Cell-division pattern and phylogenetic analyses of a new ciliate genus *Parasincirra* n. g. (Protista, Ciliophora, Hypotrichia), with report of a new soil species, *P. sinica* n. sp. found from northwest China

**CURRENT STATUS:** UNDER REVIEW

**DOI:**
10.21203/rs.2.19980/v2

**SUBJECT AREAS**
Evolutionary Biology

**KEYWORDS**
*New species, Morphology, Morphogenesis, Parasincirra, SSU rDNA phylogeny*
Abstract

**Background:** Ciliated protists, a huge assemblage of unicellular eukaryotes, are extremely diverse and play important roles in ecosystem in almost all kinds of habitats. Even though there is a growing recognition that those organisms associate with many ecological or environmental processes, their biodiversity, due to many reasons, is poorly understood and many biotopes (e.g. the soil in desert area in Asia) remain largely unknown or unconsidered. Here we document an undescribed form found in sludge soil in a halt-desert inland in China and the taxonomic/ morphogenetic surveys indicate that it represents a new genus and new species, *Parasincirra sinica* n. g., n. sp. which is supported also by molecular data.

**Results:** This new, monotypic genus *Parasincirra* n. g. is defined by having three frontal cirri, an amphisiellid median cirral row about as long as the adoral zone, one short frontoventral cirral row, cirrus III/2 and transverse cirri present, buccal and caudal cirri absent, one right and one left marginal row and three dorsal kineties. The main morphogenetic features of the new taxon are: (1) frontoventral-transverse cirral anlagen II to VI are formed in primary mode; (2) the amphisiellid median cirral row is formed by anlagen V and VI, while the frontoventral row is generated from anlage IV; (3) cirral streaks IV to VI generate one transverse cirrus each; (4) frontoventral-transverse cirral anlage II generates one or two cirri, while the posterior one will be absorbed in late stages, that is, no buccal cirrus is formed; (5) the posterior part of the parental adoral zone of membranelles is renewed; (6) dorsal morphogenesis follows a typical *Gonostomum*-pattern; and (7) the macronuclear nodules fuse to form a single mass. Based on the SSU rDNA information, analyses of the phylogenetic relationship inferred from Bayesian inference and maximum likelihood analyses were unable to outline the exact position of this new form among some other species of related genera which are generally assigned in the family Amphisiellidae Jankowski, 1979. The morphological/ morphogenetical differences between the new genus/species and *Uroleptoides* Wenzel, 1953/ *Parabistichella* Jiang et al., 2013, as well as other amphisiellids, clearly support the validity of the establishment of this new genus *Parasincirra*. 

Background
With characteristic of highly differentiated organelles and unique cytological/genetic feature, i.e. dimorphic nuclei (macro- and micronucleus), specialized sexual process (conjugation), ciliates become prevalent model in ecological, evolutionary and genetic studies [1–6]. However, the above works are seriously limited by insufficient understanding of the global diversity of ciliates. As the most diverse linages of ciliates, the subclass Hypotrichia Stein, 1859 has been the subject of morphological and phylogenetic research [7–16]. And even so, overall previous findings failed to recover the monophyly of Hypotrichia and the classification, validity, delimitation and phylogenetic relationships among and within these groups remains problematic [17, 18].

Among these, the order Stichotrichida is one of the most confused and diverse ciliate groups both in taxonomy and phylogeny [19]. One of its largest members, Amphisiellidae, is characterised by possessing an amphisiellid median cirral row derived from two or three rather than one frontoventral-transverse cirral anlagen and represents a species-rich family. Most amphisiellid species are known to occur in terrestrial habitats, although some in marine [8, 20-21]. In the present study, we present a new species in this family collected from sludge soil in a flood drain in Lanzhou, China. Observations of its morphology and morphogenesis, both in vivo and after protargol staining, demonstrate that it represents a novel genus, Parasincirra n. g. of the family Amphisiellidae. The SSU rDNA of the new isolate was sequenced and the based phylogenetic studies were also investigated. These studies that focused on the new species living in the sludge niche provide additional evidence that amphisiellid species show versatility on their morphological and genetic features during its adaptation to sludge environment.

Results

Establishment of the new genus Parasincirra n. g.

Diagnosis. Amphisiellidae with elongate body. Three frontal cirri. Amphisiellid median cirral row about as long as adoral zone. One short frontoventral cirral row. Cirrus III/2 and transverse cirri present. One right and one left marginal row. Three dorsal kineties. Caudal cirri and buccal cirrus lacking.
**Etymology.** Composite of the Greek prefix *para-* (close to; related; deviating) and the posterior part (*-sincirra*) of the genus name *Hemisincirra* Hemberger, 1985. This indicates that *Parasincirra* has a cirral pattern similar to the *Hemisincirra*. This similarity should indicate a relationship to *Hemisincirra*, but whereas the new genus lacks a buccal cirrus *Hemisincirra* possesses one. Like *Hemisincirra*, *Parasincirra* has a feminine gender.

