly on live observation. Therefore, information on its infraciliature, as well as its SSU rRNA gene sequence, remains unavailable.

In the present study, two novel species of trachelocercids and one poorly known Geleia species were isolated from the intertidal zone of sandy beaches at two locations in China, Zhanjiang and Qingdao (Fig. 1a). All three species were investigated both in vivo and following protargol impregnation. In addition, the molecular phylogeny of each species was analysed based on SSU rRNA gene sequence data.

**METHODS**

**Sample collection, observation and identification.** Trachelocerca chinensis sp. n. was collected on 15 December 2009 from a mangrove wetland in Zhanjiang, southern China (21° 12’ N 110° 25’ E), where the water temperature was 20 °C and the salinity about 14‰ (Fig. 1b). Tracheloraphis dragescoï sp. n. was sampled on 29 April 2009 from the intertidal zone of the No. 1 Bathing Beach in Qingdao, China (36° 06’ N 120° 34’ E), where the water temperature was 18 °C and the salinity about 30‰ (Fig. 1c). Geleia acuta was collected from the intertidal zone of Diaosuyuan sandy beach in Qingdao, China (36° 04’ N 120° 27’ E), where the water temperature was 23 °C and the salinity about 20‰ (Fig. 1d). Sampling methods largely followed Xu et al. (2013b). Briefly, sand (top 5 cm layer) or sediment plus seawater was taken from the site. Cells were picked out using a micropipette under a dissecting microscope, and live ciliates were observed in vivo using an oil-immersion objective and differential interference microscopy. The infraciliature was revealed using the protargol impregnation method (Wilbert, 1975). Drawings of impregnated specimens were made with the help of a camera lucida. Counts and measurements were performed under magnifications ranging between ×100 and ×1000. Terminology and systematics are according to Foissner & Dragesco (1996a) and Lynn (2008), respectively.

**DNA extraction and gene sequencing.** DNA extraction was performed using the DNeasy Tissue kit (Qiagen) according to Gao et al. (2013). Primers used for SSU rRNA gene amplification were universal eukaryotic primers (forward 5’-AACCTGGTTGATCCT-GGAG-3’ or 5’-GAAACTGCGAATGGCTC-3’; reverse 5’-TGAT-CCTTCTGAGGTGCCTAC-3’; Elwood et al., 1985; Medlin et al., 1988). PCR amplification and sequencing of the SSU rRNA gene were performed according to Fan et al. (2013) and Lv et al. (2013).

**Phylogenetic analyses.** Other than three newly obtained SSU rRNA gene sequences, the sequences used in the present analyses were downloaded from the GenBank database (for accession numbers, see Fig. 1). Alignment of the SSU rRNA gene sequences was initially performed according to Fan et al. (2010a) following the default parameters in the GUIDANCE web server (Penn et al., 2010b). Ambiguous columns in the alignment that fell below a confidence score of 0.7, as calculated by GUIDANCE, were removed, as in Fan et al. (2014). Spirostomum ambiguum, Eufolliculina uhligii, Blepharisma americanum, Stentor amethystinus and Stentor roeseli were used as outgroup taxa. The resulting curated alignment included 1565 characters of 49 taxa. A Bayesian inference (BI) analysis was performed using the GUIDANCE algorithm (Penn et al., 2010a) following the default parameters in the GUIDANCE web server (Penn et al., 2010b).
RESULTS AND DISCUSSION

Family Trachelocercidae Kent, 1881
Genus Trachelocerca Ehrenberg, 1840
Trachelocerca chinensis sp. n. (Figs 2 and 3; Table 1)

Diagnosis. Extended cells in vivo about 600–800 × 40–60 μm; head inconspicuous; 26–30 somatic kineties; single nuclear group composed of about four to six macronuclei and two micronuclei; colourless cortical granules.

Type locality and ecology. A mangrove wetland in Zhanjiang (21° 12’ N 110° 25’ E), southern China, where the water temperature was 20 °C and the salinity about 14‰ (Fig. 1b).

