

Pauciramus yunnanensis, gen. et sp. nov., a novel freshwater red alga from China with proposal of the Ottiales ord. nov. (Nemaliophycidae, Rhodophyta)*

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Abstract Species of the red algal order Acrochaetiales mostly inhabit marine environments, with only two freshwater taxa *Audouinella* and *Ottia*. A new genus and species are described for freshwater red alga *Pauciramus yunnanensis* from Ailao Mountain, Yunnan, China. It is closely related to *Ottia* and a new order Ottiales was proposed for these genera. *Pauciramus* has unique combination of morphological characteristics including the following: plants caespitose and densely pulvinate, slender uniseriate filaments with well-developed rhizoids, rarely branched, cylindrical vegetative cell with a single, ribbon-shaped and parietal chloroplast, reproduction by tetrasporangia, and dense sporangial branchlet only at the upper portion of filaments. Phylogenetic analysis of sequence data from the plastid ribulose-1,5-bisphosphate carboxylase-oxygenase large-subunit (*rbcL*), small subunit gene of the ribosomal cistron (SSU) and barcode region near the 5' end of the mitochondrial cytochrome oxidase subunit I (COI-5P) indicated that: the new taxon, *P. yunnanensis*, was in a well-supported clade with *Ottia meiospora*, and this clade was sister to order Palmariales and Acrochaetiales. To adhere to the principle of monophyly, a new freshwater order Ottiales including *Ottia* and *Pauciramus* is proposed. Despite the high sequence interspecific divergences and obvious morphological differences between genera *Ottia* and *Pauciramus*, seems impractical to establish a new family for a monospecific genus. Therefore, we temporarily classified *Pauciramus* into the family Ottiaceae, and made necessary revisions to the description to accommodate this genus.

Keyword: China; 5' end of the mitochondrial cytochrome oxidase subunit I (COI-5P); Ottiales; *Pauciramus*; ribulose-1,5-bisphosphate carboxylase-oxygenase large-subunit (*rbcL*); small subunit gene of the ribosomal cistron (SSU)

1 INTRODUCTION

Red algae (Rhodophyta) primarily inhabit marine environments, with freshwater red algae accounting for only 3% of the species diversity (Sheath and Vis, 2015). These freshwater taxa are particularly well represented in the subclass Nemaliophycidae, including four orders: Acrochaetiales, Balbianiales, Batrachospermales, and Thoreaales (Kumano, 2002; Lam et al., 2016). The order Acrochaetiales currently includes four families with 220 accepted species and is the only order of subclass Nemaliophycidae that has both freshwater and marine taxa, with approximately 10% of species occurring in freshwater environment (Guiry and Guiry, 2021). The freshwater

taxa are only distributed in the genera *Audouinella* Bory and *Ottia* Entwisle, J. R. Evans, M. L. Vis, & G. W. Saunders (Harper and Saunders, 2002; Kumano, 2002; Entwisle et al., 2018). *Ottia* belongs to the monogeneric family Ottiaceae with only one species *Ottia meiospora* (Skuja) Entwisle, J. R. Evans, M. L. Vis, & G. W. Saunders. This genus and family were recently established based on morphological characteristics and DNA sequence data; this taxon is

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an endophyte of other freshwater rhodophytes and characterized by uniseriate branched filaments, cells with 1–2 parietal, cup, or ribbon-shaped chloroplasts, without pyrenoids, and monosporangia in small clusters (Entwisle et al., 2018). Skuja (1944) first described *O. meiospora* (as *Balbiania meiospora* Skuja) from a collection of *Nothocladus lindaueri* Skuja from the Bay of Islands, New Zealand. Garbary (1987) transferred *Balbiania* (only includes two species *B. meiospora* and *B. investiens* (Lenormand ex Kützing) Sirodot) to *Audouinella* due to no obvious morphological difference between *Balbiania* and *Audouinella*. Subsequently, based on molecular comparisons and further study of morphology, *B. investiens* was returned to the genus *Balbiania* with a new family (Balbianiaceae) and order (Balbianiales) established. By contrast, *Audouinella* (*Balbiania*) *meiospora* remained tentatively assigned to *Audouinella* (family Audouinellaceae) due the lack of DNA sequence data (Harper and Saunders, 1998; Sheath and Müller, 1999). Soon after, Skinner and Entwisle (2001) identified an Australian endophyte of *Nothocladus* from northern New South Wales and Little Yarra River in Victoria and attributed collection to *B. investiens*. Entwisle et al. (2018) reinvestigated this Australian taxon. Their phylogenetic analyses confirmed that it belonged in the order Acrochaetales, and Australian collections have a similar morphology *B. meiospora* as reported by Skuja (1944). To accommodate this red algal endophyte, a new genus and family were proposed (Entwisle et al., 2018).

