Morphological and molecular characterization of *Parafurgasonia zhangi* spec. nov. and *Chilodonella acuta* Kahl, 1931 (Protozoa, Ciliophora), from a soil habitat of Saudi Arabia

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The morphology and infraciliature of two soil ciliates, *Parafurgasonia zhangi* spec. nov. and *Chilodonella acuta* Kahl, 1931, collected from Saudi Arabia, were investigated by observations of both living cells and specimens after standard staining methods. *P. zhangi* differs from its congeners by the combination of the following features: excretory pore quite near posterior end of paroral membrane, 16 or 17 somatic kineties with about 11 kinetids in each one on dorsal side, paroral membrane gently curved and composed of about 15 dikinetids, and hypostomial organelle composed of four or five files of kinetids with four monokinetic each. The diagnosis of *Chilodonella acuta* was renewed to include characteristics revealed by the silver impregnation method: cells *in vivo* measuring 33–45×18–26 μm, dorsal hump and tail-like podite present, two contractile vacuoles, seven left and five right kineties, 9–11 nematodesmal rods, and dorsal brush containing about 11 basal bodies. Phylogenetic analyses based on small-subunit rRNA gene sequences showed that *P. zhangi* was closer to species of the Colpodidiidae rather than the Furgasoniidae represented by *Furgasonia blochmanni*, and *Chilodonella acuta* clustered with its congener *Chilodonella uncinata* but was a well-outlined species of the genus.

**INTRODUCTION**

Soil ciliates, with the total number of known species approaching 1000, are distributed worldwide. They play an important role in nutrient cycling of terrestrial food webs (Acosta-Mercado & Lynn, 2004; Chao *et al.*, 2006). With the ability to form resistant resting cysts, soil ciliates are adapted well to various soil habitats even including desert sand dunes (Foissner *et al.*, 2002, 2008; Verni & Rosati, 2011). Compared with the large number of investigations on marine ciliate fauna of the Saudi Arabian coasts (Al-Rasheid, 1996, 1997, 1999, 2001; Al-Farraj, 2008; Chen *et al.*, 2013), ciliates from soil habitats of Saudi Arabia remain poorly known. However, some recent reports have shown that a quite wide diversity of ciliates might exist in dry habitats (Al-Farraj, 2011; Berger *et al.*, 2006; Fan *et al.*, 2014a; Foissner *et al.*, 2008).

In the present paper, the morphological description of two ciliates, isolated from a dry soil of Saudi Arabia, was documented based on observations of specimens *in vivo* and after various staining methods. Molecular characterization was also applied to contribute to species circumscription and to further our understanding of the phylogeny of the Nassophorea and Phyllopharyngea.

**METHODS**

**Sample collection, observation and identification.** The sampling location is within an area of farmland (26° 22′ 01″ N 44° 46′ 03″ E) that is situated about 12 km from Zulfi city, Riyadh, Saudi Arabia. The farmland was natural and not subjected to fertilizer. Some vegetation was observed around the sampling location. A sample of about 0.5 kg of a composite dry soil was collected directly from the upper 5 cm surface and preserved in a plastic jar without further treatment. The sample was processed with the non-flooded Petri dish method (Foissner, 1987) in the laboratory. Living cells were isolated and observed *in vivo* using differential interference contrast microscopy. Silver carbonate (Ma *et al.*, 2003) and protargol (Wilbert, 1975) staining methods were used to reveal the infraciliature. Direct-fluorescence microscopy with fluorescent taxol (Flutax) (He *et al.*, 2006) was used to reveal the silverline system (the cortical microtubules). Drawings were made with the help of a camera lucida. Measurements were made under ×100–1250 magnification. Terminology and classification mainly follow Foissner (1999) and Lynn (2008), respectively.

**DNA extraction and gene sequencing.** Extraction of genomic DNA was carried out following the methods described by Gong *et al.* (2007). To minimize PCR amplification errors, high-fidelity TaKaRa

**Abbreviations:** BI, Bayesian inference; ML, maximum-likelihood; SSU, small subunit.

The GenBank/EMBL/DDBJ accession number for the SSU rRNA gene sequence of *Chilodonella acuta* is KJ452458.
ExTaq polymerase was used to amplify the small-subunit (SSU) rRNA gene using universal oligonucleotide primers (forward 5'-GAAA-CTGGGATGTCCTC-3'; reverse 5'-TGATCCCTCTGCAAGTTCCA-CCTAC-3') designed by Medlin et al. (1988) and Elwood et al. (1985). Cloning and sequencing were performed as reported by Yan et al. (2013).