Type specimens. A protargol-impregnated slide containing the holotype specimen (Fig. 2h, i) marked with an ink circle is deposited in the Laboratory of Protozoology, Ocean University of China, China (no. XY09121501). A paratype slide is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2014.8.27.1.

Etymology. chinensis. N.L. fem. adj. chinensis of or pertaining to China, referring to the fact that this organism was first discovered in China.

Description. Fully extended cells about 600–800 × 40–60 μm in vivo; body flexible and flattened ribbon-like, with two ends narrow; head small and inconspicuous; tail distinctly narrowed and claviform (Figs 2a–c and 3a–c). Endoplasm greyish and opaque due to packed inclusions, approx. 1 × 2 μm, with two ends transparent (Fig. 3a, b). Nuclear apparatus in centre of trunk, containing about four to six macronuclei and two micronuclei, forming a tight cluster, approx. 10 μm in diameter (Figs 2f, g and 3g, k, l). Cortical granules round, approx. 1 μm in diameter, colourless, distributed between ciliary rows and in glabrous stripe (Figs 2d and 3d). Locomotion by gliding between sand grains and organic debris. Cell surface densely ciliated with glabrous stripe, about as wide as two somatic kineties (Figs 2e, h, i and 3b–j). Entire infraciliature consisting of dikinetids. Twenty-six to thirty somatic kineties on trunk, with cilia about 10 μm long. Anterior and posterior secant system formed on left side of glabrous stripe, where some kinetics abut bristle kinety (Figs 2e, h, i and 3j). Oral infraciliature consisting of circumoral kinetics, several dikinetids of which are loosely spaced above glabrous stripe on left side (Figs 2e and 3h; double arrowheads); many irregularly distributed dikinetids located between circumoral kinetics and glabrous stripe (Figs 2e, h and 3h, j; arrow).

Comparison with related species (Fig. 4; Table 2). Since there is no information on the oral ciliation of most species within the Trachelocercidae, their generic classification based on the new generic definition remains questionable (Foissner & Dragesco, 1996a, b). Consequently, a comparison between the novel species and related forms should not be limited to the genus Trachelocerca. Of about 70 trachelocercids, 34 species possess a single nuclear group (Xu et al., 2011c) and, considering the number of macronuclei and their general morphology, six of these species should be compared with the novel form.

Tracheloraphis vermiformis Raikov, 1962 resembles Trachelocerca chinensis sp. n. in possessing a similar number of macronuclei and similar body shape, i.e. a narrow glabrous stripe, inconspicuous head and claviform tail. It differs from the latter, however, in its shorter body (350–650 μm vs 600–800 μm) and its larger number of somatic kineties (70 vs 26–30) (Fig. 4a–c, e, f; Table 2; Raikov, 1962).

Tracheloraphis indisincta Wright, 1982 and Tracheloraphis niveus Wright, 1982 have similar body shapes and numbers of macronuclei to the novel form, but they can be distinguished from it by possessing more somatic kineties (39–47 and 36–47 vs 26–30) (Fig. 4o–r, t–w; Table 2; Wright, 1982).

The original description of Tracheloraphis enigmaticus Dragesco, 1960 is very brief, and no redescription has been made since it was first reported, meaning that the identity of this species remains questionable. Based on the original description, however, it can be distinguished from the novel species by having a broader glabrous stripe (as wide as eight somatic kineties vs as wide as two somatic kineties) and a remarkably long and thin neck (vs gradually narrowed neck) (Fig. 4j–l; Table 2; Dragesco, 1960).

Although Trachelocerca stephail (Dragesco, 1965) Dragesco, 2002 and Trachelocerca bodiani (Dragesco, 1963) Dragesco, 2002 have similar numbers of macronuclei and similarly narrow glabrous stripes to Trachelocerca chinensis sp. n., they can be separated from the novel species by having fewer somatic kineties (16–20 and 10–12 vs 26–30) and different body shape (having a conspicuous head and round tail vs an inconspicuous head and distinctly narrowed tail) (Fig. 4d, g–i, o–r, t; Table 2; Dragesco, 2002).
Genus *Tracheloraphis* Dragesco, 1960

*Tracheloraphis dragescoi* sp. n. (Figs 5 and 6; Table 1)

**Diagnosis.** Extended cells *in vivo* about 600–1000 × 30–60 μm; head conspicuous with 14–22 somatic kineties; single nuclear apparatus located in the middle of body.