Freshwater taxa of Acrochaetales are widely distributed in China. Since Jao (1940) first reported Acrochaetales species in freshwater environments from China, 10 species have been reported, all of which belong to the genus *Audouinella* (Shi, 2006). However, four have been assigned to other taxa or the sporophyte stages of other freshwater taxa using molecular analyses (Pueschel et al., 2000; Chiasson et al., 2005, 2007; Necchi and Oliveira, 2011; Chen et al., 2014), and the remaining six species are still to be investigated with molecular data to support their distinctiveness. Xie and Shi (2004) reported *B. meiospora*, endophyte of batrachspermalean taxa, using morphology from a collection of Zhejiang (Hangzhou, Yuhuang Mountain, CK547, collected by C.-C. Jao in 1954) and Guizhou (Huangguoshu, KC830108, collected by Yong YAO et al. in 1983). Whether this Chinese collection is *Ottia meiospora* requires further confirmation. In this study, we discovered an interesting uniseriate freshwater red

alga from Ailao Mountain, Yunnan (Fig.1). Phylogenetic analyses demonstrate its position within the family Ottiaceae clade and requires a new systematic arrangement with the establishment of a new genus and order.

2 MATERIAL AND METHOD

2.1 Samples collection

Algal specimens were collected in April 2019 from Shimenxia Scenic Area of Ailao Mountain, Yunnan, China (23°58'22"N, 101°31'25"E, above sea level (ASL): 1 987.7 m Fig.1). The clear stream flowing from the valley of Ailao Mountain passes through nearly 1 km of rocky canyons (Shimenxia Scenic Area) surrounded by dense forests. The spring water formed many small streams, which flowed down from the cliffs of the canyon and merged into the river. The algae only grow on the wet and smooth rocks, hit by drops of mountain spring water, on the banks of the river.

The samples were washed with sterile water to remove debris and epiphytes. Specimens for DNA analysis were preserved in silica desiccant. Some voucher specimens were preserved in 4% formaldehyde (holotype: SXU-YN19053) for morphological analyses and the remaining portions were pressed as herbarium voucher specimens (isotype: SXU-YN19054, SXU-YN19055). Voucher specimens were deposited in Herbarium of Shanxi University (SXU), Shanxi University, Taiyuan, Shanxi Province, China. Herbarium acronym follows Thiers (2021).

2.2 Morphological and DNA analyses

Morphological analyses were conducted according to previous literature (Skuja, 1944; Skinner and Entwisle, 2001; Kumano, 2002; Xie and Shi, 2004; Shi, 2006; Entwisle et al., 2018). Vegetative and reproductive structures were photographed and examined using an Olympus BX-43 microscope equipped with a charge-coupled device (DP72; Olympus, Tokyo, Japan).

For DNA extraction, tissue dried in silica was homogenized by grinding in liquid nitrogen with a mortar and pestle, and total DNA was extracted following the protocol described by Saunders (1993) with modifications following Vis and Sheath (1997). Polymerase chain reaction (PCR) amplification of the plastid-encoded ribulose-1,5-bisphosphate carboxylase-oxygenase large-subunit gene (*rbcL*),

