Taxonomy and phylogeny of two species of the genus *Deviata* (Protista, Ciliophora) from China, with description of a new soil form, *Deviata parabacilliformis* sp. nov.

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The morphology and morphogenesis of a soil hypotrichous ciliate, *Deviata parabacilliformis* sp. nov., isolated from northern China, were investigated. *D. parabacilliformis* measures about 75–210×25–60 μm *in vivo*, with an elongate and flexible body. It possesses one right marginal row, two to four left marginal rows and three dorsal kineties. The main morphogenetic features of *D. parabacilliformis* are: (i) the oral primordium originates *de novo*; (ii) anlage IV of the opisthe originates from parental frontoventral row V, anlage V originates *de novo*, and anlage VI forms from frontoventral row VI; and (iii) anlage I of the proter originates from the anterior portion of the parental paroral, anlage II originates from the buccal cirrus, anlage III originates from the parabuccal cirri, anlage IV originates from parental frontoventral row IV and anlage V forms from the anterior of parental frontoventral row VI. The morphology of an edaphic population of another species of the genus *Deviata*, *Deviata bacilliformis* (Gelei 1954) Eigner 1995, was also investigated. This work also provides the first record of SSU rRNA gene sequences for species of the genus *Deviata*. Molecular phylogenetic analysis suggests that *Deviata* is not monophyletic, and its position is poorly resolved due to weak phylogenetic signal of the 18S marker in the Stichotrichida.

INTRODUCTION

The ciliated protozoa are a species-rich and morphologically diverse group. Morphological identification is challenging for these organisms, especially for the highly complicated hypotrichs. This situation is being improved, however, as a result of staining techniques which can reveal the infraciliature and other characteristics precisely (Chen et al., 2013a, b; Jiang et al., 2013a, b; Li et al., 2013; Pan et al., 2013; Shao et al., 2013a; Singh & Kamra, 2013; Singh et al., 2013; Fan et al., 2014; Gao & Katz, 2014; Huang et al., 2014; Jung et al., 2014).

Abbreviations: BI, Bayesian inference; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession numbers for the SSU rRNA gene sequences of *Deviata bacilliformis* and *Deviata parabacilliformis* are KJ766110 and KJ766111, respectively.

The genus *Deviata* was established by Eigner (1995) with *Deviata abbrevescens* as the type species. Diagnostic features of the genus include: non-dorsomarginalian hypotrichia with more than one long cirral row on right and left lateral sides of the body; one of the cirral rows right of the adoral zone terminates in the centre of the ventral surface (Berger, 2011); typical parental (old) cirral rows are absent; and multiple deviations within anlagen develop during morphogenesis (Eigner, 1995). According to Berger (2011), there are currently eight recognized species in the genus. Except for the type species *D. abbrevescens*, detailed morphogenetic processes are not known. Furthermore, there are currently no SSU rRNA gene sequence data for species of the genus *Deviata* and the phylogenetic position of the genus is uncertain (Berger, 2011).

This paper describes the morphology and morphogenesis of a novel *Deviata* species, *Deviata parabacilliformis*...
sp. nov., isolated from a soil sample collected from China. In addition, the morphological redescription of a Chinese population of *Deviata bacilliformis* is provided, and phylogenetic analyses based on SSU rRNA gene sequences are also carried out for both species.

**METHODS**

**Morphological studies.** Soil samples were collected from the suburbs of Tianjin city, China (Fig. 1a), on 14 November 2012. *D. bacilliformis* was collected from a grassy area at the Xiaohanzhuang service area off the Binbao Expressway (G2501) (39° 19′ N 117° 09′ E) (Fig. 1b) while *D. parabacilliformis* sp. nov. was collected from a ditch about 100 m from the Xiaohanzhuang service area (39° 19′ N 117° 09′ E) (Fig. 1c). Ciliates were made to excyst and emerge from the soil samples by employing the non-flooded Petri dish method (Foissner, 1987). They were then isolated and cultures were established at room temperature (about 25 °C) in Petri dishes containing mineral water with squeezed rice grains to enrich the bacterial food (Shao et al., 2013). Living cells were observed under an Olympus BX51 DIC microscope and photographed using a digital camera. The protargol silver staining method of Wilbert (1975) was used to reveal the infraciliature. Measurements of the stained specimens were carried out with an ocular micrometer. Drawings of stained specimens were performed at ×1250 with the aid of a camera lucida. To illustrate the changes occurring during morphogenetic processes, old (parental) ciliary structures are depicted by contour whereas new structures are shaded black (Lv et al., 2013). Terminology and classificatory system are used (mainly) according to Berger (2011).

