Morphology and phylogenetic analysis of two oxytrichid soil ciliates from China, *Oxytricha paragranulifera* n. sp. and *Oxytricha granulifera* Foissner and Adam, 1983 (Protista, Ciliophora, Hypotrichia)

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The morphology and infraciliature of two hypotrichous ciliates, *Oxytricha paragranulifera* n. sp. and *Oxytricha granulifera* Foissner and Adam, 1983, collected respectively from the surface of a sandy soil in the Huguang mangrove forest, Zhanjiang, China, and the surface of soil in a forest beside Ziwu Road, Xian, north-west China, were examined. *O. paragranulifera* n. sp. is characterized by an elongate body with slightly tapered anterior end, two macronuclear nodules and two micronuclei, paroral and endoral in *Stylonychia*-pattern, colourless cortical granules distributed in clusters or irregular short rows, adoral zone occupying 37% of the body length, marginal rows almost confluent posteriorly, six dorsal kineties and three caudal cirri, caudal cirri and dorsal bristles almost indistinguishable when viewed *in vivo*. The well-known *O. granulifera* Foissner and Adam, 1983 was also redescribed and can be separated from the novel species by having cortical granules arranged along dorsal kineties and marginal rows on both sides (vs grouped in clusters as well as in short irregular rows), paroral and endoral in *Oxytricha*-pattern (vs in *Stylonychia*-pattern), macronuclear nodules obviously detached (vs adjacent) and a non-saline terrestrial habitat (vs saline terrestrial). The separation of these two taxa is also firmly supported by the molecular data, which show a significant difference between the two in their SSU rRNA gene sequences (similarity 97.1%). Phylogenetic analyses based on SSU rRNA gene sequence data suggest a close relationship within the Oxytrichidae assemblage between *O. paragranulifera* n. sp. and *O. granulifera*.

INTRODUCTION

There is consensus among most taxonomists (Song, 1990, 2001; Song & Wilbert, 1997a, b, 2002; Berger, 1999; Foissner, 1999; Song & Warren, 1999; Foissner et al., 2002, 2008; Küppers et al., 2011; Chen et al., 2013b; Lv et al., 2013; Shao et al., 2013a, b; Shi et al., 2002; Singh & Kamra, 2013; Singh et al., 2013) that the following characters are important for species separation in oxytrichids: (i) body size, shape and colour; (ii) characteristics of cortical granules; (iii) whether dorsal cilia and caudal cirri are indistinguishable or distinguishable from marginal cirri; (iv) relative length of buccal apparatus and DE value; (v) characteristics of macronuclear nodules and micronuclei; (vi) the pattern of the buccal and somatic ciliature; (vii) morphometric data relating to the ciliature; and (viii) habitat.

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

The GenBank/EMBL/DDBJ accession numbers for the SSU rRNA gene sequences of *Oxytricha granulifera* and *Oxytricha paragranulifera* n. sp. are KJ081199 and KJ081200.
Oxytricha is one of the oldest genera of hypotrichs (Berger, 1999). Its systematics, however, have traditionally been highly confused, not least because a properly defined type species was not fixed until relatively recently (Berger, 1999). The status of Oxytricha, along with three other oxytrichid genera with flexible bodies and 18 frontoventral transverse cirri, was recently reviewed by Shao et al. (2011), who defined it as follows: flexible 18-cirrus oxytrichids with undulating membranes in the Oxytricha pattern and adoral zone in the shape of a question mark; frontoventral cirri in a V-shaped pattern; one left and one right marginal row; five or six dorsal kineties in the Oxytricha-pattern, that is, one or two dorsomarginal kineties and simple kinety three; fragmentation present; caudal cirri on dorsal kineties 1, 2 and 4. The genus Oxytricha currently includes approximately 48 morphospecies, several of which have overlapping species-level characters with respect to their living morphology, infraciliature, morphometric data and habitat. In November 2010 and April 2012, two oxytrichid ciliates were isolated in China from the surface of a sandy soil and the surface of soil in a forest. Subsequent observations demonstrated them to be members of the genus Oxytricha.

