Morphology, morphogenesis, and molecular phylogeny of a soil ciliate, *Gonostomum kuehnelti* Foissner, 1987 (Ciliophora, Hypotrichia), from northwestern China

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Morphology, morphogenesis, and molecular phylogeny of a soil ciliate, *Gonostomum kuehnelti* Foissner, 1987 (Ciliophora, Hypotrichia), from northwestern China

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**ABSTRACT**

The morphology and morphogenesis of the hypotrichous soil ciliate *Gonostomum kuehnelti* Foissner, 1987, isolated from northwestern China, was investigated based on observations of live and protargol-stained specimens. Photomicrographs and some detailed morphological information, e.g. cortical granules and the number of dorsal kineties, are provided in this study. The morphogenesis of our population agrees in most details with that of the type population. The main events during binary fission are as follows: (1) the parental adoral zone of membranelles is retained completely; (2) six streaks of the undulating membranes and cirral anlagen are segmented in a 1: 2: 2: 4: 4 pattern from left to right, and form three frontal, two frontoventral, one buccal, two frontoterminal, three postoral ventral, two pretransverse ventral, and two transverse cirri, respectively; and (3) the marginal rows and dorsal kineties develop intrakinetally. In addition, the SSU rDNA sequence of *G. kuehnelti* is provided. Phylogenetic analyses based on SSU rDNA sequences show that the genus *Gonostomum* is non-monophyletic and *G. kuehnelti* is placed within the core clade of *Gonostomum*.

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**KEYWORDS**

Gonostomatidae; ontogeny; protozoa; SSU rDNA; taxonomy

**Introduction**

have led to a better understanding of systematic and evolutionary relationships among hypotrichs, although the available molecular data are still very limited for this group (Gao et al. 2016; Bharti et al. 2017; Wang et al. 2017b; Lyu et al. 2018).

The genus Gonostomum was established by Sterki (1878) with G. affine (original combination: Oxytricha affinis Stein, 1859) as the type species. To date, 18 species have been classified in this genus (Song 1990; Berger 2011; Bharti et al. 2015; Foissner 2016; Lu et al. 2017). Gonostomum kuehnelti was originally discovered in an organically farmed field near the village of Seekirchen near Salzburg, Austria (Foissner 1987). Subsequently, three populations of G. kuehnelti have been described from Austria, Turkey and India, respectively (Eigner 1999; Çapar 2007; Kamra et al. 2008). However, some morphogenetic features have not been clearly described, and SSU rDNA sequence data are lacking for G. kuehnelti.

In the present study, we describe the morphology and morphogenesis of a G. kuehnelti population collected from northwestern China. The SSU rRNA gene of G. kuehnelti is sequenced for the first time and the molecular phylogeny of genus Gonostomum is investigated.

Materials and methods

Sampling and cultivation

Gonostomum kuehnelti was isolated from the surface layer of soil (upper 0–5 cm) in an apple forest in Maiji District (N34°30′06″, E106°04′15″), Tianshui City, Gansu Province, China, 6 June 2016 (Figure 1). The soil temperature and water content were 24.8 °C and 12.9%, respectively.

Ciliates were made to excyst by employing the non-flooded Petri dish method (Foissner et al. 2002). Raw cultures were established at room temperature (about 25°C) using Petri dishes filled with filtered soil percolate. Rice grains were added to support microbial growth (Deng et al. 2018). Although we could not establish a clonal culture, the specimens used for morphological and molecular analyses were very likely conspecific because no other Gonostomum morphospecies were found during live observations or in the protargol preparations.

Morphology and morphogenesis

Isolated cells were observed in vivo using bright field and differential interference contrast microscopy. The protargol staining method of Wilbert (1975) was used to reveal the infraciliature and the nuclear apparatus. Counts and measurements of stained specimens were performed at a magnification of 1,000×. Drawings of stained cells were made with drawing device. To illustrate the changes occurring during morphogenesis, the old ( parental) ciliary structures are depicted by contour whereas new structures are shaded black. For general and specific terms, see Berger (2011).