**Phylogenetic analyses.** Other than the SSU rRNA gene sequence of Chilodonella acuta, sequences used in the present analyses were obtained from the NCBI GenBank database. Sequences were first aligned using the GUIDANCE algorithm (Penn et al. 2010a) with default parameters in the GUIDANCE web server (Penn et al., 2010b). Ambiguous columns in the alignment below the confidence score of 0.7 calculated by GUIDANCE were removed. The final alignment including 1723 sites and 64 taxa was used to reconstruct phylogenetic trees. Loxophyllum jinni, Entodinium caudatum, Polyplostron multi-vesiculatum, Strombidium purpureum and Oxytricha granulifera were chosen as the outgroup taxa. The most appropriate model for phylogenetic analysis was selected by both Modeltest version 3.4 (Posada & Crandall, 1998) and MrModeltest version 2.2 (Nylander, 2004). Maximum-likelihood (ML) analyses were carried out using RAxML-HPC2 version 7.2.8 (Stamatakis et al., 2008) on the CIPRES Science Gateway (Miller et al., 2010) using the GTR + G model as the optional choice. Support came from a majority-rule consensus tree of 1000 bootstrap replicates. Bayesian inference (BI) analysis was performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway using the GTR + I + G model as selected by MrModeltest version 2.2 according to the Akaike information criterion. Markov chain Monte Carlo simulations were run with two sets of four chains for 3 000 000 generations with a sample frequency of 100 generations, and the first 7500 trees were discarded as burn-in. All remaining trees were used to calculate posterior probabilities using a majority rule consensus.

**RESULTS**

**Family Furgasoniidae Corliss, 1979**

**Genus Parafurgasonia Foissner & Adam, 1981**

*Parafurgasonia zhangi* spec. nov. (Figs 1 and 2; Table 1)

**Diagnosis.** Member of *Parafurgasonia* from soil habitat; cells in vivo measuring 48–63 × 19–31 µm, ellipsoidal in outline; contractile vacuole above mid-body with excretory pore at level of anterior end of postoral kineties and near posterior end of paroral membrane; 16 or 17 somatic kineties with about 11 kinetids in dorsal somatic kineties; paroral membrane gently curved and composed of about 15 dikinetids, hypostomial organelle composed of four or five files with about four monokinetids each.

Type locality. Farmland near Zulfi city, Riyadh, Saudi Arabia (26° 22’ 01” N 44° 46’ 03” E).

Eymology. The species name is after Professor Zhang, in dedication to our respected colleague, the deceased eminent protozoologist Professor Zuoren Zhang, East China Normal University, in recognition of his significant contributions to the study of protozoa.

Type slide. The holotype slide with silver carbonate-stained specimens has been deposited in the Laboratory of Protozoology, East China Normal University, China (registry no. FXP2013051601).

**Description.** Size in vivo about 48–63 × 19–31 µm. Body slightly to distinctly (up to 2:1) flattened laterally, ventral view elongate oval, right margin slightly cambered, while left margin straight (Figs 1a and 2a–d). Buccal field subapically located, with buccal cavity depressed (Figs 1a and 2a). Oral basket composed of about eight pharyngeal rods (one cell examined in vivo), extending from buccal opening to right posterior part of cell, about 32 µm long (Figs 1e and 2f). Cells colourless, cytoplasm filled with 1–5 µm diameter fat globules (Figs 1a and 2a–c). Contractile vacuole at anterior side of equator, pump every 11–14 s, about 10 µm across when fully extended, with conspicuous excretory pore invariably located quite near posterior end of paroral membrane (Figs 1e, f and 2b, c, j). Extrusomes in resting state about 5–7 µm long, fusiform and obliquely attached to pellice, 20–30 µm long when fully extruded (Figs 1a, e and 2a, b, e). Macronucleus approximately globular, in vivo about 9–13 µm in diameter, usually in middle third or posterior half of cell; micronucleus, attached to macronucleus tightly (Figs 1b, c and 2i, k). Somatic cilia about 6–8 µm long. No mucocysts observed.

Creeps or swims moderately fast by rotation about main body axis. Phototaxis when observed under microscope (Fig. 2d).