In the present paper, their morphology is described and their SSU rRNA gene sequences are analysed.

**METHODS**

**Sampling and cultivation (Fig. 1).** Oxytricha paragranulifera n. sp. was collected from the surface of a sandy soil in the Huguang town mangrove forest, Zhanjiang, China (21°06′ N 110°18′ E), on 25 November 2010, when the soil temperature was 24 °C, salinity 25.5% and pH 7.3. Oxytricha granulifera was collected from the surface of soil in the centre of Zhuque National Forest Park, Xi’an, China (33°55′ N 108°32′ E), on 30 April 2012, when the soil temperature was 28 °C, salinity 3% and pH 8. Ciliates were stimulated to excyst and emerge from the soil samples by employing the non-flooded Petri dish method described by Foissner (1987) and updated by Foissner et al. (2002). Isolated specimens were maintained as non-clonal cultures in Petri dishes at room temperature (20 °C) using boiled freshwater with rice grains to enrich bacterial food organisms.

**Morphology.** Living cells were observed with bright-field and differential interference contrast microscopy (Chen et al., 2013a). The protargol silver-staining method described by Wilbert (1975) was used to reveal the infraciliature (Pan et al., 2013). Measurements of stained specimens were carried out with an ocular micrometer (Paiva...
et al., 2012). Drawings of stained specimens were performed at ×1250 magnification with the aid of a camera lucida (Foissner, 2012).

**Terminology.** General terminology is mainly according to Berger (1999); for explanation of terms specific for hypotrichs (e.g. pseudorow, mixed row, DE value), see Berger & Foissner (1997), Berger (1999, 2006, 2008, 2011), Foissner & Stoeck (2011) and Foissner & Al-Rasheid (2006). For the designation of the frontoventral-transverse cirri, the numbering system by Wallengren (1900) is used [for details see p. 16 of Berger (1999)]. The term ‘18-cirrus hypotrich’ means a hypotrich with 18 frontoventral-transverse cirri [e.g. p. 27 in Berger (2008)].

**DNA extraction, PCR amplification and sequencing.** One or more cells was isolated from the ciliate cultures and then washed three times with sterilized fresh/saline water (0.22 μm filtered). These cells were then transferred to a 1.5 ml microfuge tube with a minimum volume of water. Genomic DNA of O. paragranulifera n. sp. and O. granulifera was extracted using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer’s instructions (Gao et al., 2013; Yi et al., 2012). PCR amplification of the SSU rRNA gene was performed according to Li et al. (2013) and Li et al. (2012), with primers 18s-F (5’-AACCTGGGTATGCTGCAG-3’) and 18s-R (5’-TGATCCCTTGAGTTCACCT-3’) (Medlin et al., 1988).

**Phylogenetic analyses.** Using the online program Muscle 3.7 (Edgar, 2004), the SSU rRNA gene sequences of O. paragranulifera n. sp. and O. granulifera were aligned with 51 hypotrichid sequences obtained from the GenBank database. Three urostylid species were selected as the outgroup taxa. These sequences were edited manually using BioEdit 7.0.0 in order to remove ambiguous gaps (Hall, 1999). The program MrModeltest version 2.0 (Nylander, 2004) selected GTR + I (+ = 0.6678) + G ( = 0.5202) as the best model with the Akaike information criterion, which was then used for Bayesian inference (BI) and maximum-likelihood (ML) analyses. BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), using BioEdit 7.0.0 in order to remove ambiguous gaps (Hall, 1999). The program MrModeltest version 2.0 (Nylander, 2004) selected GTR + I (+ = 0.6678) + G ( = 0.5202) as the best model with the Akaike information criterion, which was then used for Bayesian inference (BI) and maximum-likelihood (ML) analyses. BI analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). ML trees were reconstructed using RaxML-HPC2 (Stamatakis, 2006) at the CIPRES website (http://www.phylo.org/). MEGA 4.0 (Tamura et al., 2007) was used to visualize tree topologies.