**Type species.** *Parasincirra sinica* n. sp.

**Type locality:** Sludge soil (36º3’N; 103º49’E) in Lanzhou, China.

**Remarks.** We do not assign *Hemisincirra interrupta* (Foissner, 1982) Foissner in Berger, 2001 and *H. vermicularis* Hemberger, 1985 to our new genus *Parasincirra* although both species also lack a buccal cirrus. The main reasons are that either the ontogenetic or the molecular information remains unknown.

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**Description of *Parasincirra sinica* n. sp.**

**Diagnosis.** Size in vivo 90–160 × 20–40 µm. Body slender, fusiform to worm-shaped, with pointed posterior end. Cortical granules about 0.5 µm across, colourless and grouped around dorsal ciliary organelles. Contractile vacuole located slightly ahead of mid-body. Two to six (mostly four) macronuclear nodules. Adoral zone composed of 14–19 membranelles. Amphisiellid median cirral row terminating behind cytostome level, composed of invariably four cirri. Three frontal and one parabuccal cirri; frontoventral row consistently with two cirri; two to four transverse cirri. One left and one right marginal row, composed of 34–52 and 34–53 cirri respectively. Three bipolar dorsal kineties. Soil habitat.
**Type locality.** Flood drain, Lanzhou (36°03'N; 103°49'E), China.

**Etymology.** The species-group name *sinica* means the species was first discovered in China.

**Morphological description.** The body colourless to greyish, non-contractile but highly flexible, and thus cell outline variable, i.e., sigmoidal or curved (Fig. 2d). Generally slender, almost fusiform to worm-shaped. Anterior end narrowly rounded and posterior end more or less tapered to form a pointed tail that is more flexible and contractile than the rest of the cell (Fig. 2a, d, g, h), and unrecognisable in protargol preparations (Fig. 2e, f, i, j). Dorsoventrally flattened up to 2:1. Cells were 90–160 × 20–40 μm in living cells (n=6), with an average of 120 × 30 μm in prepared cells, with a ratio of length to width of about 3.5:1–7.5:1 in vivo and on average 4:1 in protargol preparations. Cortical granules colourless, round, about 0.5 μm in diameter, around dorsal ciliary organelles, visible also in protargol preparations (Fig. 2c, j). One contractile vacuole measuring about 13 μm in diameter in diastole, positioned near left margin, contracting at intervals of 10 s (Fig. 2g, h). Cytoplasm often packed with numerous small lipid droplets. Usually four (2-6) macronuclear nodules arranged along mid-line, or slightly left of it, behind buccal vertex and one to three, on average two, micronuclei attached, or near to, macronuclear nodules. Macronuclear nodules ellipsoid to ellipsoid, about 9–19 × 4-10 μm in size (after protargol staining) (Fig. 2j). Micronuclei about 2.9 × 2.4 μm in size (after protargol staining). Locomotion mainly by slowly crawling on substrate and debris, sometimes jerking back and forth. When suspended, cells often swim continuously in circles. Infraciliature as shown in Fig. 2 (b, e, f, i-l). Adoral zone of membranelles (AZM) shaped as in other amphisiellid species, terminates at 11–20% (average about 16%) of body length, comprising 14–19 membranelles. Cilia of distal membranelles about 13 μm long. Buccal cavity small, endoral and paroral bending strongly and optically intersecting with each other at their lower middle regions (Fig. 2b, e, i, k).

Most somatic cirri relatively fine with cilia about 12–16 μm long. Consistently three relatively stout frontal cirri in an almost transverse pseudo row immediately behind several distal adoral
membranelles, cilia about 15 μm long. Amphisiellid median cirral row (ACR) short and consisting of four cirri; commences at about the level of the rightmost frontal cirrus (about 6% of body length), or slightly lower, terminates at about level of buccal vertex (about 21% of body length). Parabuccal cirrus (cirrus III/2) located at the level of the middle region of the paroral and endoral. Frontoventral row in region between parabuccal cirrus and ACR, consistently composed of two cirri; commences at about the level of the second cirrus in ACR (about 8% of body length) and terminates ahead of the third cirrus in ACR (about 10% of body length). Three, rarely two or four, slightly subterminal transverse cirri, cilia of which about 16 μm long. Consistently one left and one right marginal row, each with 34–52 and 34–53 cirri, respectively. Right marginal cirral row begins dorsolaterally at anterior end of cell while left marginal cirral row begins at level of posterior end of adoral zone, both of them terminate caudally, but are not confluent (Fig. 2b, e, i, k, l).

Three dorsal kineties arranged in Gonostomum-pattern, with cilia about 3 μm in length, composed of about 13, 15 and 15 dikinetids, respectively, and arrange in a gradient; that is, kinety 3 commences apically, kinety 2 starts slightly behind kinety 3, while kinety 1 starts slightly behind kinety 2. All of them terminate at the posterior body end (Fig. 2f, j).

**Morphogenesis during binary fission**

**Stomatogenesis.** Cortical morphogenesis in Parasincirra sinica n. sp. mainly occurs in two zones: an anterior field for the proter and a posterior field for the opisthe.