**Type locality.** The intertidal zone of the No. 1 Bathing Beach in Qingdao (36° 06′ N 120° 34′ E), China, where the water temperature was 18 °C and the salinity about 30% (Fig. 1c).

**Type specimens.** A protargol-impregnated slide containing the holotype specimen (Fig. 5h, i) marked with an ink circle is deposited in the Laboratory of Protozoology, Ocean University of China, China (no. XY09042902). A paratype slide is deposited in the Natural History Museum, London, UK, with registration number NHMUK 2014.8.27.2.

**Etymology.** *dra.ges*co.i. N.L. fem. n. *dragescoi* named in honour of our respected French colleague, the eminent ciliatologist Dr Jean Dragesco, in recognition of his significant contributions to the study of ciliates.

**Description.** Fully extended cells about 600–1000 × 30–60 μm *in vivo*, filiform in shape; flexible and contractile (Figs 5a–c and 6a–e). Cell distinctly tripartite, with neck, tail and trunk regions (Figs 5a–c and 6a–e). Head conspicuous claviform; tail wedge-shaped (Figs 5a and 6a–c). Endoplasm greyish and opaque due to multiple refractile inclusions, about 3–5 μm in diameter (Fig. 6k). Nuclear apparatus including four or six macronuclei and two micronuclei. (h, i) General infraciliature of the holotype specimen to mark the circmoral kinety, bristle kinety, glabrous stripe and somatic kineties; the arrow indicates irregularly distributed dikinetids between the circmoral kinety and the glabrous stripe; arrowheads show the anterior secant system forming on the left side of the glabrous stripe. BK, Bristle kinety; CK, circmoral kinety; GS, glabrous stripe; Ma, macronuclei; Mi, micronuclei; NG, nuclear group; SK, somatic kineties. Bars, 350 μm (a), 15 μm (e) and 25 μm (h, i).

![Fig. 2. *Trachelocerca chinensis* sp. n. from life (a–d) and after protargol impregnation (e–i). (a) Typical individual, noting the inconspicuous head, claviform tail and the single nucleary apparatus located in the middle of body. (b, c) Different body shapes. (d) Distribution of cortical granules (arrows) between the ciliary rows and in the glabrous stripe, noting the anterior secant system (arrowheads) forming on the left side of the glabrous stripe. (e) Infraciliature of the anterior part, showing the circmoral kinety, bristle kinety and glabrous stripe; the arrow marks irregularly distributed dikinetids between the circmoral kinety and glabrous stripe; double arrowheads indicate several loosely spaced dikinetids of the circmoral kinety on the left side; arrowheads show the anterior secant system forming on the left side of the glabrous stripe. (f, g) Nuclear apparatus including four or six macronuclei and two micronuclei. (h, i) General infraciliature of the holotype specimen to mark the circmoral kinety, bristle kinety, glabrous stripe and somatic kineties; the arrow indicates irregularly distributed dikinetids between the circmoral kinety and the glabrous stripe; arrowheads show the anterior secant system forming on the left side of the glabrous stripe. BK, Bristle kinety; CK, circmoral kinety; GS, glabrous stripe; Ma, macronuclei; Mi, micronuclei; NG, nuclear group; SK, somatic kineties. Bars, 350 μm (a), 15 μm (e) and 25 μm (h, i).
long (Figs 5d and 6f, l). Micronuclei difficult to detect. Cortical granules elongated oval, about $0.2 \times 1 \mu m$, colourless, distributed between ciliary rows and in glabrous stripe (Figs 5e and 6g, h). Locomotion by gliding between sand grains and organic debris.