**Topology testing.** A constraint tree in which the genus Oxytricha was monophyletic was defined and loaded into PAUP 4.0 (Carnoldi & Martin, 2005), using ML criteria and heuristic search with tree bisection-reconnection and 10 random sequence addition replicates. Site-wise likelihoods were calculated in PAUP 4.0 under the GTR + I + G model with parameters as suggested by MrModeltest for all trees. The constraint tree was then compared to the unconstrained best topology and 100 random ML topologies using the approximately unbiased (AU) test (Shimodaira, 2002) as implemented in the CONSEL software package (Shimodaira & Hasegawa, 2001; Huang et al., 2012, 2014).

**RESULTS**

**Oxytricha paragranulifera n. sp. (Table 1, Figs 2 and 3)**

**Diagnosis.** Body elongate with slightly tapered anterior end, about 80–110 × 35–50 μm. Two macronuclear nodules and two micronuclei. Cortical granules colourless, densely grouped in clusters or irregular short rows. One contractile vacuole located at about mid-body near left margin. Adoral zone occupies 37% of body length in vivo and is composed of about 27 membranelles on average. Paroral and endoral in the Stylonchia-pattern. Invariably 18 frontoventral-transverse cirri. Marginal rows almost confluent posteriorly. Six dorsal kineties and three caudal cirri. Caudal cirri and dorsal bristles almost indistinguishable when viewed in vivo.

**Etyymology.** The species name paragranulifera (pa.ra.gra.nu.li’fe.ra. Gr. pref. para- beside, along; N.L. fem. adj. granulifera little seed bearing) refers to the species Oxytricha granulifera; N.L. fem. adj. paragranulifera similar to Oxytricha granulifera.

**Type locality.** Ciliates were collected in November 2010 from the surface of a sandy soil in Huguang town mangrove forest, Zhanjiang, Guangdong Province, China (21° 07’ N 110° 14’ E), when the soil temperature was 24 °C, pH 7.3 and salinity 25.5‰.

**Type specimen deposit.** A slide (no. PY10112501A) containing the holotype specimen (Fig. 2d, e) and a paratype slide (no. PY10112501B) with protargol-impregnated specimens have been deposited in the Laboratory of Protozoology, Ocean University of China, China.

**Description (Table 1, Figs 2 and 3).** Body about 80–110 × 35–50 μm in vivo, non-contractile and flexible, usually slender oval to elliptical with anterior end usually narrow, posterior broadly rounded; right margin slightly convex, left margin strongly convex (Figs 2a and 3a–c, e). Dorsocaudally flattened (about 3:1), ventral side flat, dorsal side convex in middle portion. Cytoplasm colourless to greyish, containing numerous shining globules and crystals. Cells frequently with many lipid droplets (about 2–4 μm across) and food vacuoles (about 2–5 μm across). Cortical granules colourless, round, about 1 μm across, densely grouped in clusters and irregular short rows on dorsal side, while slightly sparsely arranged in irregular short rows on ventral side (Figs 2b and 3i). Contractile vacuole about 15 μm across, located at the mid-body, near left body margin, contracting at intervals of about 1 min (Figs 2a and 3d). Invariably two macronuclear nodules, usually arranged closely in line at about the mid-body and slightly left of the midline; individual nodules ellipsoidal (length : width ratio about 2 : 1), with small to moderately large nucleoli. Usually one micronucleus attached to each macronuclear nodule (Figs 2a, e and 3a, l).

Locomotion by continuous rapid crawling on bottom of Petri dish and surface of water. When suspended, cells often swim continuously in circles.