In the opisthe, the first evidence of stomatogenesis during cell division is the appearance of groups of basal bodies on the cell surface, that is, the opisthe’s oral primordium, which is located in the end of ACR, indicating that parental basal bodies are incorporated in the primordium (Fig. 3a). These groups subsequently merge by further proliferation of basal bodies forming a single anarchic field and then the new adoral membranelles organise posteriad (Figs. 3b, 5e). The anlage for the undulating membranes (anlage I) is formed to the right of the oral primordium (Figs. 3c, 5e). Later, the left frontal cirrus develops from the anterior end of the UM-anlage (Figs. 4a, 5n). During the later stages, the differentiation of membranelles is completed, forming the new oral structure for the opisthe.
Subsequently, UM-anlage gives rise to the leftmost frontal cirrus, new endoral and paroral for the opisthe (Figs. 4a, b, 5n).

In the proter, several of the proximal membranelles dedifferentiate into sparsely distrusted basal bodies, then the basal bodies differentiate into membranelles (Fig. 3c-e). The parental undulating membranes dedifferentiate into UM-anlage, then the basic development of the UM-anlage follows a similar pattern to that in the opisthe (Figs. 3b-e, g, 4a, 5d, e, m).

**Development of the frontoventral-transverse cirri.** The development of the somatic ciliature begins with the formation of the frontoventral-transverse cirral anlagen (FVT-anlagen). At the beginning, FVT-anlagen appear as a small group of basal bodies (Fig. 3a). Apparently, the parental frontoventral cirri are disaggregated and joined in the formation of the FVT-anlagen. Later, five FVT-anlagen are formed to the right of the UM-anlage in the proter as primary primordia (Figs. 3b, 5d). Then, the FVT-anlagen fragment in the middle to form two sets of anlagen, one set for the proter and the other for the opisthe (Figs. 3d, e, 5h, i).

Subsequently, cirri segregate from anterior to posterior in the following manner: anlage I develops the frontal cirrus I/1 (leftmost frontal cirrus); anlage II produces the middle frontal cirrus; anlage III generates a parabuccal cirrus and the rightmost frontal cirrus; anlage IV contributes two cirri forming a short frontoventral cirral row; anlage V produces the posterior two cirri in ACR, and anlage VI forms the anterior two cirri in ACR; while anlagen IV to VI produce one transverse cirrus each (Figs. 4a, b, 5m, p). Finally, the new cirri move towards their final positions.

**Development of marginal rows and dorsal kineties.** Within every parental marginal row a few cirri near the anterior end, and a few others below the mid-body, differentiate to form two separate anlagen

The dorsal kineties develop by intrakinetal basal body proliferation, i.e. two anlagen develop in each parental row. Subsequently, the new marginal cirri/kineties develop and replace the old ones (Figs. 3d-h, 4a-c, 5g, h, j, m).
**Division of nuclear apparatus.** The nuclear apparatus divides in the usual way for hypotrichs hence no need to describe in detail (Figs. 3f, h, 4c, 5k).

**SSU rRNA gene sequence and phylogenetic analyses**

The sequence of the 18S rRNA gene of *Parasincirra sinica* n. sp. (GenBank accession number: MN472864) is 1731 bp long and has a G + C content of 45.70%. Phylogenetic trees inferred from the SSU rDNA sequences using two different methods (ML and BI) show similar topologies. Therefore, only the ML topology is shown, with nodal support from both methods (Fig. 7).

Molecular phylogenetic analyses result in a clade containing four polytomies represented by *Parasincirra sinica* n. sp., two *Uroleptoides* species and *Parabistichella variabilis* Jiang et al., 2013 with high support (83 % ML, 1.00 BI, Fig. 7). They also confirm the polyphyly of other amphisiellids including species belonging to the type genus *Amphisiella* Gourret & Roeser, 1888.

**Discussion**

**Comparison with similar genera**

Amphisiellidae were divided into three groups by Berger (2008) [20]. Group I comprises marine taxa (*Amphisiella, Caudiamphisiella* Berger, 2008, *Maregastrostyla* Berger, 2008 and *Spiroamphisiella* Li et al., 2007). All of these species possess a buccal cirrus and a very prominent ACR which commencing at about the level of the distal end of the adoral zone of membranelles and terminating beyond the midbody. Hence the new genus, *Parasincirra* n. g., can be distinguished from others in group I.

The group II genera (*Lamtostyla* Buitkamp, 1977 and *Uroleptoides*) possess a buccal cirrus whereas *Parasincirra* n. g. lacks a buccal cirrus. *Parasincirra* n. g. can therefore be easily distinguished by these morphological characteristics.