**DNA extraction, PCR amplification and sequencing.** Cells of *D. parabacilliformis* sp. nov. and *D. bacilliformis* were isolated and washed three times using sterilized water to remove bacterial contamination. These cells were then transferred to a 1.5 ml microfuge tube with a minimum volume of water. Genomic DNA was extracted using a Dneasy Blood & Tissue kit (Qiagen) following the manufacturer’s instructions (Gao et al., 2013; Huang et al., 2014), except that one-quarter of the suggested volume for each solution was used. PCR amplification of the SSU rRNA gene was performed according to Yi and Song (2011), with the universal primers Euk A (5′-AACCTGGTTGATCCTGCCAGT-3′) or 82F (5′-GAAACTGGCCATGTTGTCACCT-AC-3′) (Medlin et al., 1988) and Euk B (5′-TGATCCTTCTGACGTCACCCT-AC-3′) (Medlin et al., 1988). Phylogenetic analyses. The SSU rRNA gene sequences of *D. parabacilliformis* sp. nov. and *D. bacilliformis* were aligned with sequences of 52 other taxa obtained from the GenBank database, with *Protocruzia adherens* and *Protocruzia contrax* as the outgroup species, using the online program Muscle 3.7 (http://www.phylogene.fr/) (Edgar, 2004). Subsequently, these sequences were manually refined by removing ambiguous gaps in BioEdit 7.0.0 (Hall, 1999). The program MrModeltest v.2.0 (Nylander, 2004) selected GTR +I (=0.4460) + G (=0.4529) as the best model with Akaike’s information criterion, which was then used for Bayesian inference (BI). BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) which was run for 10 000 000 generations with the first 2500 trees being discarded as burn-in. Remaining trees were used to generate the consensus tree and to calculate the posterior probabilities. The maximum-likelihood (ML) tree and 1000 bootstrap replicates were reconstructed using the RAxML online program (Stamatakis et al., 2008) with the model GTR +I+ G. TreeView v1.6.6 (Page, 1996) and Mega 4.0 (Tamura et al., 2007) were used to view and edit tree topologies.

**RESULTS**

**Deviata parabacilliformis** sp. nov.

**Diagnosis.** Edaphic *Deviata*, body measuring 75–210 × 25–60 μm (*n*=14) *in vivo*, body elongate and flexible. Pellicle colourless, cortical granules lacking. Usually two macro-nuclear nodules, contractile vacuole close to left body margin about in mid-body. Adoral zone occupies about 15–25 % of body length *in vivo* and is composed of, on average, 21 membranes. Three frontal cirri, one buccal cirrus and usually one parabuccal cirrus. Two long fronto-ventral rows. One right marginal row and two to four left marginal rows. Three dorsal kinetics, dorsal cilia about 5 μm long, caudal cirrus lacking.

**Type locality.** Ditch beside the Binbao Expressway about 100 m from the Xiaohanzhuang service area in the suburb of Tianjin city (39° 19′ N 117° 09′ E) (Fig. 1a).

**Type slides.** The holotype slide (no. LFC20121702a) and five paratype slides (nos. 20121702b–d) with protargol-stained...
specimens are deposited in the Laboratory of Hydrobiology, Hebei University, China.

**Etymology.** The species name *parabacilliformis* (pa.ra.ba. cil.li.for’mis. N.L. fem. adj.) is a composite of the prefix *para-* (Greek preposition, beside, like) and the species name *bacilliformis*, and refers to the similarity of this species to *D. bacilliformis*.