Infraciliature as shown in Figs 2(c–e) and 3(m–p). Adoral zone occupies 37% of cell length (Fig. 2d) and is composed of 25–29 membranelles. Distal portion of adoral zone extends slightly posteriorly onto right side of cell, that is, a DE value 0.23 in the holotype specimen [Fig. 2d; for explanation of DE value, see p. 18 of Berger (2006)]. Paroral and endoral optically parallel (Figs 2c and 3m). Frontoventral-transverse ciliature comprising 18 cirri: three slightly enlarged frontal cirri with cilia about 15 μm
Table 1. Characterization of *O. paragranulifera* n. sp. (upper line) and *O. granulifera* Foissner and Adam, 1983 (lower line)

Data are based on protargol-impregnated specimens. — Data unavailable.

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<td>7</td>
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Description of two hypotrichous ciliates from soil

long; single buccal cirrus near anterior end of undulating membranes; frontoventral cirri arranged in a short, mixed, asymmetrical, V-shaped row; cirrus III/2 slightly ahead of the level of cirrus VI/3; cirrus III/2 closer to the remaining frontoventral cirri than to the paroral and endoral; three postoral ventral cirri located intermediately behind the vertex and distinctly separated from the two pretransverse ventral cirri; postoral ventral cirrus IV/2 arranged more...
anteriorly than V/4; five transverse cirri in a hook-shaped row, with cilia about 20 μm long; two pretransverse ventral cirri, cirrus VI/2 located at about the same level as the leftmost transverse cirrus, cirrus V/2 closer to cirrus VI/2 than cirrus V/3; distance between cirrus V/3 and V/4 shorter than that between cirrus V/3 and V/2 (Figs 2c, d and 3g, j, o). The posterior ends of marginal rows are almost confluent; left row with 18–25 cirri, right row with 18–25 cirri; cilia of marginal cirri about 10 μm long (Figs 2d, e and 3f).

Six dorsal kineties; leftmost two (dorsal kineties 1 and 2) bipolar, each comprising about 20 pairs of basal bodies; third and fifth dorsal kineties start at about the anterior end of cell and terminate at 4/5 and 2/5 of cell length, respectively; fourth dorsal kinety commences near the equatorial level of the cell and stretches to the posterior end; rightmost dorsal kinety (dorsal kinety 6) composed of two or three dikinetids (Figs 2e and 3p). Dorsal cilia about 3 μm long in vivo. Three caudal cirri, thin, located at posterior body margin, narrowly separated, one each on dorsal kineties 1, 2 and 4 (Figs 2e and 3h); cilia of caudal cirri about 12 μm long in vivo and thus almost indistinguishable from marginal cirri (Figs 2a and 3a, j).

Fig. 2. Morphology of *O. paragranulifera* n. sp. (a) Ventral view of a representative individual. (b) Distribution of cortical granules on dorsal side. (c) Ventral view of anterior portion, to show the paroral and endoral, and frontoventral cirri. (d, e) Ventral (d) and dorsal (e) view of the holotype, to show the general infraciliature. AZM, Adoral zone of membranelles; BC, buccal cirri; CC, caudal cirri; E, endoral; FC, frontal cirri; FVC, frontoventral cirri; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; P, paroral; PTVC, pretransverse ventral cirri; PVC, postoral ventral cirri; RMR, right marginal row; TC, transverse cirri; 1–6, dorsal kineties. Bars, 35 μm (a, d, e), 15 μm (b).

**Oxytricha granulifera** Foissner and Adam, 1983 (Table 1, Fig. 4)

**Description.** Body about 90–130 × 30–50 μm in vivo, length:width ratio approximately 3:1 in vivo, 1.8:1 on average in protargol preparations. Body flexible but not contractile, usually slender oval to elliptical with anterior end usually narrow, posterior broadly rounded (Fig. 4a, b). Dorsoventrally flattened (about 3:1), ventral side flat, dorsal side convex in middle portion; right cell margin slightly convex to slightly concave, left margin strongly convex, usually widest in front of mid-body. Cytoplasm colourless to greyish, containing numerous shining globules and crystals (Fig. 4c). Cells with many lipid droplets (about 4–5 μm across) and food vacuoles (about 5–10 μm across). Cortical granules colourless, round, about 1 μm across, arranged along dorsal kineties and marginal rows on both sides (Fig. 4d). Contractile vacuole located at mid-body, near left body margin, contracting at intervals of about 10 s (Fig. 4b, c). Invariably two macronuclear nodules, usually arranged at anterior and posterior 1/3, as well as slightly left of the midline (Fig. 4f). Two micronuclei, one each beside the two macronuclear nodules (Fig. 4f). Locomotion by slow to moderately fast crawling on substrate, but usually motionless.