Cell surface densely ciliated with unciliated zone on left side, which is almost as wide as the body (Figs 5h, i and 6p, q). Entire infraciliature consisting of dikinetids. Fourteen to twenty-two somatic kineties on trunk, with cilia about 7–10 $\mu m$ long. Anterior ends of ciliary rows curved to right and composed of densely spaced dikinetids (Figs 5h, i and 6p, q). Anterior and posterior secant system formed on left side of glabrous stripe, where some kineties abut bristle kinety (Figs 5i and 6p). Glabrous stripe bordered by bristle kinety, kinetids of which are more widely spaced and irregularly arranged than those of the somatic kineties (Figs 5g, i and 6m, o, p). Oral ciliature consisting of circumoral kinety, which is interrupted by two or three inserted brosse kineties (Figs 5h, i and 6i, j, p).

**Comparison with related species (Fig. 7; Table 3).** As with *Trachelocerca chinensis* sp. n., the lack of information about the oral ciliature of the Tracheloceridae means that the comparison between the novel species and related forms should not be limited to the genus *Tracheloraphis*. Of the approximately 70 trachelocerids, eight species should be compared with the novel form considering the number of macronuclei and their general morphology.

*Tracheloraphis hamatus* Wright, 1982 resembles *Tracheloraphis dragescoi* sp. n. in body size, width of the glabrous stripe and the number of macronuclei. It can be distinguished from the latter, however, since it possesses a
**Table 1.** Morphometric data from *Trachelocerca chinensis* sp. n. (upper line), *Tracheloraphis dragescoi* sp. n. (middle line) and *Geleia acuta* (lower line)

Data are based on protargol-impregnated specimens.

<table>
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<tr>
<th>Characteristic</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
<th>n</th>
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<td>46.1</td>
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<td>14.9</td>
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<td>42.4</td>
<td>5.0</td>
<td>11.7</td>
<td>15</td>
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<td>4.1</td>
<td>16.3</td>
<td>15</td>
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<td>7.3</td>
<td>8</td>
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**Fig. 4.** Morphology of some forms closely related to *Trachelocerca chinensis* sp. n. (a–c, e, f) *Tracheloraphis vermiformis* (from Raikov, 1962). (d, g–i) *Trachelocerca bodiani* (from Dragesco, 2002). (j–l) *Tracheloraphis enigmaticus* (from Dragesco, 1960). (m, n, s) *Tracheloraphis niveus* (from Wright, 1982). (o–r, t) *Trachelocerca stephai* (from Dragesco, 2002). (u–w) *Tracheloraphis indistincta* (from Wright, 1982). Arrowheads show cortical granules. CK, Circumoral kinety; GS, glabrous stripe; Ma, macronuclei; Mi, micronuclei; NG, nuclear group; SK, somatic kineties. Bars, 300 µm (a, k), 200 µm (g), 60 µm (h, i, o, p) and 600 µm (s, w). Reproduced with permission.
different type of cortical granules (globose, less than 0.5 \( \mu m \) in diameter vs oval, about 0.2 \( \times \) 1 \( \mu m \)) and fewer somatic kineties (10–14 vs 14–22) (Fig. 7n, s, t, u, v; Table 3; Dragesco, 2002; Wright, 1982).

*Tracheloraphis africanus* Dragesco, 1965 has a similar body size and numbers of somatic kineties and macronuclei to the novel species. However, it differs from the latter in having no cortical granules (vs present) but rod-shaped granules, about 3 \( \mu m \) long, scattered in the superficial cytoplasm, which, according to the original report, may be bacteria but are not mucocytes (vs absent) (Fig. 7f–h; Table 3; Dragesco, 1965; Dragesco & Dragesco-Kerneés, 1986).

*Tracheloraphis gracilis* Dragesco, 1960 has similar numbers of macronuclei and oval cortical granules to *Trachelocerca chinensis* sp. n.