Group III comprises two genera, i.e. *Lamtostylides* Berger, 2008 and *Paramphisiella* Foissner, 1988. These species possess a buccal cirrus and have only one cirrus (cirrus III/2) left of the ACR. In *Parasincirra* n. g., however, there is no buccal cirrus and one frontoventral cirri left of the ACR. So, the new genus also differs from species of this group.
Up to now, six genera, that is, *Afroamphisiella* Foissner et al., 2002, *Cossothigma* Jankowski, 1978, *Hemisincirra*, *Mucotrichidium* Foissner et al., 1990, *Terricirra* Berger & Foissner, 1989 and *Tetrastyla* Schewiakoff, 1892, are *incertae sedis* in Amphisiellidae. With reference to the general infraciliature, *Hemisincirra* appears to be a close form to *Parasincirra* n. g., however, the type species of *Hemisincirra* has a buccal cirrus (vs. absent in *Parasincirra* n. g.). *Afroamphisiella* can be distinguished from *Parasincirra* n. g. since it possesses a buccal cirrus (vs. absent) and lacks transverse cirri (vs. present). *Cossothigma* can be separated from the new genus through its trachelostylic body shape and oral apparatus (vs. elliptical to elongate elliptical in shape and oral apparatus in *Oxytricha*-pattern), and the probable presence of caudal cirri (vs. absent in the new genus). *Mucotrichidium* differs from the new genus in possessing a buccal cirrus, postperistomial cirrus and caudal cirri (vs. absent in *Parasincirra* n. g.). *Terricirra* and *Tetrastyla* can also be separated from *Parasincirra* n. g.; *Terricirra* possesses a buccal cirrus (vs. absent in *Parasincirra* n. g.).

**Comparison of *Parasincirra sinica* n. sp. with similar species**

Species assigned to *Hemisincirra* have an infraciliature which is very similar to that of our new species; namely, three frontal cirri, a short amphisiellid median cirral row, fewer transverse cirri and a lack of caudal cirri.

Considering its somatic ciliature, our new species resembles *Hemisincirra interrupta* and *H. vermicularis* most in that these species also lack buccal cirrus. Discrepancies between *Parasincirra sinica* n. sp. and *H. interrupta*, however, are that the latter possesses fewer dorsal kineties (one vs. three), and more macronuclear nodules (about 30 vs. two to six) as well as more cirri in amphisiellid median cirral row (six to eight vs. invariably four). *Hemisincirra vermicularis* differs from *Parasincirra sinica* n. sp. in having more macronuclear nodules (about ten vs. two to six) and contractile vacuoles (four vs. one), and fewer dorsal kineties (one vs. three) [20].

In terms of the somatic ciliature, *Lamtostyla decorata* Foissner et al., 2002, *L. perisincirra* (Hemberger, 1985) Berger & Foissner, 1987, *L. islandica* Berger & Foissner, 1988, *Uroleptoides magnigranulosus* (Foissner, 1988) Berger, 2008 and *U. longiseries* (Foissner et al., 2002) Berger, 2008
closely resembles *Parasincirra sinica* n. sp. and thus should be compared. *Parasincirra sinica* n. sp. differs from *Lamtostyla decorata* in: (i) smaller body size (90-140 x 20-40 μm vs. 100-220 x 20-35 μm); (ii) buccal cirrus and pretransverse cirri absent (vs. present); and (iii) fewer transverse cirri (two to four vs. five to nine) [20].

Discrepancies between *Parasincirra sinica* n. sp. and *Lamtostyla perisincirra* are also observed in (i) larger body size (90-140 x 20-40 μm vs. 50-80 x 20-30 μm); (ii) cell outline fusiform (vs. parallel body margins with both ends broadly rounded); (iii) buccal cirrus absent (vs. present); (iv) larger number of cirri in ACR (four vs. six to eight); and (v) cortical granules present (vs. absent) (vs. continuous) [20].

*Parasincirra sinica* n. sp. appears to be a close form to *Lamtostyla islandica*, but the former can be recognised by: (i) larger body size (90-140 x 20-40 μm vs. 60-80 x 20-25 μm) in vivo; (ii) cell outline fusiform (vs. parallel body margins with both ends broadly rounded); (iii) buccal cirrus absent (vs. present); (iv) cortical granules present (vs. absent); and (v) arrangement of endoral and paroral (at about same level vs. overlapping only by about half of their length) [20].

*Uroleptoides magnigranulosus* has a close relationship to *Parasincirra sinica* n. sp. in the SSU rDNA tree of our present work. *Parasincirra sinica* n. sp., however, can be recognised by: (i) buccal cirrus absent (vs. present) and (ii) lower number of cirri in ACR (four vs. 12-19) and transverse cirri (two to four vs. constantly five) [20].

The dissimilarity between *Parasincirra sinica* n. sp. and *Uroleptoides longiseries* exists in buccal cirrus absent (vs. present in the latter) and fewer cirri in ACR (four vs. 24-54 in the latter) in the former[20].