Infraciliature as shown in Fig. 4(e–h). Adoral zone occupies 39% of cell length on average in protargol preparations, composed of 28–38 adoral membranelles. Distal portion of adoral zone extends slightly posteriorly on to the right side of cell, that is, a DE value about 0.30 [Fig. 4e; for explanation of the DE value, see p. 18 of Berger (2006)]. Paroral and endoral intersect each other optically behind the buccal cirrus (Fig. 4e). Three slightly enlarged frontal
cirri with cilia about 15 μm long; single buccal cirrus near anterior end of paroral and endoral; frontoventral cirri arranged in a short, mixed, asymmetrical, V-shaped row; cirrus III/2 slightly ahead of level of cirrus VI/3; cirrus III/2 closer to remaining frontoventral cirri than to paroral and endoral (Fig. 4e). Three postoral ventral cirri located intermediate behind vertex and distinctly separated from the two pretransverse ventral cirri; postoral ventral cirrus IV/2 arranged more anteriorly than V/4. Five transverse cirri in a hook-shaped row, with cilia about 20 μm long; two pretransverse ventral cirri, cirrus VI/2 located near rightmost transverse cirrus, cirrus V/2 closer to cirrus VI/2 than cirrus V/3; distance between cirri V/3 and V/4 less than that between cirri V/3 and V/2 (Fig. 4g). Posterior ends of marginal rows are almost confluent; left row with 23–31 cirri, right row with 26–32 cirri; cilia of marginal cirri about 10 μm long (Fig. 4g).

Six dorsal kineties; leftmost two (dorsal kineties 1 and 2) bipolar, each comprising about 20 pairs of basal bodies; third and fifth dorsal kineties start at about the anterior end of cell and terminate at 4/5 and 2/5 of cell length, respectively; fourth dorsal kinety commences near the equatorial level of cell and stretches to the posterior end; rightmost dorsal kinety (dorsal kinety 6) composed of two
or three dikinetids (Fig. 4h). Dorsal cilia about 3 µm long in vivo. Three caudal cirri, thin, located at the posterior body margin, slightly separated, one each on dorsal kineties 1, 2 and 4.

**Phylogenetic analyses**

The SSU rRNA gene sequence of our *O. granulifera* isolate is 1620 bp long and has a DNA G+C content of 45.68%, and that reported previously for *O. granulifera* (GenBank accession no. AF164122) is 1774 bp long and has a DNA G+C content of 45.55%. The similarity between them is 99.7%, indicating that they are conspecific. The topologies of the ML and BI trees were similar; therefore, only the ML tree is shown (Fig. 5). In both analyses, *O. paragranulifera* n. sp. and *O. granulifera* fall into the Oxytrichinae assemblage. However, *Oxytricha* is not a monophyletic group: 14 species fall into nine clades. Furthermore, the monophyly of the genus *Oxytricha* is rejected by the AU test (P=0.003). *O. granulifera* clustered with *Oxytricha* sp. 1 MD-2012 with high support (ML/BI, 83/0.93), and *O. paragranulifera* n. sp. was sister to *Onychodromopsis flexilis* with low and high support (ML/BI, 77/0.96).

**DISCUSSION**

*Oxytricha paragranulifera* n. sp. (Table 1, Figs 2 and 3)

**Comparison with congeners.** In terms of its slender oval to elliptical body shape and slightly tapered anterior end, two macronuclear nodules and indistinguishable dorsal cilia and caudal cirri, 23 congeners resemble *O. paragranulifera* n. sp. We therefore compared *O. paragranulifera* n. sp. with each of these.