### Table 2. Morphometric comparison of *Trachelocerca chinensis* sp. n. with some closely related forms

<table>
<thead>
<tr>
<th>Species</th>
<th>Body length (( \mu m ))</th>
<th>SK (n)</th>
<th>Ma (n)</th>
<th>Source</th>
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<td>600–800</td>
<td>26–30</td>
<td>4–6</td>
<td>Present work</td>
</tr>
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<td><em>Tracheloraphis vermiiformis</em></td>
<td>350–650</td>
<td>70</td>
<td>2–6</td>
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<td><em>Tracheloraphis niveus</em></td>
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<td>36–47</td>
<td>4</td>
<td>Wright (1982)</td>
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<td><em>Tracheloraphis enigmatica</em></td>
<td>600</td>
<td>ND</td>
<td>5</td>
<td>Dragesco (1960)</td>
</tr>
<tr>
<td><em>Trachelocerca bohiai</em></td>
<td>400</td>
<td>10–12</td>
<td>4 or 5</td>
<td>Dragesco (2002)</td>
</tr>
</tbody>
</table>

Ma, Macronuclei; SK, somatic kineties; ND, no data available.

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**Fig. 5.** *Tracheloraphis dragescoi* sp. n. from life (a–c, e) and after protargol impregnation (d, f–i). (a) Typical individual, noting the single nuclear apparatus located in the middle of the body. (b, c) Different body shapes. (d) Macronuclei forming a tight cluster. (e) Distribution of cortical granules (arrowheads) between the ciliary rows and in the glabrous stripe. (f, g) Infraclariature of the posterior body end, showing somatic kineties, bristle kinety and glabrous stripe occupying almost the entire left side. (h, i) Anterior right (h) and left (i) sides of the holotype specimen, indicating the circumoral kinety, somatic kineties, brosse, glabrous stripe and bristle kinety; arrowheads mark the anterior secant system forming on the left side of the glabrous stripe. B, Brosse; BK, bristle kinety; C, (protein) crystal; CK, circumoral kinety; GS, glabrous stripe; Ma, macronuclei; NG, nuclear group; NU, nucleolus; SK, somatic kineties. Bars, 350 \( \mu m \) (a–c), 25 \( \mu m \) (f, g) and 50 \( \mu m \) (h, i).
**Fig. 6.** *Tracheloraphis dragescoi* sp. n. from life (a–h, k) and after protargol impregnation (i, j, l–q). (a, b) Typical individual, noting the single nuclear apparatus located in the middle of body. (c–e) Different body shapes. (f, l) Macronuclei forming a tight cluster. (g, h) Distribution of cortical granules (arrowheads) between the ciliary rows and in the glabrous stripe. (i, j) Infraciliature of the anterior body end, showing the circumoral kinety, brossé and bristle kinety. (k) Arrowheads indicate refractile inclusion. (m) Infraciliature of the left middle region, noting the bristle kinety and glabrous stripe. (n, o) Infraciliature of the posterior end, marking the somatic kineties, bristle kinety and the glabrous stripe occupying almost the entire left side. (p, q) Infraciliature of the anterior left (p) and right (q) sides, indicating the circumoral kinety, somatic kineties, brossé, glabrous stripe and bristle kinety; arrows show the anterior secant system forming on the left side of the glabrous stripe. B, Brossé; BK, bristle kinety; C, (protein) crystal; CK, circumoral kinety; GS, glabrous stripe; Ma, macronuclei; NG, nuclear group; SK, somatic kineties. Bars, 300 μm (a–e), 25 μm (n) and 50 μm (p, q).

dragesci sp. n., but it can be separated from the novel form by having a differently shaped tail (rounded vs wedge-shaped) and fewer somatic kineties (12 or 13 vs 14–22) (Fig. 7c–e, i; Table 3; Dragesco, 1960).

*Tracheloraphis prenanti* Dragesco, 1960 can be separated from *Tracheloraphis dragescoi* sp. n. in having more somatic kineties (20–26 vs 14–22) and macronuclei (6–8 vs 4) (Fig. 7q, r, w; Table 3; Dragesco & Dragesco-Kerneis, 1986).