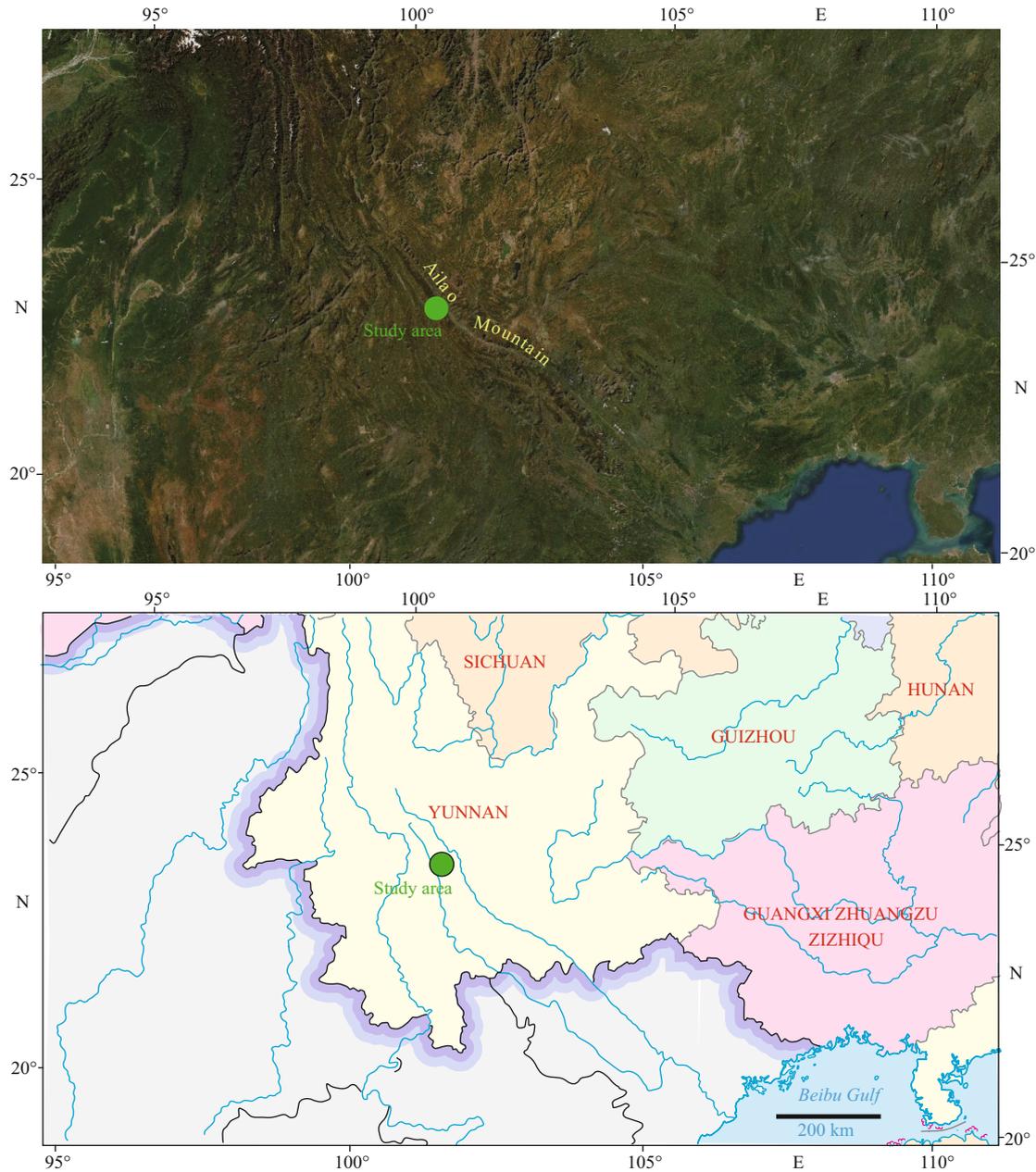


Fig.1 Map of China showing the location of the study area Ailao Mountain in Yunnan, China

Map review No. GS(2020)4634.

barcode region near the 5' end of the mitochondrial cytochrome oxidase subunit I gene (COI-5P) and small subunit gene of the ribosomal cistron (SSU) were chosen for amplification in 20- μ L volumes containing 11.3- μ L ddH₂O, 2- μ L 10 \times buffer, 2.0- μ L 2.5-mmol/L dNTPs, 0.2- μ L Taq DNA polymerase (all from Sangon Biotech Co., Ltd., China), 2.0 μ L of each primer (10 mmol/L), and 0.5 μ L of genomic DNA. The *rbcL* gene (1 532 bp) was PCR amplified using the F-57 \times R-753, F-993 \times R-*rbcLS* primers (Freshwater and Rueness, 1994) and F160 \times *rbcLR* primers (Vis et al., 1998) using the following cycle:

95 $^{\circ}$ C for 3 min, 40 cycles of 94 $^{\circ}$ C for 1.5 min, 37 $^{\circ}$ C for 2 min, 72 $^{\circ}$ C for 3 min, a final hold for 2 min at 72 $^{\circ}$ C; the COI-5P gene (664 bp) was PCR amplified using the GazF1 and GazR1 primers (Saunders, 2005) using the following cycle: 94 $^{\circ}$ C for 2 min, 35 cycles of 94 $^{\circ}$ C for 30 s, 47 $^{\circ}$ C for 30 s, 72 $^{\circ}$ C for 1 min, a final hold for 7 min at 72 $^{\circ}$ C; and the SSU gene (1 572 bp) was PCR amplified using G01 \times G10, G02 \times G14, G04 \times G13, and G06 \times G17 primers (Harper and Saunders, 2001) using the following cycle: 94 $^{\circ}$ C for 4 min, 38 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 30 s, 72 $^{\circ}$ C for 1 min 30 s, a final hold for 7 min at 72 $^{\circ}$ C.

2.3 Sequence determination and data analysis

The PCR products were purified using a SanPrep column DNA gel purification kit (Sangon, China) and then were submitted to BGI Tech Corporation (Beijing, China) for sequencing using an ABI 3730XL sequencer with PCR primers. Contigs were assembled and edited using Sequencher 4.10.1. The sequence data of *rbcL* (MW874643), COI-5P (MW874644) and SSU (MW879164) markers were deposited in GenBank.

For phylogenetic analyses, sequence data for subclass Nemaliophycidae were downloaded from GenBank (Supplementary Table S1 for analysis based on *rbcL*, Supplementary Table S2 for analysis based on *rbcL*+SSU+COI-5P). The subclass Corallinophycidae was used as outgroup for phylogenetic analyses (Scott et al., 2013).