DNA extraction, PCR amplification, and sequencing

Two cells were washed three times in distilled water before being processed for DNA extraction using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the
manufacturer’s instructions. The SSU rDNA was amplified using Q5® Hot Start high-fidelity 2x master mix DNA polymerase with primers 18S-F (5ʹ-AACCTGGTTGATCCTGCCAGT-3ʹ) and 18S-R (5ʹ-TGATCCTTCTGCAGGTTCACCTAC-3ʹ) (Medlin et al. 1988). Cycling parameters for PCR amplifications were as follows: 30 s initial denaturation at 98°C; followed by 18 cycles of 98°C for 10 s, 69°C for 40 s with touchdown of 1°C for each cycle, 72°C for 90 s; 18 cycles of 98°C for 10 s, 51°C for 40 s, 72°C for 90 s; and a final extension at 72°C for 4 min. Purified PCR product of the appropriate size was inserted into the pClone007 Blunt simple vector (TSINGKE, Qingdao Co., Ltd.) and sequenced in both directions at TSINGKE incorporated company.

**Phylogenetic analyses**

In addition to the SSU rDNA sequence of *Gonostomum kuehnelti* obtained in this study, 84 sequences of representative hypotrichs downloaded from the GenBank database were included in the phylogenetic analyses. Six oligotrichs, *Strombidium apolatum*, *S. cuneiforme*, *Strombidinopsis acuminata*, *Parastrombidinopsis minima*, *Schmidingerella tarakaensis*, and *Tintinnopsis tocatinensis* were used as outgroup species. Sequences were aligned online using MUSCLE on the GUIDANCE (Penn et al. 2010) web server (http://guidance.tau.ac.il) with default parameters. Both ends of the alignments were trimmed.
and ambiguous columns were removed with set parameters (score below 0.655), resulting in a matrix of 1,738 characters. Maximum likelihood (ML) analyses with 1,000 bootstrap replicates were performed using RAxML-HPC2 on XSEDE v8.2.9 (Stamatakis et al. 2008) on the CIPRES Science Gateway with the GTR+CAT model selected by Modeltest v3.4 (Posada and Crandall 1998). Bayesian inference (BI) analyses were carried out under MrBayes (Ronquist and Huelsenbeck 2003) on XSEDE v3.2.6 on the CIPRES Science Gateway using the GTR+I + G model selected by Akaike Information Criterion in MrModeltest v2 (Nylander 2004). Markov chain Monte Carlo simulations were run with two sets of four chains for 4,000,000 generations, with a sample frequency of 100 generations and a burn-in of 10,000. All remaining trees were used to calculate posterior probabilities (PP) using a majority rule consensus. Tree topologies were visualised using MEGA 5.0 (Tamura et al. 2013).

Following the morphological classification, the approximately unbiased (AU) test (Shimodaira 2002) was performed using CONSEL v0.1 (Shimodaira and Hasegawa 2001). The ML tree was compared with the constrained ML tree based on the hypothesis that species of *Gonostomum* form a monophyletic group.

**Deposition of voucher slides**

Nine voucher slides (registration no. ZHR-2016060602/A-I) have been deposited in the Laboratory of Microbiota, College of Life Science, Northwest Normal University, Lanzhou, China.

**Results**

**Chinese population of *Gonostomum kuehnelti* Foissner, 1987**

**Morphological description**

Body 75–100 × 30–45 μm *in vivo*, length: width ratio ca. 2.5–3.5:1 *in vivo* (n = 10), 2–3:1 on average in fixed and stained cells. Flexible but non-contractile, usually slender to ellipsoidal, both ends narrowly rounded; left margin strongly convex, right margin slightly to strongly convex (Figures 2(a–c) and 3(a,b)). Cells colourless to grey; cytoplasm filled with numerous food vacuoles, 6–8 μm in size, containing single-celled green algae, 2–5 μm in size. (*Figure 3(f)*). Cortical granules rod-shaped, 0.5 × 1 μm *in vivo*, sparsely distributed, colourless and usually ejected under cover slip pressure, hence can be easily overlooked (Figures 2(d) and 3(e)). Contractile vacuole about 10–15 μm across, located at about mid-body, slightly left of midline. Collecting canals lacking (Figures 2(a–c) and 3(a,b)). 7–16 spherical or ellipsoidal macronuclear nodules, often forming two more or less distinct groups slightly left of midline (Figures 2(g) and 3(i)). Locomotion by slowly rotating clockwise or gliding, sometimes crawling on substrate.