**Morphogenetic comparison**

One of the most remarkable morphogenetic features in *Parasincirra sinica* n. sp. is that the rightmost frontoventral row is formed by two anlagen, which is a specific character for amphisiellids and called the amphisiellid median cirral row. Hitherto, accounts of morphogenesis are available for only several taxa in Amphisiellidae with the main morphogenesis comprising a diversity of processes:

1. the parental adoral zone of membranelles is completely retained in some
genera/species, e.g. Amphisiella, Lamtostyla, Lamtostylides, Paramphisiella and Hemisincirra inquieta Hemberger, 1985, while it is partly renewed in other taxa, e.g. Parasincirra n. g.;

2. ventral cirri develop from five (e.g. Lamtostylides and Paramphisiella), six (e.g. Amphisiella, Parasincirra n. g., Spiroamphisiella, Hemisincirra inquieta, Terricirra, Mucotrichidium and most Lamtostyla species) or seven (e.g. Lamtostyla salina Dong, et al, 2016) FVT-anlagen;

3. FVT-anlage II generates the buccal cirrus (in Amphisiella, Spiroamphisiella, Lamtostyla, Lamtostylides, Paramphisiella, Afroamphisiella, Hemisincirra inquieta, Terricirra and Mucotrichidium) or not (in Parasincirra n. g.);

4. the amphiisiellid median cirral row is formed by two (in Amphisiella, Hemisincirra inquieta, Parasincirra n. g., Lamtostyla, Lamtostylides, Mucotrichidium and Paramphisiella) or three (in Terricirra and Spiroamphisiella) anlagen;

5. caudal cirri are formed in some taxa, i.e. Spiroamphisiella, Paramphisiella and Mucotrichidium, while not formed in other genera, i.e. Amphisiella, Parasincirra n. g., Lamtostyla, Lamtostylides, Afroamphisiella, Hemisincirra inquieta and Terricirra;

6. no transverse cirri is formed in Afroamphisiella and Paramphisiella while transverse cirri are formed in Amphisiella, Parasincirra n. g., Lamtostyla, Lamtostylides, Terricirra, Mucotrichidium, Hemisincirra inquieta and Spiroamphisiella [20, 22–24].

**Phylogenetic analyses**

Molecular phylogenetic analyses cannot resolve the relationship of the four polytomies represented by Parasincirra, Uroleptoides and Parabistichella genera (Figs. 6, 7). Whether or not the assignments of the latter two genera to the family Amphisiellidae need further clarification [17, 20, 21, 23, 25–29], the new genus Parasincirra has the critical character of the family Amphisiellidae of an ACR that
originates from two separate anlagen, and apparently it should be assigned in this family (exactly, group II in Amphisiellidae according to Berger, 2008) [19, 20]. Furthermore, the genera within this clade share the same three enlarged frontal cirri and one marginal cirral row on each side pattern. The main difference between Parabistichella and Parasinccira is the number and origin of the frontoventral rows; that is, the most remarkable morphogenetic features in Parasinccira is the long frontoventral row (= amphisiellid cirral row) formed by two anlagen while it is formed by just a single anlage in Parabistichella. The main difference between the species of the genera Uroleptoides and Parasinccira, meanwhile, is whether or not the buccal cirrus appears [20, 25–27]. We speculate that the presence or absence of the buccal cirrus near the anterior end of the undulating membranes might be determined by the degree of food sufficiency, considering it might serve as a food-collecting mechanism. The species that isolated from the sludge soil might undergo small food pressure and therefore the buccal cirrus was absorbed by the cell. Alternatively, the reason why these obvious differences in morphological characters are not reflected in the phylogeny might simply be that there is currently insufficient data to figure out how those lineages are related [30]. Besides, the phylogenetic relationship between P. sinica and the most morphologically similar genera, Lamtostyla and Hemisinicirra, also need further investigation due to the remote position of the former and the lacking information of the latter. Thus, we retain the original classification of the present species in a new genus since neither Lamtostyla nor Hemisinicirra are very well defined (none of the two type species is characterised ontogenetically) [20].

Moreover, other amphisiellid species, even for the type genus Amphisiella, are also not resolved well in our phylogenetic analyses, as has also been the case in previous studies (Fig. 7) [23, 25, 28], which might indicate that the critical character of the family Amphisiellidae (the ACR originating from two separate anlagen) is an apomorphic trait within the amphisiellid hypotrichs that might evolve independently under different conditions. Given the species being sampled and the deficient data on the morphogenesis and SSU rDNA sequences, there is insufficient evidence to unravel a robust phylogeny for this complex group [21, 23, 25–28, 31]. Future integrated taxonomic studies that based on morphological, genetic and ecological approaches therefore are needed in amphisiellids.
biodiversity survey in order to gain a better understanding of evolutionary relationships among them. Overall, our studies have shown a better insight into the diversity of amphisiellid ciliates in sludge soil.