*O. granulifera* Foissner and Adam, 1983 has a very similar body size and body shape to *O. paragranulifera* n. sp., but can be distinguished from the latter by having cortical granules arranged along the dorsal kineties and marginal rows on both sides (vs grouped in clusters as well as in short irregular rows). Furthermore, the paroral and endoral intersect each other optically (vs in parallel...
**Oxytricha fallax** Stein, 1859 can be separated from presence of symbiotic algae (vs absent) (Berger, 1999).

**Oxytricha chlorelligera** Kahl, 1932 is similar to **Oxytricha granulifera** n. sp. by having a broadly rounded (vs adjacent) macronuclear nodules, the **Oxytricha**-pattern (vs **Stylonychia**-pattern) of its paroral and endoral and the absence (vs presence) of cortical granules (Berger, 1999).

Compared with the well-known **Oxytricha granulosa** Schmitz, 1986, **O. paragranulifera** n. sp. has a smaller body (80–110 vs 155–280 μm), colourless cortical granules which are grouped in clusters as well as in short irregular rows (vs yellow–green in colour and arranged in 14–18 longitudinal rows), fewer membranelles (25–29 vs 44–50), more dorsal kinetics (six vs four or five), paroral and endoral in the Stylonychia-pattern (vs in the **Oxytricha**-pattern) and two (vs one) micronuclei (Berger, 1999).

**Oxytricha hymenostoma** Stokes, 1887 differs from **O. paragranulifera** n. sp. by having a broadly rounded (vs

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**Fig. 5.** ML phylogenetic tree inferred from the SSU rRNA gene sequences of 53 hypotrichs showing the positions of **O. paragranulifera** n. sp. and **O. granulifera**. The new sequences from the present study are indicated in bold and by arrows. Numbers near nodes are non-parametric bootstrap values for ML analysis and posterior probability values for BI. Asterisks indicate disagreements in the topology of the BI tree. All branches are drawn to scale. Bar, 0.01 expected substitutions per site.
slightly tapered) anterior end, slightly separated (vs well separated) postoral and pretransverse cirri, dorsal kinety 4 starting almost at the anterior end (vs commencing at the mid-body) and obviously detached (vs adjacent) macronuclear nodules (Berger, 1999).

Compared with O. paragranulifera n. sp., Oxytricha longicirrata Kahl, 1932 has its paroral and endoral in the Oxytricha-pattern (vs in the Stylonychia-pattern), a lack of cortical granules (vs their presence), obviously detached (vs adjacent) macronuclear nodules and distinctly separated (vs confluent) marginal rows posteriorly (Berger, 1999).

Considering the somatic ciliature, body shape and the appearance of the undulating membranes, O. paragranulifera n. sp. is similar to Oxytricha aeruginosa Wrzesniowskiego, 1866, although the former can be recognized by its colourless (vs russet and black) cortical granules, smaller body size (80–110 vs 120–165 μm) and adjacent (vs obviously detached) macronuclear nodules (Berger, 1999).

O. paragranulifera n. sp. differs from Oxytricha longissima Dragesco and Njine, 1971 in having a smaller body (80–110 vs 250 μm), transverse cirri slightly (vs distinctly) displaced anteriorly, fewer right and left marginal cirri (18–25 and 18–25 vs 40–54 and 37–42, respectively) and adjacent (vs obviously detached) macronuclear nodules (Berger, 1999).

In terms of its somatic ciliature and body shape, O. paragranulifera n. sp. is most similar to Oxytricha multiseta Dragesco, 1966. The former, however, can be recognized by the number of transverse cirri (consistently five vs six or seven) and adjacent (vs obviously detached) macronuclear nodules (Berger, 1999).