Four alignments were constructed using BioEdit v7.2.1 (Hall, 1999) with default gap penalties and refined by eye, including individual gene regions *rbcL* (*rbcL*1: 87 from 53 species, 1 282 aligned sites for analysis based on *rbcL*) (*rbcL*2: 56 from 53 species, 1 282 aligned sites for analysis based on *rbcL*+SSU+COI-5P), SSU (51 from 50 species, 1 791 aligned) and COI-5P (44 from 44 species, 664 aligned). In addition, a combined alignment was generated (57 taxa, 3 737 aligned sites; *rbcL*=1–1 282, SSU=1 283–3 073, and COI-5P=3 074–3 737) using PhyloSuite (Zhang et al., 2020). Pairwise distance comparison of sequence variation was conducted using MEGA5 software (Supplementary Table S3) (Tamura et al., 2011), and Box-plot of genetic distance based on *rbcL*, SSU, and COI-5P gene sequences was constructed using OriginPro 2021 (OriginLab Corporation, Northampton, MA, USA). The best-fit model for *rbcL* were determined by ModelFinder (Kalyaanamoorthy et al., 2017) with AIC criterion (GTR+F+R5 for maximum likelihood (ML) and GTR+F+I+G4 for Bayesian Inference (BI)); and for the concatenated *rbcL*+SSU+COI-5P alignment, the appropriate models of sequence evolution were selected using PartitionFinder2 (Lanfear et al., 2016), with all algorithm and AIC criterion (for ML: Subset (1)(2)(3)=GTR+I+G; for BI, lset applyto (1)(2)(3): nst=6, rates=invgamma). ML phylogenies were inferred using IQ-TREE (Nguyen et al., 2015) with 5 000 ultrafast bootstraps (for *rbcL*, under the GTR+R5+F model and for *rbcL*+SSU+COI-5P under Edge-linked partition model), as well as the Shimodaira-Hasegawa-like approximate likelihood-

ratio test (Guindon et al., 2010). Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al., 2012) with 2 parallel runs, 2 000 000 generations, in which the initial 25% of sampled data were discarded as burn-in (for *rbcL* under GTR+I+G+F model; and for *rbcL*+SSU+COI-5P under partition model). The resulting phylogenetic trees were edited using Figtree1.4.2 (<https://tree.bio.ed.ac.uk/software/figtree/>).

3 RESULT

3.1 Molecular analysis

Three sequences (*rbcL*-1 282 bp, COI-5P-664 bp, SSU-1 791 bp) were obtained from the samples SXU-YN19053. Unfortunately, despite the best efforts, we were unable to grow this alga in culture, and the sequencing of LSU (large subunit gene of the ribosomal cistron) failed with repeated attempts using a variety of standard primer sets and protocols. Four alignments of the subclass Nemaliophycidae were generated. The *rbcL*1 data set (for analysis based on *rbcL*) is 1 282 base pairs with 658 constant sites and 624 variable sites; The *rbcL*2 data set (for analysis based on *rbcL*+SSU+COI-5P) is 1 282 base pairs with 670 constant sites and 612 variable sites; the COI-5P data consisted of 664 characters, of which 296 are constant sites and 368 are variable sites; and alignment of SSU gene is 1 791 bp, which included 1 134 constant sites and 654 variable sites.

The phylogenetic trees generated from ML and BI analyses based on *rbcL* or the concatenated *rbcL*+SSU+COI-5P alignment were closely congruent, such that only the trees inferred from ML were presented here with ML bootstrap support values and BI posterior probability on the nodes (Figs.2 & 3). Both trees showed the Ailao Mountain specimen was in a well-supported clade with *Ottia meiospora* (90/0.82 for *rbcL* tree and 95/1.00 for *rbcL*+SSU+COI-5P tree) with high interspecific divergences (13.40%, 163 bp for *rbcL*, and 0% among the three samples of *O. meiospora*, *O. meiospora* only have *rbcL* and LSU sequences), and sister to the order Palmariales and other families of the Acrochaetales (96/1.00 for *rbcL* tree and 97/1.00 for *rbcL*+SSU+COI-5P tree), making the order Acrochaetales polyphyletic. The great genetic distance between SXU-YN19053 and *O. meiospora* indicate that the specimen in this study represent distinct species and genus, and *Pauciramus yunnanensis* is proposed.