**Description.** About 75–210 × 25–60 μm *in vivo*, length/width ratio about 4.5:1; flexible, elongate and elliptical with both ends usually narrow; right margin slightly convex, left margin strongly convex (Fig. 2a, d, e). Buccal field about one-sixth to one-quarter of body length (Fig. 2a, b, i). Macronuclear nodules situated left of midline in the middle of body, and usually elongated ellipsoidal (Fig. 2c, h, j). Micronucleus difficult to recognize *in vivo* and in protargol preparations, but usually near anterior part of each macronuclear nodule (Fig. 2c). Cortical granules absent. Colourless cytoplasm, typically filled with numerous granular inclusions (approximately 2–4 μm), which give cells a dark, opaque appearance (Fig. 2d–g). Contractile vacuole equatorial without collecting canals (Fig. 2g). It is difficult to recognize the contractile vacuole due to numerous granular inclusions in the cytoplasm. Locomotion moderately rapid, usually gliding on surface of Petri dishes searching for food without pause. When freely swimming, rotates about its main body axis.

Adoral zone of membranelles roughly in *Gonostomum* pattern (Fig. 2a, b, i). Adoral zone occupies about 15–25% of body length, and has 20–23 membranelles (Table 1). Cilia in membranelles about 10–12 μm *in vivo*. Buccal cavity flat and narrow with endoral posterior to paroral and, apparently, longer (Fig. 2b, i). Pharyngeal fibres about 20–45 μm long and conspicuous after protargol impregnation, extending obliquely backwards.

Cirral pattern generally constant (Fig. 2b, c; Table 1) and all cirri rather long and conspicuously fine, with frontal cirri and marginal cirri about 10–15 μm *in vivo* (Fig.
Table 1. Morphometrical characterization of *D. bacilliformis* (italics) and *D. parabacilliformis*

All measurements are in micrometres. All data are based on protargol-impregnated specimens. *n*, Number of specimens investigated.

<table>
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<th>Character</th>
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<th>Max.</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
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2a). Buccal cirrus located in front of anterior end of endoral (Fig. 2b). Consistently three frontal cirri and usually one or, rarely, two parabuccal cirri behind right frontal cirrus (Fig. 2b). Frontoventral row IV usually composed of one or two cirri (Fig. 2b), commencing behind the right of parabuccal cirri; frontoventral row V begins behind frontoventral row IV and terminates about in mid-body; frontoventral row VI begins close to anterior end of cell and terminates near the rear end. Invariably, only one right marginal row (Fig. 2b, c). Three or, rarely, two or four widely spaced left marginal cirral rows. Usually, frontal cirri are each composed of 12 (3 × 4) basal bodies while buccal cirrus and parabuccal cirrus are each composed of eight (2 × 4) basal bodies and frontoventral and marginal cirri composed of two basal bodies each.

Dorsal cilia about 5 μm long in vivo and arranged in three kineties. Kineties 1 and 3 are relatively widely spaced (Fig. 2c, h). Kinety 2 is almost bipolar while the other two are slightly shorter at both ends (Fig. 2c).

**Morphogenesis.** Stomatogenesis commences with the de novo formation of opisthe’s oral primordium in the postoral area between frontoventral row V and left marginal row I (Figs 3a and 4a). With the proliferation of basal bodies, oral primordium lengthens posteriorly (Figs 3b and 4b) and differentiates into new adoral membranelles in the right anterior portion. Later, three streaks are generated (opisthe’s anlagen I–III) (Figs 3c and 4c). Subsequently, anlage VI for opisthe forms in the middle portion of parental row VI (Figs 3d and 4d).