Sixteen or 17 somatic kineties, which are composed of only monokinetids, and forming distinct anterior suture and small unciliated posterior pole area (Figs 1b, c and 2h, i). Three kineties in postoral field shorter: right two bearing about 3–5 kinetids, the left one containing 5–7 basal bodies; the other kineties longer, comprising 10–12 kinetids. Two associated fibres of each kinetid forming tick-like structure in silver staining and Flutax-labelling specimens (Figs 1b, c, f and 2g–j). Extrusomes more clearly visible after staining, a few of them scattered between kineties, while most of them arranged inter-kineties and each one close to nearby kinetid as counterpart (Figs 1f and 2j, k). Such counterparts show heteromorphy in all well-stained specimens (n=14) as illustrated in Figs 1(f) and 2(i): in anterior half, extrusomes located ahead of its counterpart kineties, while, in posterior half, located behind kinetids.

Oral opening elliptical, 2–3 × 1–2 µm in silver carbonate-prepared specimens, pharyngeal rods inconspicuous (Figs 1b, f and 2h, j). Hypostomial organelle close underneath oral opening, quadrate, oriented obliquely to main body axis, usually composed of four or five files each having about four basal bodies (Figs 1b, f and 2g, i). Paroral membrane extending right and above oral opening, gently curved and continuous with first somatic kinety, composed of about 13–17 dikinetids (Figs 1b and 2h, j). Cytopyge extending from mid-body to posterior end of cell (Figs 1b, f and 2h, j).
Silverline system composed of three to five polygenes between each two somatic kineties (Figs 1d and 2g).

**SSU rRNA gene sequence.** The sequence was published previously by Zhang et al. (2014) as *Parafurgasonia* sp. with GenBank accession number KC832955.

**Family Chilodonellidae Deroux, 1970**

**Genus Chilodonella Strand, 1928**

**Chilodonella acuta** Kahl, 1931 (Figs 3 and 4; Table 2)

Since the original description based on a German population comprises only a brief summary of its living characters, a redescription and improved diagnosis are supplied here based on an investigation of specimens from the Saudi Arabia population, both in vivo and following protargol staining.

**Improved diagnosis.** Size in vivo usually about 35–20 μm; having a dorsal hump and a tail-like podite; two contractile vacuoles; seven left and five right kineties, equatorial fragment seldom present; 9–11 nematodesmal rods; dorsal brush containing about 11 basal bodies; terrestrial or freshwater habitat.

**Deposition of voucher specimens.** Two voucher slides with protargol-impregnated specimens have been deposited in the Laboratory of Protozoology, East China Normal University, China (registry no. FXP20130515-01), and in the Natural History Museum, London, UK (registry no. NHMUK 2014.4.14.1).

**Description.** Cells in vivo measuring 33–45×18–26 μm. Body acontractile, with obtuse rostrate protrusion at anterior left, and with obtuse angle at posterior end (Figs 3a and 4a–c). One variant individual observed to have another protrusion at mid-right (Fig. 4e). Dorsoventrally flattened about 1.5–2:1, ventral side flat, anterior end...
slightly turn up to dorsal side (Fig. 3b). Dorsal hump projecting above ventral surface distinctly, gradually thickening from anterior to posterior, outline elliptical to bean-shaped, with a tail-like podite of variable size at the posterior end (Figs 3a, b and 4b, f, g). Cytoplasm rather transparent, containing many variously sized food granules (Figs 3a and 4a–e). Two contractile vacuoles, each about 3 \( \mu \)m across: one located in the anterior 2/5 of the right side, the other located in the posterior 2/5 of the left side (Figs 3a and 4a, b). Usually single, seldom two, ellipsoidal macronucleus, about 12 \( \times \) 9 \( \mu \)m across, central heteromeric, located in posterior half of body (Figs 3e and 4k, m, n). Micronucleus globular, 2–3 \( \mu \)m across, near macronucleus (Figs 3e and 4 m). Somatic cilia 4–6 \( \mu \)m long; cilia of dorsal brush longer, up to 10 \( \mu \)m (Figs 3a and 4b).

Glides moderately quickly on substrates, or on surface of water. Cells can also adhere tightly to a substrate when sucked by a pipette.