To adhere to the principle of monophyly, a new

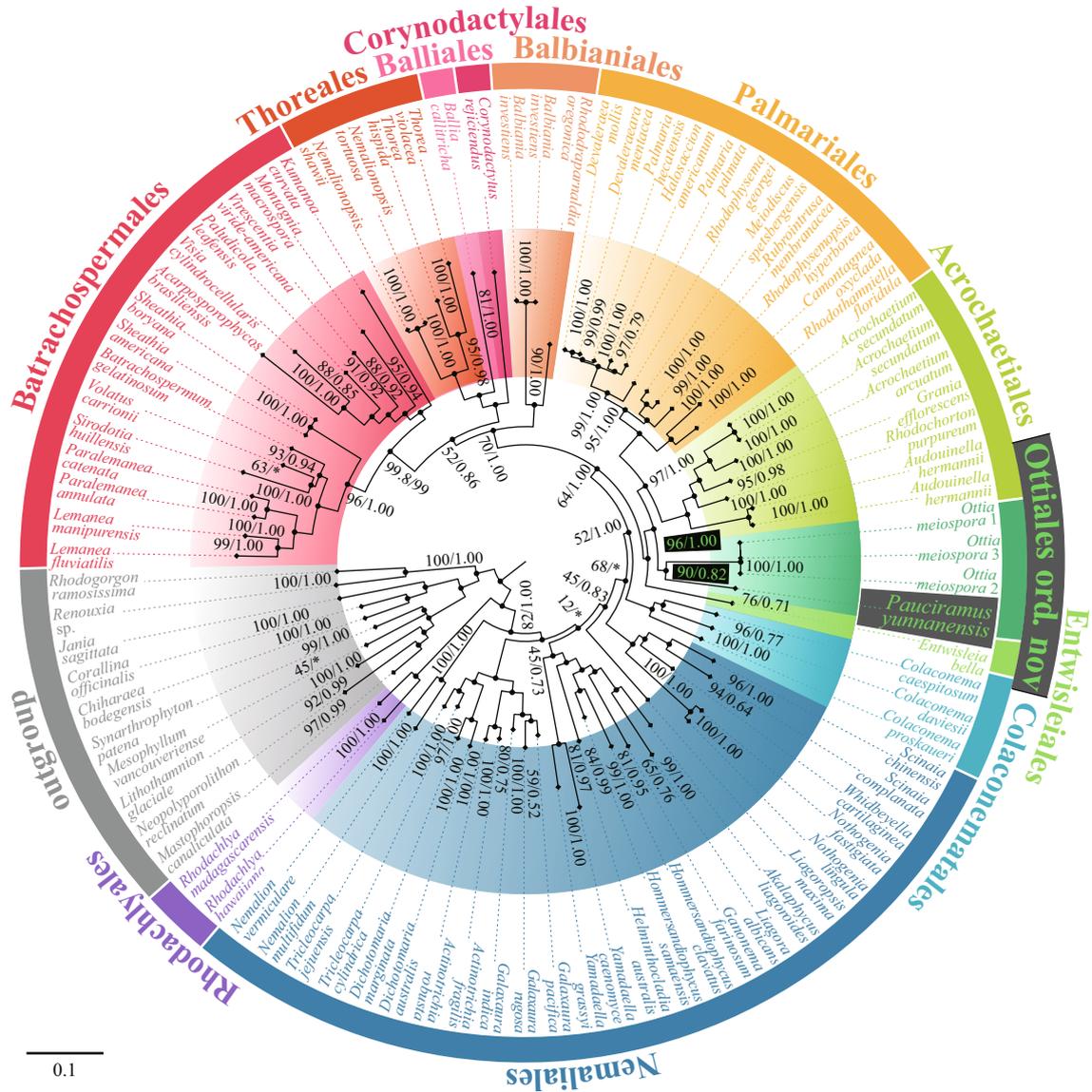


Fig.2 Maximum likelihood (ML) phylogeny of subclass Nemaliophycidae estimated from a concatenated alignment of the *rbcL*

Different colors represent different orders. Numbers at the branches indicate the bootstrap (BP, left) support for ML and posterior probabilities (PP, right) for Bayesian inference (BI). “*” denotes the branch differed in the BI topology. Specimen from this study is indicated in black box.

order including the genera *Ottia* and *Pauciramus* is proposed. The genetic distance of Acrochaetales-Palmariales complex also supports this taxonomic decision (Supplementary Figs.S1–S3 and Supplementary Table S3). The genetic distance levels between *Ottia*-*Pauciramus* complex and the other taxa of Acrochaetales-Palmariales complex (including order Acrochaetales, order Palmariales, and the only freshwater group *Audouinella*) are significantly higher than the genetic distance between these taxa. This means that *Ottia*-*Pauciramus* complex has reached the order level in terms of genetic distance. In addition, we also obtained some interesting results through these genetic distance

analyses. The results show that the *rbcL* sequence has better distinguishability at different taxonomic levels of order — 10.77%–15.21% (131–185 bp), family — 6.09%–11.18% (74–136 bp), and genus — 0.99%–9.21% (12–112 bp), but the genus *Audouinella* seems to be a special case with generally large genetic distance, almost reaching the order level (10.44%–14.88%, 127–181 bp) (Supplementary Fig.S1). SSU results (Supplementary Fig.S2) show that *Ottia*-*Pauciramus* complex is significantly different from other taxa (3.38%–4.20%, 53–66 bp), and the divergences of taxa in order Palmariales (0.38%–2.04%, 6–32 bp) is greater than order Acrochaetales (0.32%–0.64%, 5–10 bp). COI-5P (Supplementary