Based on the original illustration, the nuclear apparatus of *Tracheloraphis enigmaticus* Dragesco, 1960 consists of five macronuclei and two micronuclei, but these do not form a clustered nuclear group as in *Tracheloraphis dragescoi* sp. n. These two species can therefore be distinguished from each other (Fig. 7x–z; Table 3; Dragesco, 1960).

The original description of *Tracheloraphis striatus* Raikov, 1962 is based only on staining, and no information on its live morphology is available. It differs from the novel species, however, in having fewer somatic kineties (12–14 vs 14–22) (Fig. 7m, o; Table 3; Raikov, 1962).

The original report of *Tracheloraphis remanei* Dragesco, 1960, meanwhile, is based only on live observation, and this species has never been redescribed. Although no
information on its infraciliature is available, based on the original illustration, it can be distinguished from *Tracheloraphis dragescoi* sp. n. in that it has a narrower glabrous stripe (about one-third of the body width vs about as wide as the body) and a smaller ratio of body length to width (about 11:1 vs about 20:1) (Fig. 7a, b; Table 3; Dragesco, 1960).

Although no information on the infraciliature of *Trachelocerca schulzei* Dragesco, 1960 is available, according to the original illustration, it can still be separated from the novel species in that it has a larger ratio of body length to width (about 35:1 vs about 20:1) (Fig. 7j–l, p; Table 3; Dragesco, 1960).

**Table 3.** Morphometric comparison of *Tracheloraphis dragescoi* sp. n. with some closely related forms

<table>
<thead>
<tr>
<th>Species</th>
<th>Body length (μm)</th>
<th>SK (n)</th>
<th>Ma (n)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tracheloraphis dragescoi</em> sp. n.</td>
<td>600–1000</td>
<td>14–22</td>
<td>4</td>
<td>Present work</td>
</tr>
<tr>
<td><em>Tracheloraphis prenanti</em></td>
<td>400–750</td>
<td>20–26</td>
<td>6–8</td>
<td>Dragesco &amp; Dragesco-Kernéis (1986)</td>
</tr>
<tr>
<td><em>Tracheloraphis africanus</em></td>
<td>700</td>
<td>17 or 18</td>
<td>4</td>
<td>Dragesco &amp; Dragesco-Kernéis (1986)</td>
</tr>
<tr>
<td><em>Tracheloraphis gracilis</em></td>
<td>400–800</td>
<td>12 or 13</td>
<td>4–6</td>
<td>Dragesco (1960)</td>
</tr>
<tr>
<td><em>Tracheloraphis enigmaticus</em></td>
<td>600</td>
<td>ND</td>
<td>5</td>
<td>Dragesco (1960)</td>
</tr>
<tr>
<td><em>Tracheloraphis striatus</em></td>
<td>500–700</td>
<td>12–14</td>
<td>ND</td>
<td>Raikov (1962)</td>
</tr>
<tr>
<td><em>Tracheloraphis remanei</em></td>
<td>1000</td>
<td>ND</td>
<td>5 or 6</td>
<td>Dragesco (1960)</td>
</tr>
<tr>
<td><em>Trachelocerca schulzei</em></td>
<td>650</td>
<td>ND</td>
<td>4</td>
<td>Dragesco (1960)</td>
</tr>
</tbody>
</table>
Family Geleiidae Kahl, 1933
Genus Geleia Kahl in Foissner, 1998
Geleia acuta (Dragesco, 1960) Foissner, 1998
(Figs 8 and 9; Table 1)

The original description of this organism by Dragesco (1960) was rather brief and was based only on live observation. In addition, no redescription has yet been made, and no information is available on its infraciliature. Consequently, an improved diagnosis and a redescription based on observations of the Qingdao population are presented here.

Improved diagnosis. Body about 250–500 \( \times \) 20–35 \( \mu m \) in vivo, flattened ribbon-like; fossa conspicuous; two or three macronuclei and one micronucleus; cortical granules brown, about 0.3 \( \mu m \) in diameter; 25–40 somatic kineties; 30–40 adoral polykineties; intrabuccal kinety comprising 21–31 dikinetids.