Anlage VI for proter develops at the next stage but, because we were not able to discern the relevant stage, we could not define whether it develops de novo or from anlage VI for opisthe (Figs 3d and 4d). Marginal cirri anlagen for opisthe are recognizable within parental marginal rows (Fig. 3d, e). In proter, parental paroral, buccal cirrus and parabuccal cirrus contribute to construction of anlage I, II and III, respectively (Figs 3d and 4d). In opisthe, anlage IV develops from posterior-most one or two cirri of parental frontoventral row V (Figs 3d, f and 4e, f) while anlage V of opisthe originates de novo (Fig. 3d). Meanwhile, two separate dorsal anlagen occur within each parental kinety (Fig. 3e).

In a middle divider, the posterior portion of parental endoral is resorbed entirely (Figs 3f and 4f). The proter’s anlage V forms within the anterior portion of parental frontoventral row VI (Figs 3f and 4g), while anlage IV develops from a disaggregated cirrus of parental frontoventral row IV (Figs 3f and 4e). Meanwhile, marginal cirri anlagen for proter form within the anterior portion of parental marginal rows (Fig. 3f).

Finally, anlage I originates the left frontal cirrus (frontoventral row I) and undulating membranes, anlage II generates middle frontal and buccal cirri (frontoventral row II), anlage III develops into right frontal cirrus and parabuccal cirrus (frontoventral row III), while anlagen IV, V and VI contribute frontoventral rows IV, V and VI, respectively (Figs 3g, h and 4g–i, k).

Juvenile dorsal kineties develop from two separate anlagen within each parental kinety, no caudal cirri are formed (Figs 3e and 4j). All parental cirri are resorbed completely once the process of division is complete.

Division of nuclear apparatus proceeds in the ordinary way: the two macronuclear nodules completely fuse into a single mass prior to division, and in late dividers split into proter and opisthe macronuclei (Fig. 3i). The division of micronuclei was unclear in this study.

**Morphology of a Chinese population D. bacilliformis** (Gelei 1954) Eigner 1995

Cells elongate and flexible, about 70–160 × 25–60 μm in vivo, on average 135 × 30 μm (n= 14). Cytoplasm colourless, packed with 1–3 μm numerous granular inclusions (Fig. 5a–c). Usually two macronuclear nodules (Fig. 5f, g), contractile vacuole near the left body margin in the middle of the cell, without collecting canals (Fig. 5c). Pellicle colourless and no cortical granules. Adoral zone occupies about 15–20 % of body length in vivo and composed of 16–28 membranelles. Three frontal cirri, one buccal cirrus and one parabuccal cirrus (Fig. 5d, Table 1). Cirri long and conspicuously thin, about 10–15 μm long in vivo. One dorsal kinety (Fig. 5g), dorsal cilia about 5 μm long with no caudal cirrus. Two long frontoventral rows (frontoventral rows V and VI, longer than the length of adoral zone of...
membranelles), three right marginal rows and four left marginal rows. A single specimen from over 100 individuals examined had two short frontoventral rows (we considered this to be an abnormal cell so it was not included in Table 1) (Fig. 5e). Frontoventral and marginal cirri composed of just two basal bodies, except for the anterior one to three cirri of the cirral rows (which are composed of four basal bodies each); frontal cirri composed of nine basal bodies each and buccal and parabuccal cirri of six basal bodies each.

Movement moderately rapid, usually creeping, sometimes freely swimming with rotation around the long axis of the body.

**SSU rRNA gene sequence analyses**

The SSU rRNA gene sequence of *D. parabacilliformis* (GenBank accession number KJ766111) is 1690 bp long and has a DNA G+C content of 45.44 mol%, while that of *D. bacilliformis* (GenBank accession number KJ766110) is 1695 bp long and has a DNA G+C content of 45.90 mol%. They shared 97.9% sequence similarity. The topologies of the ML and BI trees were basically congruent (Fig. 6). In the phylogenetic trees, *D. parabacilliformis* is closely related to the assemblage *Strongylidium*, *Pseudouroleptus* and *Oxytricha* with weak data support. The sister relationship between *D. bacilliformis* and *Perisincirra paucicirrata* is moderately/highly supported (71% ML, 1.00 BI).