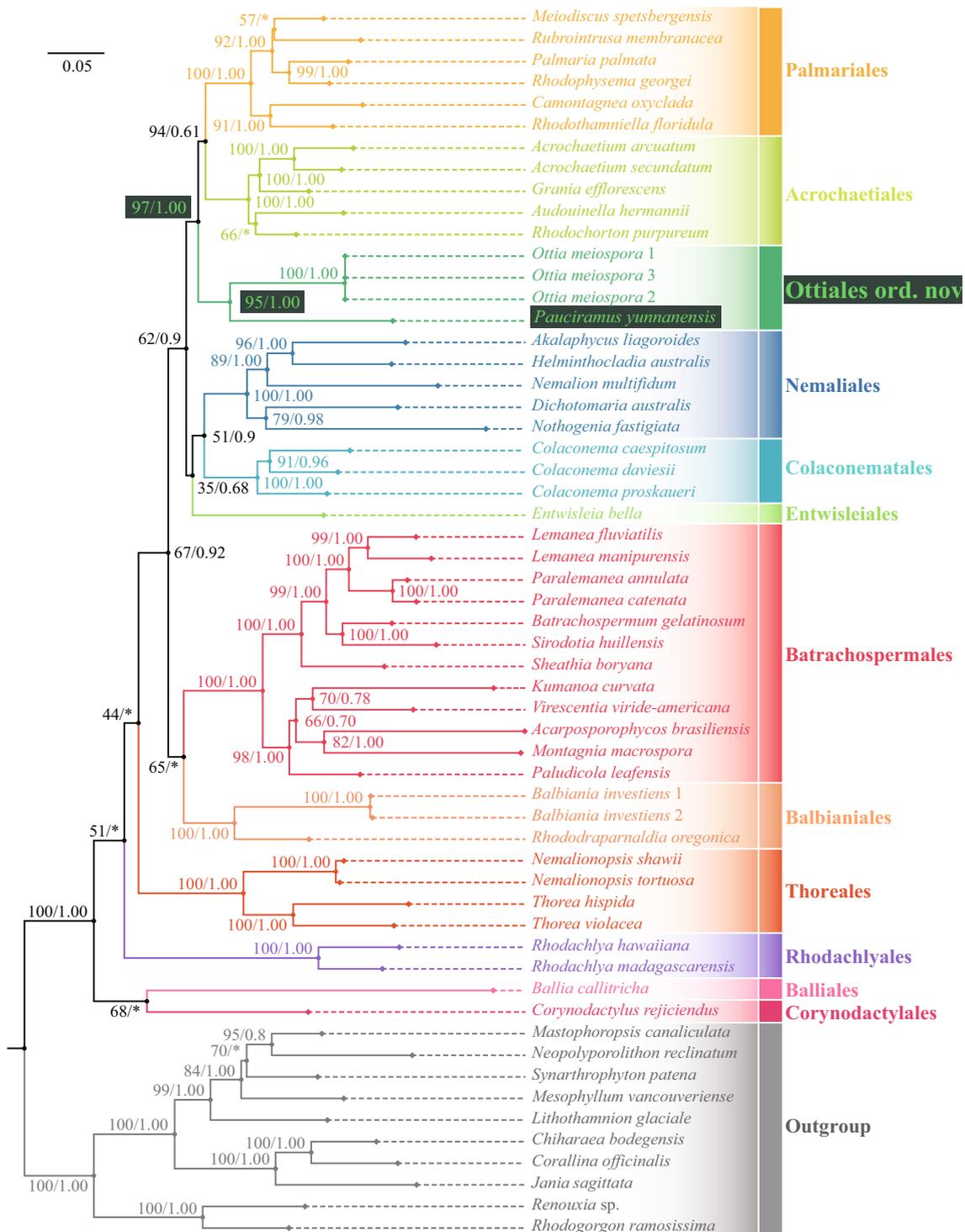


Fig.3 Maximum likelihood (ML) phylogeny of subclass Nemaliophycidae estimated from a concatenated alignment of the *rbcL*+*SSU*+*COI*-5P markers

Different colors represent different order. Numbers at the branches indicate the bootstrap (BP, left) support for ML and posterior probabilities (PP, right) for Bayesian inference (BI). “*” denotes the branch differed in the BI topology. Specimen from this study is indicated in black box.

Fig.S3) generally has a large level of difference among all taxa of Palmariales-Acrochaetiales complex (13.03%–23.29%, 80–143 bp), and there is no hierarchical difference related to the classification level.

3.2 Morphological observation

The vegetative structures of the specimen (Fig.4) were caespitose, densely pulvinate, consisting of uniseriate, slender filaments, and attached to the