Ventral somatic kineties in two fields, five kineties of right field arched, innermost one terminates anteriorly at level of cytostome, others extend to preoral field, outermost one rather loosely ciliated, terminates at left of preoral anterior end, while other four extend further backwards (Figs 3e and 4j). Equatorial fragment present in only two specimens (among 25 individuals examined), containing about nine basal bodies (Figs 3e and 4l). Kineties of left field of differing length; five terminate at preoral row, shorten from right to left, while innermost short two situated in subequatorial area (Figs 3e and 4j). Buccal infraciliature as shown in Figs 3(e) and 4(j). Preoral kinety long, composed of 32–34 basal bodies, transverse to main body axis, extends from front of cytostome to rostrate projecting, straight except curved part around cytostome. Two arched circumoral kineties, located around cytostome and overlap partially, outer circumoral kinety composed of 16–19 basal bodies, inner circumoral kinety containing 12–14 basal bodies. Oral basket opening subapically in body midline,
containing 9–11 nematodesmal rods, directed slightly leftwards and dorsally, usually only anterior part recognizable in living and protargol-impregnated specimens (Fig. 4i–k). Dorsal brush near anterior end of dorsal hump, composed of 7–12 basal bodies (Figs 3f and 4h, k).

**Table 1.** Morphometric characterization of *Parafurgasonia zhangi* spec. nov.

All data are based on silver carbonate-stained specimens. Measurements are given in μm. Means are arithmetic means.

<table>
<thead>
<tr>
<th>Character</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
<th>n</th>
</tr>
</thead>
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<td>18</td>
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<td>9.3</td>
<td>22.1</td>
<td>18</td>
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<td>Macronucleus, diameter</td>
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<td>15</td>
<td>11.5</td>
<td>1.9</td>
<td>16.5</td>
<td>18</td>
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<td>0.5</td>
<td>18.7</td>
<td>13</td>
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<td>0.3</td>
<td>10.8</td>
<td>14</td>
</tr>
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<td>0.2</td>
<td>13.2</td>
<td>14</td>
</tr>
<tr>
<td>Anterior somatic end to paroral membrane, distance</td>
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<td>3.0</td>
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<td>15</td>
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<tr>
<td>Somatic kineties (n)</td>
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<td>17</td>
<td>16.1</td>
<td>0.4</td>
<td>2.2</td>
<td>14</td>
</tr>
<tr>
<td>Basal bodies in the dorsal brush (n)</td>
<td>10</td>
<td>12</td>
<td>11.4</td>
<td>0.7</td>
<td>6.1</td>
<td>10</td>
</tr>
<tr>
<td>Basal bodies in paroral membrane (n)</td>
<td>13</td>
<td>17</td>
<td>15.3</td>
<td>1.2</td>
<td>8.2</td>
<td>22</td>
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</tbody>
</table>

**SSU rRNA gene sequence.** The SSU rRNA gene sequence of *Chilodonella acuta* has been deposited in the GenBank database with the length, DNA G+C content and accession number as follows: 1548 bp, 48.54 mol%, KJ452458. Pairwise comparison reveals that *Chilodonella*

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**Fig. 3.** *Chilodonella acuta* Kahl, 1931 from life (a–d) and after protargol staining (e, f). (a) Ventral view of a typical individual; the arrow marks the podite on the dorsal side. (b) Lateral view, showing the oral basket, macronucleus and the tail-like podite (arrowhead). (c, d) Ventral (c) and dorsal (d) views (from Kahl, 1931). (e) Ventral view of infraciliature. (f) Dorsal view of cell, to show the dorsal brush, dorsal hump (arrowhead) and tail-like podite (arrow). DB, Dorsal brush; EF, equatorial fragment; ICK, inner circumoral kinety; LK, left kineties; OCK, outer circumoral kinety; PK, paroral kinety; RK, right kineties. Bars, 20 μm.
acuta and Chilodonella uncinata differ from each other in 37 nt positions, with a sequence identity of 98%.