Description of the Qingdao population. Cell size in vivo mostly about 250–500 \( \times \) 20–35 \( \mu m \). Body flattened and ribbon-like, with posterior end forming pointed tail (Figs 8a–c and 9a, b); Cell flexible and contractile (Figs 8a–c and 9a, b, h). Fossa located between apical beak and buccal field (Figs 8a and 9a, d, i; arrowhead). Buccal field about 30–40 \( \mu m \) long (Figs 8a and 9a, b, h, i). Somatic cilia about 10–12 \( \mu m \) long. Cytoplasm packed with colourless refractile inclusions, about 1–3 \( \mu m \) in diameter. Cortical granules brown, about 0.3 \( \mu m \) in diameter, distributed along ciliary rows (Figs 8d and 9g). Locomotion mainly by sluggish gliding between sand grains and organic debris or remaining motionless at the bottom of the Petri dish.

Infraciliature as shown in Fig. 8(h, i). One preoral kinety comprising 6–10 dikinetids located in fossa and within preoral suture (Figs 8g, h and 9k). Somatic kineties extending to posterior end of cell (Fig. 8g, h). Oral structures comprising three parts: adoral polykineties that form a ciliary field on left side of oral cavity composed of up to four dikinetids (Figs 8g and 9j, k); intrabuccal kinety on right side of buccal field composed of 21–31 dikinetids (Figs 8g and 9j, k); and paroral kinetids, which form a...
ciliary field on right side of buccal field parallel to intrabuccal kinety, composed of about 29 rows of closely spaced trikinetids (Figs 8g and 9j, k). Usually two or three macronuclei; one micronucleus (Figs 8f and 9e).

Remarks. This species was first described by Dragesco (1960) as ‘400 \( \mu \)m long, general colour brown with brown pigments, body flattened, caudal end pointed, and a fossa located above buccal field’. Considering these characteristics, the Qingdao population corresponds well to the original report, and the two populations can be considered as conspecific.

Molecular phylogeny based on sequences of the SSU rRNA gene (Fig. 10)

The length, DNA G+C content and GenBank accession numbers of the SSU rRNA gene sequences of the three species are as follows: *Trachelocerca chinensis* sp. n., 1637 bp, 47.34 mol%, KJ768667; *Tracheloraphis dragescoi* sp. n., 1561 bp, 47.92 mol%, KJ768668; *Geleia acuta*, 1470 bp, 50.07 mol%, KJ768666.

The ML and BI trees have similar topologies and therefore only a single topology is presented with support values generated from both analyses (Fig. 10). As described in previous studies (Yan et al., 2013, 2014), the family Trachelocercidae is a monophyletic group (80% ML, 1.00 BI), being a sister clade to the family Kentrophoridae (90% ML, 0.97 BI). Within the Trachelocercidae, the genera *Trachelocerca* and *Tracheloraphis* are closely related but overlapping with regard to the positions of their congeners: that is, none of them belongs to a clearly separated monophyletic clade. Three populations of *Tracheloraphis huangi* nest within the genus *Trachelocerca* with full support (100% ML, 1.00 BI). *Trachelocerca chinensis* sp. n. clusters with *Trachelocerca chinensis* (GenBank accession no. FJ463746), and then forms a sister group with the three populations of *Tracheloraphis huangi*. According to personal communications, *Trachelocerca cf. ditis* (represented by sequence FJ463746) is actually a population of *Trachelocerca chinensis* sp. n., and it differs from the latter in three nucleotides. The morphological data, i.e. cell size after protargol impregnation (158–272 \( \mu \)m in *Trachelocerca cf. ditis* vs 178–273 \( \mu \)m in *Trachelocerca chinensis* sp. n.) and the numbers of somatic kineties on the head and trunk (11–15 and 27–31 in *Trachelocerca cf. ditis* vs 13 or 14 and 26–30 in *Trachelocerca chinensis* sp. n.), overlap as well. Thus, two populations of *Trachelocerca chinensis* sp. n. cluster together. In addition, *Tracheloraphis dragescoi* sp. n.