Oxytricha quadricirrata Blatterer and Foissner, 1988 can be separated from O. paragranulifera n. sp. by its distinctly separated (vs confluent) marginal rows posteriorly, its cortical granules being arranged along the dorsal kinetics and marginal rows (vs in clusters and in short irregular rows) and fewer membranelles (19–21 vs 25–29), transverse cirri (four vs five), right marginal cirri (14–17 vs 18–25) and left marginal cirri (13–18 vs 18–25) (Berger, 1999).

Oxytricha variabilis Grolie`re, 1975 differs from O. paragranulifera n. sp. in the number of micronuclei (four vs two), obviously detached (vs adjacent) macronuclear nodules and in having five (vs three) postoral ventral cirri and five (vs six) dorsal kinetics (Berger, 1999).

Oxytricha auripunctata Blatterer and Foissner, 1988 can be separated from O. paragranulifera n. sp. in having one to five (vs invariably two) micronuclei; four to five (vs three) caudal cirri, distinctly separated (vs confluent) marginal rows posteriorly; orange-yellow (vs colourless) cortical granules; paroral and endoral arranged in the Oxytricha-pattern (vs in the Stylonychia-pattern) and obviously detached (vs adjacent) macronuclear nodules (Berger, 1999).

Oxytricha arabica Foissner et al., 2008 can be separated from O. paragranulifera n. sp. in having five (vs six) dorsal kinetics and an absence of cortical granules (vs their presence) (Foissner et al., 2008).

Oxytricha lanceolata Shibuya, 1930 has a similar body size and shape to O. paragranulifera n. sp. but can be separated from the latter by the absence of cortical granules (vs their presence), four (vs six) dorsal kinetics and obviously detached (vs adjacent) macronuclear nodules (Berger, 1999).

Oxytricha matriensis Ramirez-Montesinos and Perez-Silva, 1966 differs from O. paragranulifera n. sp. in the number of right marginal cirri [about 17 (data from drawing) vs 18–25] and left marginal cirri [about 15 (data from drawing) vs 18–25], the probable absence of caudal cirri (vs their presence) and obviously detached (vs adjacent) macronuclear nodules (Berger, 1999).

Oxytricha proximata Shibuya, 1930 differs from O. paragranulifera n. sp. in its obviously detached (vs adjacent) macronuclear nodules, four (vs five) transverse cirri and in the rectangular arrangement (vs a V-shaped arrangement) of its frontoventral cirri (Berger, 1999).

Oxytricha longigranulosa Berger and Foissner, 1989 resembles O. paragranulifera n. sp. in its dorsal ciliature; however, the former can be distinguished by the arrangement of cortical granules (in short lines vs in clusters and short irregular lines), body shape (elliptical vs mostly slender oval) and in the distinctly separated (vs confluent) marginal rows posteriorly (Berger, 1999).

Compared to O. paragranulifera n. sp., Oxytricha pseudofusiformis Dragesco and Dragesco-Kernéis, 1986 has a smaller body size (35–46 μm after protargol impregnation vs 80–110 μm in vivo), fewer cirri in the frontal area (five to seven vs eight), membranelles (12–18 vs 25–29), right marginal (7–11 vs 18–25) and left marginal cirri (seven to nine vs 18–25) as well as only one (vs two) micronucleus and transverse cirri that are distinctly (vs slightly) displaced anteriorly (Berger, 1999).

Oxytricha tenella Song and Wilbert, 1989 can be separated from O. paragranulifera n. sp. in having a smaller body (50–70 vs 80–110 μm); cortical granules arranged irregularly (vs in clusters and short irregular lines); paroral and endoral arranged in the Oxytricha-pattern (vs in the Stylonychia-pattern), as well as postoral and pretransverse cirri slightly separated (vs well separated) (Berger, 1999).

Oxytricha rubripuncta Berger and Foissner, 1987 differs from O. paragranulifera n. sp. in having two to four (vs two) micronuclei, cirrus III/2 located between cirri IV/3 and VI/3 (vs between cirri VI/3 and VI/4), paroral and endoral arranged in the Oxytricha-pattern (vs in the Stylonychia-pattern), reddish (vs colourless) cortical granules, obviously detached (vs adjacent) macronuclear nodules and dorsal kinety 5 bipolar (vs terminating at the mid-body) (Berger, 1999).