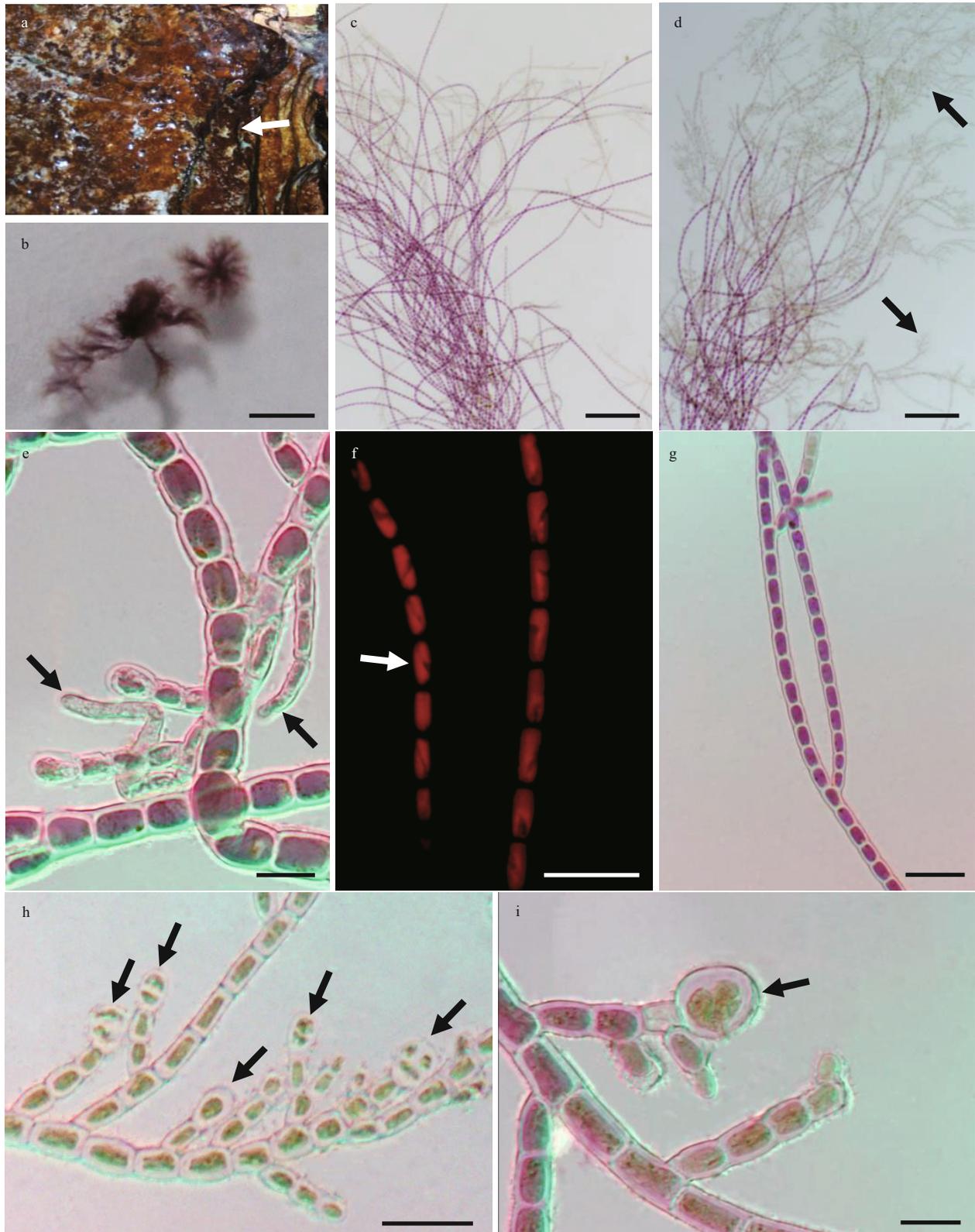


Fig.4 Morphological structures of *Pauciramus yunnanensis*, gen. et sp. nov.

a. habitat in the wet and smooth rocks on the banks of the river of Ailao Mountain. Densely pulvinate thallus of *P. yunnanensis* (white arrow); b. vegetative caespitose thalli; c. sparsely branched slender filaments; d. dense sporangial branchlet (arrows) distributed at the upper portion of filaments; e. well-developed rhizoids (arrows) forming from basal cells of filaments; f–g. cylindrical vegetative cells with a single, ribbon-shaped, parietal chloroplast (arrow); h. cruciately divided tetrasporangia (arrows), terminal on the short lateral branchlets; i. cruciately divided tetrasporangium details (arrow). Scale bars: 2 mm in b, 100 μ m in c–d, 20 μ m in f–h, and 10 μ m in e, i.

Table 1 Morphological comparison of sample in this study with species of *Audouinella* and *Balbiania* previously reported from China and *Ottia meiospora*