Material And Methods

**Sample collection, isolation, and culturing**

Sludge soil samples were collected from the upper 10 cm layer within a flood drain in Lanzhou (36°3'N; 103°49'E), China on 30 April 2017 (Fig. 1). Samples were dried at room temperature (about 24 °C) immediately after collection in order to preserve them. Months later, ciliates were induced to excyst from the soil samples by employing the non-flooded Petri dish method described by Foissner (2014) [32]. They were then isolated in the laboratory using micropipettes and non-clonal cultures were established at room temperature in Petri dishes with mineral water (Nongfu Spring), with grains of rice being added in order to stimulate the growth of bacteria to act as a food source for the ciliates. We identified only one species, and relied on in vivo morphologic characteristics to assure the accuracy of that identification for all downstream analyses, even though we were unable to establish clonal cultures. Moreover, no other stichotrichid morphotypes were present in the protargol preparation.

**Morphology and morphogenetic studies**

Live observations were carried out using bright field and differential interference contrast microscopy (Olympus BX51) and photographed using a digital camera. Protargol preparation was used to reveal the ciliature and the nuclear apparatus [33]. The protargol reagent was synthesised following the protocol of Pan et al. (2013) [34]. Counts and measurements of stained specimens were performed at a magnification of 1,000×. Drawings of protargol-prepared cells were made with the assistance of a drawing device (camera lucida). To illustrate the changes that occurred during morphogenesis, parental structures are depicted by contour whereas new structures are shaded black [35, 36]. Terminology is according to Berger (2008) [29] and the systematic classification follows Lynn (2008) [19].
DNA extraction, PCR amplification, and gene sequencing

Single cells of *Parasincirra sinica* were isolated from samples, washed several times with distilled water using a micropipette in order to remove potential contamination, and then transferred to three different 1.5 mL microfuge tubes with a minimum volume of water. DNA extraction was performed with the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer’s instructions with minor modifications [37]. PCR amplification and sequencing of the SSU rDNA were performed according to Sheng et al. (2018) [38] using high fidelity Takara Ex Taq DNA polymerase (Takara Ex Taq; Takara Biomedicals) to minimise the possibility of amplification errors. The PCR products were purified using Geneclean (BIO 101 Inc., La Jolla, CA) and sequenced bidirectionally on the ABI 3700 sequencer (GENEWIZ Biotechnology Co., Ltd., Beijing, China).

Phylogenetic analyses

The SSU rDNA sequence of the new species, together with 54 representative taxa downloaded from the GenBank database, were used in the present phylogenetic analyses. The final alignment included 54 taxa and 1734 sites, with 446 variable sites and 265 parsimony information sites. Several oligotrich species (*Novistrombidium sinicum* Liu et al., 2009, *Strombidium cuneiforme* Song et al., 2018 and *S. apolatum* Wilbert et al., 2005) were selected as putative outgroups. All sequences were aligned using the GUIDANCE web server (http://guidance.tau.ac.il/) [39]. The resulting alignment was manually edited using the program BioEdit 7.0 [40]. Both Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed on the final alignment under the best-fit nucleotide substitution model of GTR+Γ that was selected by jModelTest ver. 2.1.7 [41]. The ML analysis was performed using RAxML-HPC2 on XSEDE v8.2.12 on the online server CIPRES Science Gateway [42], with 1,000 rapid bootstrap replicates and a subsequent thorough ML search. Bayesian inference was computed with MrBayes on XSEDE 3.2.6 [43], running four Markov chains sampling every 100 generations for a million generations and discarding the first 25% of trees as burn-in. The majority rule consensus tree was produced from the remaining samples with each node labelled with its
posterior probability. SeaView v.4 [44] and MEGA v5 [45] were used to visualise the tree topologies.

**Abbreviations**

18S rRNA: Small subunit ribosomal RNA; ACR: amphisiellid median cirral row; AZM: adoral zone of membranelles; BI: Bayesian inference; bp: base pairs; FVT-anlagen: frontoventral-transverse cirral anlagen; GC: Guanine-cytosine; ML: Maximum likelihood; n. g.: novum genus; n. sp.: novum species.

**Declarations**

**Acknowledgements**

Many thanks are given to Dr Jie Huang, Chinese Academy of Sciences, for her help with phylogenetic analyses.

**Funding**

This work was supported by the Natural Science Foundation of China (Project numbers: 31872190), the Researchers Supporting Project number (RSP-2019), King Saud University, Riyadh, Saudi Arabia, the Fundamental Research Funds for the Central Universities (201841005) and the Blue Life Breakthrough Program of LMBB of Qingdao National Laboratory for Marine Science and Technology (MS2018NO04). Funding agencies had no role in the design or implementation of this study or in preparation of the manuscript.

**Authors’ contributions**

MJ carried out the live observation, protargol impregnation. MJ, ZY, ZT were responsible for DNA amplification and sequencing, and the molecular phylogenetic analyses. Manuscript drafting: MJ, SC, ZY, ZT; Manuscript review and editing: SC, ZY, SW. KA.S.AR. Language revision: KA.S.AR. (all authors helped revise the manuscript. All authors read and approved the final manuscript).