Adoral zone occupies 37–45% of body length *in vivo* (n = 10) and 40% on average in protargol preparations; commencing at anterior body end, extending straight along left body margin and curving abruptly towards center of body; composed of 24 membranelles on average (Figures 2(a,b) and 3(a–c,h)). Bases of largest membranelles about 4 μm wide, cilia up to 13 μm long *in vivo* (Figures 2(e,f) and 3(h)). Anterior end of endoral almost reaching mid-region of paroral which is composed of about 10 loosely spaced basal bodies (Figures 2(e) and 3(h)). Invariably 15 frontoventral-
transverse cirri (Figure 2(f); Table 1). Three enlarged frontal cirri with cilia about 12 µm long; one buccal cirrus close to right anterior portion of paroral; two frontoventral cirri (III/2, IV/3) (Figures 2(e,f) and 3(h)); two frontoterminal cirri (VI/3, VI/4) left of anterior part of right marginal row; ‘postoral ventral’ cirri right of posterior portion of adoral zone, forming a characteristic triangular pattern, that is, middle cirrus (V/4) slightly right of anterior (IV/2) and rear (V/3) cirri (Figures 2(e,f) and 3(h)). Two pretransverse ventral cirri (V/2, VI/2) ahead of two transverse cirri (V/1, VI/1), these four cirri forming a quadrangular pattern, an arrangement also known for some other Gonostomum species; transverse cirri moderately enlarged, cilia of which are about 11 µm long (Figures 2(a,b,f) and 3(i)). Left marginal row composed of 14–20 cirri, commencing at level of buccal vertex, terminating about at level of transverse cirri;
right marginal row composed of 21–28 cirri, commencing at level of anterior frontoterminal cirrus, terminating about at level of pretransverse ventral cirri (Figures 2(f) and 3(i)).

Dorsal cilia 3–4 μm long in vivo, arranged in three bipolar dorsal kineties (Figures 2(g) and 3(e)). Kinety 1 originates about 15% down length of cell from anterior end and extends to posterior end of cell, composed of 13–19 dikinetids; kinety 2 originates subapically and extends to posterior end of body, composed of 14–19 dikinetids; kinety
3 commences apically and extends along right cell margin to posterior end of body, composed of 18–24 dikinetids (Figures 2(g) and 3(j)). Three caudal cirri, cilia of which are about 10 μm long, located at posterior body margin, closely spaced, one at end of each dorsal kinety (Figures 2(a,b,g) and 3(d,k)).

Resting cysts colourless, globular, about 40 μm in diameter. Wall not observed, cytoplasm studded with many vacuoles up to 4 μm across (Figure 3(g)).

### Notes on morphogenesis

#### Stomatogenesis

In the opisthe, cell division commences with the de novo formation of an oral primordium (OP) roughly in the midline between the buccal vertex and the pretransverse ventral cirri, that is, starting with the proliferation of basal bodies in the postoral region (Figures 4(a) and 5(a)). As the number of basal bodies increases, the OP becomes larger and the anterior portion begins to differentiate to form the adoral membranelles of the opisthe (Figures 4(b,c) and 5(b)). Simultaneously, the formation of new membranelles proceeds posteriad. In later stages, the anterior portion of the new adoral zone bends slightly rightward (Figures 4(d,f) and 5(c,e)). As is usual for the genus Gonostomum, the anlage for the undulating membranes (UM-anlage) forms to the right of the OP as a long streak of basal bodies. Thereafter, the UM-anlage splits longitudinally into two streaks, from which the endoral and paroral are formed (Figures 4(d,f,h) and 5(c,h)).