**DISCUSSION**

**Comparison of *D. parabacilliformis* with related taxa**

estevesi Paiva & Silva-Neto 2005, *Deviata quadrinucleata* (Dragesco 2003) Berger 2011, *Deviata rositae* Küppers et al. 2007, *Deviata polycirrata* Küppers & Claps 2010 and *Deviata spirostoma* (Alekperov 1988) Berger 2011, have been described in the genus *Deviata* (Berger & Foissner, 1987; Eigner, 1995; Dragesco, 2003; Paiva & da Silva-Neto, 2005; Küppers et al., 2007; Siqueira-Castro et al., 2009; Küppers & Claps, 2010; Berger, 2011). Considering its combination of various features, namely 20–23 adoral membranelles and two nuclear nodules, as well as the fact that the cilia of the dorsal kinetids are about 5 μm long in vivo, and are therefore almost indistinguishable from the marginal cirri, *D. parabacilliformis* can be clearly separated from its congeners.

Based on consistent possession of three dorsal kineties, *D. parabacilliformis* can be easily separated from species possessing one or two dorsal kineties, i.e. *D. abbrevescens*, *D. bacilliformis*, *D. brasiliensis*, *D. estevesi*, *D. quadrinucleata* and *D. rositae*. Despite *D. polycirrata* and *D. spirostoma* having three dorsal kineties, *D. parabacilliformis* can also be distinguished from them. *D. parabacilliformis* differs from *D. polycirrata* mainly by possessing fewer long cirral rows [five to seven (including two long frontoventral and three to five right marginal rows) versus 17–21], left marginal rows (two to four versus 9–13), adoral membranelles (20–23 versus 39–48), and dikinetids in dorsal kinety 1 (6–11 versus 22–32), kinety 2 (11–21 versus 30–36) and kinety 3 (6–12 versus 20–25). *D. parabacilliformis* differs from *D. spirostoma* by having only 20–23 (versus 45–50) adoral membranelles and having much shorter dorsal bristles (5 μm versus 8–12 μm).

**Fig. 4.** Different morphogenetic stages of *D. parabacilliformis* after protargol impregnation. (a, b) Early dividers, showing origin and development of opisthe's oral primordium between parental cirral row V and left marginal cirral row 1. (c) Early divider to show differentiation of adoral membranelles within oral primordium and early development of opisthe's anlagen VI (arrow) from parental cirral row VI. (d) Ventral view of a mid-stage divider showing formation of the proter's anlagen (I–III, VI) and opisthe's anlagen (I–VI). (e, f) Ventral views of two mid-stage dividers; arrows show development of anlagen IV in both proter and opisthe. (g) Ventral view of a divider in mid- to late stage; arrows show undulating membrane anlage splitting longitudinally in proter and opisthe, and arrowhead shows proter's anlage V. (h, i) Anterior portion of a proter (h) and an opisthe (i) in mid- to late stage, to show newly formed frontal cirri, buccal cirri (arrows) and parabuccal cirri (arrowheads). (j) Dorsal view of a middle stage divider, to show intrakinetal formation of new dorsal kineties. (k) General ventral view of a late divider. FC, frontal cirri; LM, left marginal cirral row; Ma, macronuclear nodules; OP, oral primordium; I–VI (italic), frontoventral anlagen; V, VI (bold), parental frontoventral rows; 1–3, dorsal kineties. Bar, 60 μm.
Comparison between the Chinese and other populations of *D. bacilliformis*

*D. bacilliformis* was first discovered by Gelei (1954) in Hungary, with other populations being reported subsequently (Israel: Berger & Foissner, 1987; Africa: Dragesco, 2003; Argentina: Küppers & Claps, 2010). Of the above populations, the Israeli population has been described in detail (Berger, 2011). The morphology of the Chinese population of *D. bacilliformis* agrees well with that of the Israeli population (Berger & Foissner, 1987) and with that of the Argentinean population (Küppers & Claps, 2010), for example with respect to the similar ciliary pattern and the presence of a single dorsal kinety and usually two macronuclear nodules. Just as in the Argentinean population, the Chinese population exhibits a bristle in the anterior-most basal body pairs of the two outermost right marginal rows (Fig. 5g, arrowheads), and also two or three isolated and barren kinetids on the middle of the ventral surface. Unlike the Israeli and Argentinean populations, however, we did not observe collecting canals of contractile vacuole in the Chinese population. Meanwhile, two African populations were described by Dragesco (2003) but these lack the short frontoventral row present in the Israeli, Argentinean and Chinese populations.