**Availability of data and materials**

Sequence data is available in GenBank (Accession Numbers: MN472864). Several permanent slides containing the protargol-impregnated holotype specimen of *Parasincirra sinica* n. sp., with registration
number of MJY2017043001 are deposited in the Laboratory of Protozoological Biodiversity and Evolution in Wetland, Shanxi Normal University.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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| Character | HT | Min | Max | Mean | M  | SD | CV | n  |
|-----------|----|-----|-----|------|----|----|----|----|
| Body, length | 106 | 81  | 152 | 119.7 | 115 | 18.3 | 15.3 | 25  |
| Body, width | 30  | 18  | 47  | 30.3 | 31  | 6.3  | 21.0 | 25  |
| Body, length: width ratio | 3.53 | 2.29 | 7.59 | 4.12 | 3.79 | 1.13 | 27.35 | 25  |
| AZM, length | 19  | 13  | 24  | 19.1 | 19  | 2.5  | 13.0 | 25  |
| AZM, length: body length ratio | 0.18 | 0.11 | 0.20 | 0.16 | 0.16 | 0.02 | 13.07 | 25  |
| AZM, number | 16  | 14  | 19  | 15.6 | 15  | 1.3  | 8.1  | 25  |
| Frontal adoral membrane lamellae, number | 5   | 5   | 5   | 5.0  | 5   | 0    | 0    | 25  |
| Ventral adoral membrane lamellae, number | 11  | 9   | 14  | 10.7 | 10  | 1.2  | 11.4 | 25  |
| PBC, number | 1   | 1   | 1   | 1.0  | 1   | 0    | 0    | 25  |
| FVR, number | 2   | 2   | 2   | 2.0  | 2   | 0    | 0    | 25  |
| ACR, cirri, number | 4   | 4   | 4   | 4.0  | 4   | 0    | 0    | 25  |
| Frontal cirri, number | 3   | 3   | 3   | 3.0  | 3   | 0    | 0    | 25  |
| Left marginal cirri, number | 44  | 34  | 52  | 41.3 | 41  | 5.3  | 12.9 | 25  |
| Right marginal cirri, number | 40  | 34  | 53  | 41.2 | 39  | 5.2  | 12.6 | 25  |
| Transverse cirri, number | 3   | 2   | 4   | 3.1  | 3   | 0.5  | 16.0 | 25  |
| Dorsal kineties, number | 3   | 3   | 3   | 3.0  | 3   | 0    | 0    | 25  |
|                           | Number | Number | Number | Number | Number | Number | Number | Number | Number | Number |
|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Dikinetids in DK1, number| 10     | 10     | 17     | 12.5   | 12     | 2.3    | 18.7   | 15     |
| Dikinetids in DK2, number| 15     | 13     | 18     | 14.9   | 15     | 1.5    | 10.3   | 15     |
| Dikinetids in DK3, number| 17     | 11     | 18     | 14.5   | 14     | 2.2    | 15.0   | 15     |
| Macronuclear nodules, number | 4  | 2      | 6      | 4.1    | 4      | 0.7    | 16.2   | 25     |
| Macronuclear nodule, average length | 10 | 9      | 19     | 13.8   | 14     | 2.8    | 20.4   | 25     |
| Macronuclear nodule, average width | 5  | 4      | 10     | 6.0    | 6      | 1.2    | 20.4   | 25     |
| Micronuclei, number      | 2      | 1      | 3      | 2.0    | 2      | 0.7    | 37.5   | 25     |
| Micronuclear nodule, average length | 3  | 2      | 4      | 2.9    | 3      | 0.4    | 14.5   | 25     |
| Micronuclear nodule, average width | 2  | 2      | 4      | 2.4    | 2      | 0.4    | 18.0   | 25     |

*aAll data are based on protargol-stained specimens. Measurements in µm. Abbreviations: ACR, Amphisiellid median cirral row; AZM, Adoral zone of membranelles; CV, coefficient of variation in %; DK, Dorsal kineties; FVR, Frontoventral cirral row; HT, holotype specimen; M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; n, sample size; PBC, parabuccal cirri; SD, standard deviation. Figures*
Figure 1

Locations of the sample sites. (a, b) Portion of Google Map, showing the location of Lanzhou, China (36°03′N; 103°49′E). (c) Showing the area surrounding the flood drain from where the sample containing Parasincirra sinica n. sp. was collected.
Figure 2

Morphology of Parasincirra sinica n. sp. from life (a, c, d, g, h) and after protargol staining (b, e, f, i–m). (a) Ventral view of a representative specimen. (b) Ventral view, to show ciliature of frontoventral area. (c) Arrangement of cortical granules on dorsal side. (d) Ventral views, to show the various body shapes. (e, f) Ventral (e) and dorsal (f) view of a typical individual, to show the ciliature and nuclear apparatus. (g, h) Ventral views of representative individuals, arrow indicates contractile vacuole. (i) Ventral view of the holotype specimen to show ventral ciliature. (j) Dorsal view to show cortical granules (arrows). (k) Ventral view of anterior portion, to show the cirri in frontoventral area and a short gap in adoral zone of membranelles (arrow). (l) Ventral view, to show transverse cirri.
(m). Detail of cortical granulation on dorsal body side (arrows). ACR, amphisiellid cirral row; AZM, adoral zone of membranelles; E, endoral; FC, frontal cirri; FVR, frontoventral cirral row; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; P, paroral; PBC, parabucal cirri; RMR, right marginal row; TC, transverse cirri; 1–3, dorsal kineties 1–3. Scale bars: A, E, F, I = 60 μm, C = 15 μm.