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**Fig. 9. Geleia acuta from life (a–d, g–i) and after protargol impregnation (e, f, j, k).** (a) Typical individual, noting the buccal field; the arrowhead marks the fossa between the apical beak and buccal field; the arrow indicates the pointed tail. (b) Different body shape. (c) Pointed tail. (d) Fossa (arrowhead). (e) Macronuclei and micronucleus. (f) Infraciliature of the posterior end, noting the somatic kineties. (g) Arrowheads show the distribution of cortical granules between the ciliary rows. (h) Contracted cell. (i) Anterior end, showing the buccal field and fossa (arrowhead). (j, k) Infraciliature of the buccal area, indicating the adoral polykineties, intrabuccal kinety, paroral kineties, somatic kineties and preoral kinety. Ap, Adoral polykineties; BF, buccal field; Ibk, intrabuccal kinety; Ma, macronuclei; Mi, micronucleus; Pk, paroral kineties; Prek, preoral kinety; SK, somatic kineties. Bars, 100 \( \mu \)m (a, b), 120 \( \mu \)m (h) and 30 \( \mu \)m (i–k).
groups with the clade Trachelocerca spp.–Tracheloraphis huangi. Therefore, both Trachelocerca and Tracheloraphis appear to be not monophyletic. Moreover, the hypothesis that Tracheloraphis is monophyletic is rejected (P=0.011, <0.05) by the AU test, while the hypothesis that Trachelocerca is monophyletic is not rejected (P=0.143, >0.05).

The topology within the Trachelocercidae is rather stable, as the positions of most trachelocerids are the same in the current study as in a previous report (Yan et al., 2014), except for Apotrachelocerca arenicola and Kovalevaia sulcata. According to Yan et al. (2014), K. sulcata occupies the basal position, while A. arenicola is the next most deeply branching taxon, which is contrary to our findings. However, the support values in both cases are rather low (52 % ML, 0.53 BI in Yan et al., 2014; 56 % ML, 0.88 BI in the current study), which means that the positions of these two taxa remain uncertain.

The generic classification of the genera within the Trachelocercidae is based mainly on oral apparatus, i.e. simple or compound circumoral kineties, and the presence or absence of brosse. One possibility is that the presence/absence of brosse is a result of convergent evolution. If this is the case then, based on the common feature, i.e. the single-rowed circumoral kinety, the genus Tracheloraphis should be a synonym of Trachelocerca. However, another genus, Kovalevaia, in which the circumoral kinety also consists of a single row, does not cluster with Tracheloraphis and Trachelocerca, but occupies a basal position within the Trachelocercidae. Given that the position of Kovalevaia is not strongly supported, it is premature to combine the genera Tracheloraphis and Trachelocerca. More data, especially additional sequences and morphogenetic data of Kovalevaia, Prototrachelocerca and Apotrachelocerca, are needed to provide an adequate resolution of the placement of these genera.

The family Geleididae is monophyletic (98 % ML, 0.99 BI), within which Parduczia orbis occupies a basal position. The genus Geleia is a fully supported monophyletic group that forms a sister clade with Parduczia. G. acuta clusters with G. fossata with high support (98 % ML, 1.00 BI), and this cluster forms a sister relationship to the group including Geleia simplex, Geleia sp. isolate K2 (GenBank accession no. AJ971535), G. sinica, G. swedmarkii and Geleia sp. RRD-2003 (AY187926). The systematic position of G.
acuta based on its SSU rRNA gene sequence confirms that this species belongs to the genus Geleia. Meanwhile, the observation that the genus Geleia is monophyletic shows that the characters used to separate Geleia and Parduczia, i.e. the composition of adoral polykineties and paroral kinety/polykineties, are reliable diagnostic features at the generic level.

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REFERENCES


Two novel trachelocercids and one geleid