In the proter, the parental adoral zone is retained completely intact and no new membranelles are formed, so changes of the oral structure are confined to the paroral and endoral. The origin of UM-anlage and the fate of the parental paroral and endoral are

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### Table 1. Morphological characterisation of Chinese population of *Gonostomum kuehnelti.*

<table>
<thead>
<tr>
<th>Character</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>M</th>
<th>SD</th>
<th>CV</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Length of body</td>
<td>75</td>
<td>110</td>
<td>87.8</td>
<td>85</td>
<td>9.8</td>
<td>11.1</td>
<td>25</td>
</tr>
<tr>
<td>Width of body</td>
<td>24</td>
<td>48</td>
<td>37.8</td>
<td>46</td>
<td>6.9</td>
<td>18.2</td>
<td>25</td>
</tr>
<tr>
<td>Length of adoral zone</td>
<td>30</td>
<td>44</td>
<td>36.5</td>
<td>39</td>
<td>3.8</td>
<td>10.4</td>
<td>25</td>
</tr>
<tr>
<td>Number of adoral membranelles</td>
<td>21</td>
<td>27</td>
<td>23.6</td>
<td>23</td>
<td>1.4</td>
<td>6.0</td>
<td>25</td>
</tr>
<tr>
<td>Number of frontal cirri</td>
<td>3</td>
<td>3</td>
<td>3.0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of buccal cirri</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>25</td>
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<tr>
<td>Number of frontoventral cirri</td>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of frontoterminal cirri</td>
<td>3</td>
<td>3</td>
<td>3.0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of postoral ventral cirri</td>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of pretransverse cirri</td>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of transverse cirri</td>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>25</td>
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<tr>
<td>Number of caudal cirri</td>
<td>3</td>
<td>3</td>
<td>3.0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of left marginal cirri</td>
<td>14</td>
<td>20</td>
<td>17.1</td>
<td>18</td>
<td>1.5</td>
<td>8.7</td>
<td>25</td>
</tr>
<tr>
<td>Number of right marginal cirri</td>
<td>21</td>
<td>28</td>
<td>24.5</td>
<td>24</td>
<td>2.0</td>
<td>8.2</td>
<td>25</td>
</tr>
<tr>
<td>Number of dorsal kineties</td>
<td>3</td>
<td>3</td>
<td>3.0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Number of basal bodies in dorsal kinety 1</td>
<td>13</td>
<td>19</td>
<td>16.0</td>
<td>19</td>
<td>1.9</td>
<td>12.0</td>
<td>22</td>
</tr>
<tr>
<td>Number of basal bodies in dorsal kinety 2</td>
<td>14</td>
<td>19</td>
<td>15.9</td>
<td>16</td>
<td>1.2</td>
<td>7.8</td>
<td>19</td>
</tr>
<tr>
<td>Number of basal bodies in dorsal kinety 3</td>
<td>18</td>
<td>24</td>
<td>20.5</td>
<td>20</td>
<td>1.7</td>
<td>8.1</td>
<td>22</td>
</tr>
<tr>
<td>Total number of dorsal dikinetids</td>
<td>48</td>
<td>60</td>
<td>53.4</td>
<td>53</td>
<td>3.2</td>
<td>6.0</td>
<td>20</td>
</tr>
<tr>
<td>Number of macronuclear nodules</td>
<td>7</td>
<td>16</td>
<td>11.2</td>
<td>13</td>
<td>1.9</td>
<td>17.1</td>
<td>25</td>
</tr>
</tbody>
</table>

All data are based on protargol-stained specimens. All measurements in μm.

Notes: AZM, adoral zone of membranelles; CV, coefficient of variation in %; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number; SD, standard deviation.
uncertain as these stages in morphogenesis are not observed. In the following stages, the development of the UM-anlage is the same as that in the opisthe (Figures 4(d,f,i) and 5(c)).