Figure 3

Early and middle stages of morphogenesis in Parasincirra sinica n. sp. after protargol staining. (a, b) Ventral views of early dividers, showing oral primordium of opisthe and frontoventral-transverse cirral anlagen. Note parental undulating membranes start to dedifferentiate (b). (c, d) Ventral views of later dividers, to show the development of oral primordium, frontoventral-transverse cirral anlagen and undulating membranes anlagen (arrows). Note the dedifferentiation of membranelles at the proximal end of the old adoral
zone of membranelles (arrow in c), and the intrakinetally formed anlagen for the marginal rows (d). (e–h) Ventral (e, g) and dorsal (f, h) views of middle dividers, to show stretched marginal anlagen and dorsal kineties anlagen, the posterior membranelles of the parental adoral zone of membranelles renewed (g) and the macronuclear nodules fusing into a single mass. Note the old dorsal dikinetids are not absorbed (arrows). OP, oral primordium; LMA, left marginal anlagen; LMR, left marginal row; Ma, macronuclear nodules; Mi, micronuclei; RMA, right marginal anlagen; RMR, right marginal row; 1–3, dorsal kineties anlagen 1–3.

Scale bars: A, G, H = 60 μm.

Figure 4
Late stages of morphogenesis in Parasincirra sinica n. sp. after protargol staining. Ventral (a, b) and dorsal (c) views, to show the frontoventral-transverse cirral anlagen differentiating into cirri, transverse cirri migrating into their final position (arrows), the old adoral zone of membranelles have been rebuilt (arrowheads). LMA, left marginal anlagen; LMR, left marginal row; RMA, right marginal anlagen; RMR, right marginal row; Ma, macronuclear nodules; Mi, micronuclei; 1–3, dorsal kineties anlagen 1–3. Scale bars = 60 μm.
Figure 5

Photomicrographs of Parasincirra sinica n. sp. during morphogenesis (after protargol staining). (a–d) Ventral views of early dividers, to indicate the oral primordium (arrows in a–c), the formation of frontoventral-transverse cirral anlagen and undulating membranes starting to dedifferentiate (arrowhead). Note the old frontal cirri remain intact (arrows in d). (e, f) Ventral views of later dividers, to show the oral primordia starting to differentiate into membranelles (arrow), formation of undulating membranes anlagen in the proter (arrowhead), and frontoventral-transverse cirral anlagen starting to separate (f). (g–i) Ventral views of later dividers, to show the dedifferentiation of membranelles at the proximal end of the old adoral zone of membranelles (arrow), the intrakinetally formed anlagen for the marginal rows (arrowhead), and stretched marginal anlagen and
frontoventral-transverse cirral anlagen (h, i). (j–l) Ventral (j) and dorsal (k, l) views of middle divider, to show frontoventral-transverse cirral anlagen differentiating into cirri (j), dorsal kineties anlagen (arrowheads), the old dorsal dikinetids (arrows) and the macronuclear nodules fusing into a single mass (k). (m, n) Ventral views of a late divider, arrows show transverse cirri migrating into their final positions in the opisthe (m) and proter (n). Note the undulating membranes anlagen longitudinally splitting into parorals and endorals. (o) Dorsal view, to show the newly formed dorsal kineties. (p) Ventral view, to demonstrate transverse cirri (arrow) migrating into their final positions. Scale bars = 15 μm.
Diagram of the infraciliature, and formation patterns of ventral cirri (dotted lines connecting cirri that develop from the same cirral streaks, arrows mark the buccal cirri) (a, c, e-j) and dorsal ciliature (b, d). (a) Parasincirra sinica. (b) Parasincirra sinica, Amphisiella annulata, Uroleptoides longiseries, Parabistichella variabilis, Bistichella cystiformans and Keronopsis helluo. (c) Lamtostyla salina. (d) Lamtostyla salina and Orthoamphisiella breviseries. (e) Amphisiella annulata. (f) Uroleptoides longiseries. (g) Parabistichella variabilis. (h) Orthoamphisiella breviseries. (i) Bistichella cystiformans. (j) Keronopsis helluo [20, 21, 23, 25, 27, 46, 47].
Maximum likelihood (ML) tree inferred from the SSU rDNA sequences showing the phylogenetic relationships of Parasincirra sinica n. sp. (in bold) and the related species (in rectangular box). Numbers near nodes are bootstrap values for maximum-likelihood and posterior probability values for Bayesian inference (BI). “*” at nodes indicates disagreement between the two methods. Fully supported (100%/1.00) branches are marked with solid circles. The scale bar corresponds to 0.01 expected substitutions per site.