Phylogenetic analyses

The topologies generated using two algorithms (BI and ML) were generally concordant; therefore, a single topology is presented with support values from both algorithms indicated on branches (Fig. 5). Phylogenetic trees support the polyphyly of the class Nassophorea, containing three separate clades: Microthoracida, Nassulida/Colpodidiida and Discotrichida. Furgasonia blochmanni occupied the basal position of the clade Nassulida/Colpodidiida in both trees (98% ML, 1.00 BI). The others formed two assemblages: three species of the Nassulidae cluster together with full support, and P. zhangi clusters with two species of the Colpodidiidae with uncertain position. In the ML tree, P. zhangi is basal to the clade of Colpodidium caudatum and the unidentified colpodiid species, while, in the BI tree, P. zhangi clusters with colpodiid species and then forms a sister clade with Colpodidium caudatum. Phylogenetic trees also support the monophyly of the class Phyllopharyngea, which comprises three subclasses, Synhymeniidia, Suctoria and Cyrtophoria, with the latter subclass having orders as illustrated in Fig. 5. Chilodonella acuta clusters with its congener Chilodonella uncinata with full support and is located in the assemblage of the well-supported monophyly of the family Chilodonellidae.
Table 2. Morphometric characterization of Chilodonella acuta
Kahl, 1931

All data are based on protargol-impregnated specimens. Measurements are given in μm. Means are arithmetic means.

<table>
<thead>
<tr>
<th>Character</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)</th>
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<td>Oral basket rods (n)</td>
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</table>

**DISCUSSION**

**Comments on Parafurgasonia zhangi**

To date, there are only three species in the genus Parafurgasonia, and all are from soil habitats and of about equal size. They are Parafurgasonia protectissima (Penard, 1922) Foissner, 1999, Parafurgasonia sorex (Penard, 1922) Foissner & Adam, 1981, and Parafurgasonia terricola Foissner, 1999.

*P. zhangi* spec. nov. can be distinguished from *P. sorex*, the type of the genus, in having fewer somatic kineties (16 or 17 vs 23–26), fewer kinetids in each dorsal kinety (10–12 vs 23–26) and an excretory pore quite near (vs fairly far away from) the buccal field. Moreover, the paroral membrane of *P. sorex* is shaped like an arched bridge and contains dikinetids arranged in parallel, while, in the novel species, the paroral membrane is slightly oblique to the longitudinal axis and contains dikinetids arranged in series. The species was first reported by Kahl (1931) with two or three contractile vacuoles, obliquely arranged dorsal kineties and four ventral kineties in each side of the buccal field. No studies on the emphasis of the infraciliature are available.

**Comments on Chilodonella acuta**

The species was first reported by Kahl (1931) with two or three contractile vacuoles, obliquely arranged dorsal kineties and four ventral kineties in each side of the buccal field. No studies on the emphasis of the infraciliature are available. Our isolation corresponds well with Kahl (1931) in general living morphology, except that the original population bore fewer somatic kineties on the ventral side (8 vs 12). Considering that the data were obtained by living observation of such small organisms, we infer that some shorter kineties might have been missed in his observations.

There is only one congener of the genus with a dorsal podite, *Chilodonella siegelae* (Macus, 1943) Jankowski, 2007. *Chilodonella acuta* can be distinguished from it by having more kineties (five right and seven left vs four right and five left kineties) and a blunt round (vs an obvious sharp one) protrusion at the anterior left (Jankowski, 2007).

**Discussion based on phylogenetic trees**

Previous studies (Fan et al., 2014b; Gao et al., 2012; Gong et al., 2009; Pan et al., 2012; Zhang et al., 2014) have considered the Phyllopharyngea and Chilodonellidae as monophyletic and Nassophorea as polyphyletic, which is strongly supported by our research. Furthermore, *Chilodonella acuta* clusters with its congener *Chilodonella uncinata* with full support values, which, along with their sequence comparisons, indicates the validity of *Chilodonella acuta* as a well-outlined and distinctive member of the genus. Our results reveal that *P. zhangi* is closer to the Colpodidiidae rather than to *F. blochmanni*, consequently indicating that the Furgasoniidae is possibly not monophyletic, but more data are clearly needed to make a final conclusion, since its hypothesized monophyly was not rejected in previous tests (Zhang et al., 2014). The Colpodidiidae was promoted as an order, Colpodidiida, by Foissner et al. (2002), which is mainly characterized by bearing no cyrtos and thus differed from Nassulidae and Furgasoniidae, the two families of the Nassulida. However, the present analyses do not support this assignment, because the unidentified colpodidi species no longer forms a well-separated assemblage with *Colpodidium caudatum*, but clusters with *P. zhangi* in the BI tree. In addition, Omar & Foissner (2012) reported that the Colpodidiida is ontogenetically nearer to the microthoracids than to the nassulids.
because of the mixokinetal origin of the adoral membranelles. We therefore conclude that further taxonomic revision of this group is far from clear and awaits more evidence.

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