Frontoventral ciliature and marginal rows
Some key stages during the ontogenetic process were not observed, so the origin of the frontoventral-transverse cirral anlagen (FVTA) is not clear. While the anarchic field of the oral primordium elongates, the buccal cirrus (II/2) disorganise and proliferates basal bodies anteriorly and posteriorly forming anlage II; a group of basal bodies originate de novo beneath cirri III/2 and IV/2, forming anlage III and IV, respectively. Anlage V seems
to be formed de novo at the top and bottom of cirri V/3 and V/4, and anlage VI originates to the right of anlage V (Figures 4(b,c) and 5(b)). We deduce that FTVA II–VI are probably primary anlagen that split into two sets during the middle stages of morphogenesis. Gradually, frontoventral-transverse cirri are formed in the following manner: anlage I produces frontal cirrus I/1 (= left frontal cirrus); anlage II produces the buccal cirrus (II/2) and frontal cirrus II/3 (= middle frontal cirrus); anlage III produces the right frontal cirrus (III/3) and anterior frontoventral cirrus (III/2); anlage IV produces the posterior frontoventral (IV/3) and anteriormost postoral ventral (IV/2) cirri, respectively; anlage V forms the left transverse cirrus (V/1), the left pretransverse ventral cirrus (V/2), and the rearmost and middle postoral ventral cirri (V/3, V/4); and anlage VI forms

Figure 5. Photomicrographs of dividers of Gonostomum kuehnelti after protargol preparation. (a,b) Ventral views of early dividers showing oral primordium, arrow marks the formation of new membranelles; (c) ventral view of late divider, arrowheads mark the left frontal cirri (I/1), arrow denotes left marginal row anlage of proter, frontoterminal cirri are encircled; (d) dorsal view showing division of the macronuclear mass; (e) ventral view of opisthe of a late divider showing development of new cirri, arrowhead marks left marginal anlage, circle denotes the frontoterminal cirri; (f) dorsal view of late divider showing new dorsal kineties 1–3 of proter, inter alia, arrowheads mark new caudal cirri; (g,h) very late divider showing proter and opisthe (g); broken lines connect cirri which originated from the same anlage, arrowheads denote buccal cirrus, arrows indicate pretransverse ventral cirri, frontoventral cirri are encircled. AZM, adoral zone of membranelles; E, endoral; FC, frontal cirri; FTC, frontoterminal cirri; LMR, new left marginal row; Ma, macronuclear nodules; OP, oral primordium; P, paroral; PVC, postoral ventral cirri; RMA, right marginal anlagen; RMR, new right marginal row; TC, transverse cirri; II, III, V, VI, frontoventral-transverse cirral anlagen; 1–3, new dorsal kineties. Scale bars: a, c, e–g = 55 μm; h = 25 μm.
the right transverse cirrus (VI/1), the right pretransverse ventral cirrus (VI/2), and rightmost two frontoventral cirri (VI/3, VI/4). Streaks I–VI generate the following numbers of cirri in both the proter and the opisthe: 1, 2, 2, 2, 4, 4. Thus, a total of 15 cirri are formed. The cirri then separate and migrate towards their final positions.

The marginal rows develop within the parental rows (Figures 4(d,f,h,i) and 5(c,e,g,h)). No parental cirri are retained after division (Figures 4(h,i) and 5(g,h)).

**Dorsal ciliature**
The dorsal kineties originate by intrakinetal proliferation. Usually, one caudal cirrus is formed at the end of each kinety (Figures 4(e,g,j) and 5(f)).

**Nuclear apparatus**
The macronuclear nodules fuse during the early stages of morphogenesis and subsequently apportions into the proter and opisthe. The species-specific number of nodules is attained by both daughters during the late stages of morphogenesis (Figures 4(e,g,j) and 5(d,f)).

**Phylogenetic analyses based on SSU rDNA sequences (Figure 6)**
The SSU rDNA sequence of *Gonostomum kuehnelti* (GenBank accession number MF445660) is 1728 bp long and has a G + C content of 45.83%. Phylogenetic trees inferred from SSU rDNA sequences using two different methods (ML and BI) resulted in similar topologies, therefore only the ML tree is shown with nodal support from both methods (Figure 6). The genus *Gonostomum* is not monophyletic and the phylogenetic relationships within this genus were not resolved as indicated by low support values in both ML and BI trees. *Gonostomum kuehnelti* grouped with *G. affine*, two sequences of *G. strenuum* and one unidentified species of *Gonostomum*, with high support (ML/BI, 84/0.90). This cluster formed a sister relationship with a clade comprising *Cotterillia bromelicola*, *G. sinicum*, one unknown *Gonostomum* species, and two environmental sequences (68% ML, 1.00 BI). As indicated by Group I of Gonostomatidae, several branches of environmental sequences were formed and clustered with *Gonostomum* species. The other two congeners included in the analyses, *G. namibiense* and *G. paronense*, grouped with four urostylids with low support. The AU test rejected the possibility that the genus *Gonostomum* is monophyletic (p = 0.021).