**Morphogenetic comparison of species of the genus *Deviata***

The morphogenetic process of five species of the genus *Deviata* has been investigated in detail, including the type species *D. abbrevescens* Eigner 1995, *D. brasiliensis* Siqueira-Castro et al. 2009, *D. bacilliformis* (Gelei 1954) Eigner 1995, *D. polycirrata* Küppers & Claps 2010 and *incertae sedis* *D. estevesi* Paiva & Silva-Neto 2005 (Berger, 2011). *D. parabacilliformis* exhibits similar ontogenetic features to the above five species in: (i) having six frontoventral cirr al anlagen (*D. abbrevescens*, *D. brasiliensis*, *D. polycirrata* and *D. estevesi*); (ii) the left and right marginal rows and the dorsal kineties originating in the conventional way (*D. abbrevescens*, *D. brasiliensis*, *D. polycirrata* and *D. estevesi*); (iii) no parental cirral rows being retained after division (*D. abbrevescens*, *D. brasiliensis*, *D. polycirrata* and *D. estevesi*); and (iv) the oral primordium in the opisthe forming *de novo* (*D. bacilliformis*). Because we were not able to identify the relevant stage, we could not deduce whether the anlage VI in the proter develops from the anlage in proter or *de novo*.

The main differences were as follows. (i) The origin of the oral primordium: while the oral primordia of *D.
Two Deviata species from China

D. parabacilliformis (versus in a complex way in separated anlagen are formed within rows 4–6 in D. estevesi contributes one frontal cirrus in 2009). (iii) The undulating membrane anlage in the proter, which is formed due to differences in the length of frontoventral rows. (ii) According to Berger (2011), this kind of difference may be indicated by bold type. Support values for nodes are for ML and BI. Nodes unresolved in the ML tree and the BI tree are indicated by an asterisk. GenBank accession numbers are given for each species. All branches are drawn to scale. Bar, 5 substitutions per 100 nt positions.

**Fig. 6.** ML tree (Final ML Optimization Likelihood: \(-12850.860558\)) inferred from the SSU rRNA gene sequences of 54 hypotrich taxa, showing the positions of D. parabacilliformis and D. bacilliformis. New sequences from the present study are indicated by bold type. Support values for nodes are for ML and BI. Nodes unresolved in the ML tree and the BI tree are indicated by an asterisk. GenBank accession numbers are given for each species. All branches are drawn to scale. Bar, 5 substitutions per 100 nt positions.

D. abbrevescens and D. brasilienisi originate within the parental frontoventral row IV, that of D. parabacilliformis originates de novo/intrakinetically varies within a population. According to Berger (2011), this kind of difference may be due to differences in the length of frontoventral rows. (ii) The origin of anlage V in the proter, which is formed in frontoventral row VI in D. parabacilliformis but in frontoventral row V in D. brasilienisi (Siqueira-Castro et al., 2009). (iii) The undulating membrane anlage in the proter contributes one frontal cirrus in D. parabacilliformis but develops a row of cirri in D. estevesi. (iv) Each two separated anlagen are formed within rows 4–6 in D. estevesi (versus in a complex way in D. parabacilliformis). (v) Anlage V in the opisthe forms de novo in D. parabacilliformis but from parental frontoventral row VI in D. polycirrata (as deduced by Küppers & Claps, 2010).

The origin of anlagen IV and V of the proter and anlage V of the opisthe in D. abbrevescens is rather complicated (Berger, 2011), because some parental cirri in other rows are involved in the formation of the anlagen.

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