Oxytricha durhamiensis Berger, 1999 can be separated from O. paragranulifera n. sp. by having cortical granules in...
linear groups of two to five parallel to the dorsal kineties (vs in clusters and short irregular lines) and cirrus III/2 located between cirri IV/3 and VI/3 (vs between cirri VI/3 and VI/4) (Berger, 1999).

*Oxytricha oxyserina* Berger, 1999 can be distinguished from *O. paragranulifera* n. sp. in having three bipolar dorsal kineties (vs six dorsal kineties, four of which are not bipolar), distinctly separated (vs confluent) marginal rows posteriorly, and an adoral zone that is rather long relative to the body length (51 vs 37% after protergol impregnation) (Berger, 1999).

Compared to *O. paragranulifera* n. sp., *Oxytricha alfredi* Berger, 1999 has one (vs two) micronucleus and a rather long posterior-most marginal (or caudal?) cirrus (vs indistinguishable) (Berger, 1999).

Compared with *O. paragranulifera* n. sp., *Oxytricha acido-tolerans* Weisse et al., 2013 has its paroral and endoral in the *Oxytricha*-pattern (vs in the *Stylonychia*-pattern), a lack of cortical granules (vs their presence), distinctly separated (vs confluent) marginal rows posteriorly and dorsal ciliature (dorsal kineties 1 and 2 shortened anteriorly and each comprising about 10 pairs of basal bodies (vs bipolar and each comprising about 20 pairs of basal bodies) and third dorsal kinety terminating at midline (vs 4/5) of cell length (Weisse et al., 2013).

**Oxytricha granulifera** Foissner and Adam, 1983 (Table 1, Fig. 4)

**Comparison with Austrian population.** According to the original report (Foissner & Adam, 1983), our population closely resembles the Austrian population of *O. granulifera*, the main difference being the number of dorsal kineties (five in the Australian population vs six in the present population). We believe that this difference is intraspecific and therefore not significant for species-level separation, since all other key characters, i.e. body size, shape, micronuclear and micronuclear features, arrangement of cirri, morphometric data (Table 1; Foissner & Adam 1983) and biotope, are consistent with the original description. The identification of the Xi’an population is therefore not in doubt. Further, the similarity between the SSU rRNA gene sequences of our *O. granulifera* and the Australian isolate of *O. granulifera* is 99.7%, indicating that they are conspecific.

**Phylogenetic analyses**

In the present SSU rRNA gene trees, *O. granulifera* was sister to *Oxytricha* sp. 1 MD-2012, and *O. paragranulifera* n. sp. clustered with *Onychodromopsis flexilis*, *Oxytricha ottowi* 1 MD-2012, *O. ottowi* 2 MD-2012, *O. longigranulosa* and *Urosomoida lanceolata*. Each of these species has an 18 frontal-ventral-transverse cirral pattern, dorsomarginal kineties (unknown for *Onychodromopsis*) and a flexible body, and has been assigned to the Oxytrichinae based on both morphology and molecular information (Berger, 1999). The 14 oxytrichid species are distributed among nine clades, and the AU test also rejects the monophyly of the genus *Oxytricha*. This is consistent with the results of many previous studies (for example, Hu et al., 2011; Shao et al., 2011). The non-monophyly of *Oxytricha* indicates that the 18-cirrus pattern might not be an apomorphy of this genus. Additionally, the sequence of *O. paragranulifera* n. sp. was not sister to the two *O. granulifera* sequences, which demonstrated that *O. granulifera* and *O. paragranulifera* n. sp. are different species.

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**REFERENCES**


Foissner, W. (2012). *Schmidingerorthrix extraordinaria* nov. gen., nov. spec., a secondarily oligomerized hypotrich (Ciliophora, Hypotricha,