**Discussion**

**Identification of the Chinese population of Gonostomum kuehnelti Foissner, 1987**

Detailed descriptions of *G. kuehnelti* were provided by Foissner (1987), Capar (2007) and Kamra et al. (2008) for populations in Austria, Turkey and India, respectively. The Chinese population closely resembles these three populations in all key characters, i.e., body shape and colour, nuclear apparatus, pattern of paroral and endoral, number of dorsal kineties, morphometric data and biotope (Figure 15 and Table 14 in Berger 2011). There were, however, some slight differences with the Chinese population having a larger body size *in vivo* than the Austrian population (75–100 × 30–45 μm
A slightly smaller body in vivo than the Turkish population (75–100 μm vs. 100–105 μm), a smaller ratio of body length to width in vivo than the Turkish population (2.5–3.5 vs. 4.2), and a longer adoral zone of membranelles relative to the body length in vivo than the Turkish population (37–45% vs. 25%) (Table 2). We consider that these differences probably are intra-specific because the localities of these populations are very widely separated and the habitats from which they were collected variously different soil types (Foissner 1987; Eigner 1999; Çapar 2007; Kamra et al. 2008). Therefore, the identity of the Chinese isolate is not in doubt (Table 2).
Table 2. Comparison of four populations of *Gonostomum kuehnelti*.

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of body <em>in vivo</em> (μm)</td>
<td>60–90</td>
<td>-</td>
<td>100–105</td>
<td>75–100</td>
</tr>
<tr>
<td>Width of body <em>in vivo</em> (μm)</td>
<td>25–35</td>
<td>-</td>
<td>23–25</td>
<td>30–45</td>
</tr>
<tr>
<td>Length:width ratio <em>in vivo</em></td>
<td>2.5</td>
<td>-</td>
<td>4.2a</td>
<td>2.5–3.5</td>
</tr>
<tr>
<td>Length of body (protargol) (μm)</td>
<td>50–71</td>
<td>55–70</td>
<td>-</td>
<td>75–110</td>
</tr>
<tr>
<td>Width of body (protargol) (μm)</td>
<td>20–28</td>
<td>15–26</td>
<td>-</td>
<td>25–50</td>
</tr>
<tr>
<td>Length:width ratio (protargol)</td>
<td>2.4a</td>
<td>3.3</td>
<td>-</td>
<td>2.3</td>
</tr>
<tr>
<td>Cortical granules</td>
<td>1–1.5 μm long and loosely arranged, colourless</td>
<td>Colourless</td>
<td>0.5–1 μm long, loosely arranged, colourless</td>
<td>Rod-shaped, 0.5 × 1 μm in vivo, loosely arranged, colourless</td>
</tr>
<tr>
<td>Ratio of AZM to body length <em>in vivo</em></td>
<td>40%a</td>
<td>45%</td>
<td>25%</td>
<td>37–45%</td>
</tr>
<tr>
<td>Length of AZM (protargol) (μm)</td>
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<td>27–32</td>
<td>-</td>
<td>30–44</td>
</tr>
<tr>
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<td>22–28</td>
<td>22a</td>
<td>21–27</td>
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<td>Frontal cirri (number)</td>
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<td>Frontoventral cirri (number)</td>
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<tr>
<td>Postoral ventral cirri (number)</td>
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<tr>
<td>Pretransverse cirri (number)</td>
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<tr>
<td>Transverse cirri (number)</td>
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<td>Caudal cirri (number)</td>
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<td>Left marginal cirri (number)</td>
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<td>13–16</td>
<td>19a</td>
<td>14–20</td>
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<tr>
<td>Right marginal cirri (number)</td>
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<td>19–22</td>
<td>22a</td>
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<td>Dorsal kinetics (number)</td>
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<tr>
<td>Dikinetids in DK1 (number)</td>
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<td>14–18</td>
<td>13a</td>
<td>13–19</td>
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<td>Dikinetids in DK2 (number)</td>
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<td>13–16</td>
<td>16a</td>
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<td>Dikinetids in DK3 (number)</td>
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<td>17–20</td>
<td>22a</td>
<td>18–24</td>
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<tr>
<td>Macronuclear nodules (number)</td>
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<td>13–19</td>
<td>15a</td>
<td>7–16</td>
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<td>Terrestrial</td>
<td>Terrestrial</td>
<td>Terrestrial</td>
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</tbody>
</table>

Notes: 1, Austrian population; 2, Indian population; 3, Turkish population; 4, Chinese population; -: Data not available; a: Data obtained from illustrations; AZM: adoral zone of membranelles; DK1–3: dorsal kinetics.
Comparison of morphogenesis with congeners

Morphogenetic data are available for only nine species of Gonostomum (G. affine, G. algicola, G. kuehnelti, G. strenuum, G. salinarum, G. singhii, G. gonostomoidum, G. bromelicola and G. sinicum; Foissner 1987, 2016; Song 1990; Eigner 1999; Kamra et al. 2008; Berger 2011; Bharti et al. 2015; Lu et al. 2017). Based on the Chinese population reported here, the most characteristic events during morphogenesis in G. kuehnelti can be summarised as follows: (1) the parental adoral zone of membranelles is retained completely by the proter; (2) the six streaks of the undulating membranes anlage (UMA) and cirral anlagen are segmented in a 1: 2: 2: 4: 4 pattern from left to right, thus forming three frontal, two frontoventral, two frontoterminal, one buccal, three postoral ventral, two pretransverse ventral and two transverse cirri, respectively; (3) the marginal rows develop intrakinetally; and (4) the dorsal kineties originate by intrakinetal proliferation. According to Eigner (1999), anlage I (UM-anlage) for proter is formed from a long primary primordium. However, as illustrated in Figure 4(b,c), we did not observe any change in anlage I. Furthermore, since the long primary primordium is not clearly recognisable from the published data, it should be verified by further studies (Berger 2011). One of the most remarkable morphogenetic features in G. bromelicola is the unique dorsal development, i.e. its second dorsal kinety anlage undergoes fragmentation during the middle division stage and finally four dorsal kineties are formed (Foissner 2016). However, all other Gonostomum species follow the Gonostomum pattern, viz., no fragmentation of the dorsal kinety anlage and only three dorsal kineties are formed (Berger 2011). Therefore, as more ontogenetic modes become available for other species of Gonostomum, the generic diagnosis may need to be amended.

Phylogenetic analyses

Neither the family Gonostomatidae nor the genus Gonostomum is monophyletic in our SSU rDNA phylogenetic analyses (Figure 6), which is consistent with previous studies (Berger 2011; Foissner and Stoeck 2011; Bharti et al. 2015; Lu et al. 2017). The internal relationships within the family and the genus remain uncertain as tree topologies are not consistent among other studies (Bharti et al. 2015; Huang et al. 2016). Nevertheless, a well-supported core group of Gonostomum is always recovered. Gonostomum kuehnelti was placed within the core group of Gonostomum in both ML and BI trees, supporting the morphological identification. It is noteworthy that in the present phylogeny, G. namibiense and G. paronense clustered with four urostylids rather than with the core group of gonostomatid species. Given that G. namibiense and G. paronense both have the unique-tailed bodies and frontoventral cirral pairs (Bharti et al. 2015), we agree with Lu et al. (2017) that these two species may belong to the genus Apogonostomum Foissner, 2016. However, it is also possible that the present topology is biased due to limited taxon sampling as SSU rDNA sequence data of Gonostomatidae are only available for seven Gonostomum species and Cotterillia bromelicola, while 12 genera and 54 species have been assigned in this family (Berger 2011; Foissner and Stoeck 2011; Bharti et al. 2015; Foissner 2016). Moreover, several unidentified environmental sequences grouped closely with gonostomatids, indicating that a considerable proportion of species in this group are yet to be discovered. Thus, sequences of additional gonostomatid species are required in order to recover a robust phylogeny for this complex group.
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Disclosure statement

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