Morphological character	SXU-YN19053	<i>O. meiospora</i>	<i>B. meiospora</i>	<i>A. cylindrica</i> C.-C. Jao	<i>A. eugenea</i> (Skuja) C.-C. Jao	<i>A. hermannii</i> (Roth) Duby	<i>A. lanosa</i> Jao
Plant size (mm)	1–2	Microscopic	Microscopic	0.5	1–1.5	0.5–1	3
Color	Purplish red	Reddish-brown to brown	Purplish red	Blue-green	Red-violet	–	Dark purple
Lifestyle	Independent growth	Endophytic	Endophytic	Independent growth	Independent growth	Independent growth	Independent growth
Basal system	Well-developed rhizoids	Creeping filaments	Creeping filaments	Irregular prostrate	Prostrate pseudoparenchymatous	Prostrate pseudoparenchymatous	Irregular prostrate
Branch feature	Sparse branches only at upper portion	Sparse branches	Sparse branches	Branches at upper portion	Irregularly branched	Irregularly branched	–
Filaments length (cell number)	50–200	4–6	3–10	–	3–10	–	–
Vegetative cell shape	Cylindrical	Obovoid to elongate obpyriform (sometimes curved)	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Vegetative cells length, diameter (µm)	5–14, 6–8	5–8, 4–5	12–18, 8–11	11.7–13.5, 4–7	2–4 times than diameter, 9–11	2–4 times than diameter, 8–11	42–72, 9–12
Sporangial branchlet	Dense only at upper portion	Scattered throughout the thallus	Scattered throughout the thallus	Scattered	Scattered	Mostly at upper portion	Scattered
Tetrasporangia shape, length, diameter (µm)	Obovoid, 5–13, 7–15	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Monosporangia shape, length, diameter (µm)	Not observed	Clavate to subglobose, 8–11, 7–8	Obovoid, 13–14, 8–9	Cylindrical-ovate, 13–18, 7–9	Obovoid, 16–18, 12–15	Obovoid, 10–15, 7–7.5	Obovoid, 14–17, 10–14
Sexual reproduction	Not observed	Spermatangium and putative carpogonium	Only spermatangia observed	Not observed	Not observed	Not observed	Not observed
Reference	This study	Entwisle et al., 2018	Xie and Shi, 2004	Jao, 1941	Jao, 1940	Shi, 2006	Jao, 1941

– indicates that the morphological feature has not been reported in the existing literature.

substratum by well-developed rhizoids. These characteristics are close to some taxa of order Acrochaetiales such as *Audouinella*, *Balbiania*, and *Ottia*. Morphological comparison of sample in this study with species of *Audouinella* and *Balbiania* previously reported from China and *Ottia meiospora* is provided (Table 1) according to the descriptions in earlier studies (Skuja, 1944; Kumano, 2002; Xie and Shi, 2004, Shi, 2006; Skinner and Entwisle, 2001; Entwisle et al., 2018).

Our sample (SXU-YN19053) has obvious morphological differences with *O. meiospora* including slender filaments 50–200 cell stories with well-developed rhizoids (vs. 4–6 cell stories with creeping filaments in *O. meiospora*); cylindrical vegetative cells (vs. obovoid to elongate obpyriform in *O. meiospora*); sporangia branchlet only at upper portion (vs. scattered throughout the thallus in *O. meiospora*); reproduction by tetrasporangia (vs. by

monospores in *O. meiospora*), and the sexual reproduction structure, spermatangium and putative carpogonium reported in *O. meiospora* was not found in the newly collected freshwater red algae. Furthermore, *O. meiospora* is endophyte, and epiphytic/endophytic on various species of *Nothocladus* s. lat., spreading loosely throughout thallus of host (Entwisle et al., 2018), which is obviously different from sample in this study as our samples were caespitose with epilithic habit.

In China, six freshwater *Audouinella* and one *Balbiania* have been reported (Xie and Shi, 2004; Shi, 2006), and all of these species lack molecular sequences. However, these species can be easily distinguished from the samples in this study by morphology, especially the well-developed rhizoids and tetrasporangia. Furthermore, *B. meiospora* previously reported from China can be identified as *O. meiospora* based on morphology.

3.3 Taxonomic proposal

Ottiales K. P. Fang, F. R. Nan, & S. L. Xie ord. nov.

Diagnosis: Small freshwater red alga composed of uniseriate branched filaments, cells with a single or sometimes two parietal chloroplasts; chloroplast cup or ribbon-shaped, without pyrenoids; fitting one of the two following sets of characteristics: (1) Epiphytic/endophytic on various species of *Nothocladus* s. lat., consisting of basal and erect systems; cells of basal irregular in shape, fusiform to occasionally inflated in middle or ends; erect systems branched, 4–6-celled, cells obovoid to elongate obpyriform (sometimes curved), terminal hairs present; asexual reproduction by monospores; sexual reproduction with spermatangium and putative carpogonium. (2) Epilithic on rock substrata, consisting of caespitose and densely pulvinate thalli, gracile and uniseriate filaments with well-developed rhizoids, rarely branched; cells cylindrical, terminal hairs absent; asexual reproduction by tetrasporangia, sporangial branchlet only at the upper portion of filaments; sexual reproduction unknown.

Type family: Ottiaceae (Entwisle, J. R. Evans, M. L. Vis, & G. W. Saunders) emend. K. P. Fang, F. R. Nan, & S. L. Xie

Ottiaceae (Entwisle, J. R. Evans, M. L. Vis, & G. W. Saunders) emend. K. P. Fang, F. R. Nan, & S. L. Xie

Characters as for the order.

Type genus: *Ottia* Entwisle, J. R. Evans, M. L. Vis, & G. W. Saunders (2018: 82)

Taxonomic notes: Family Ottiaceae was established with only one genus and species *Ottia meiospora* based on morphological characteristics and DNA sequence data (Entwisle et al., 2018). In this study, we found a uniseriate freshwater red alga, and molecular data from the *rbcL*, SSU and COI-5P indicated that: the new taxon was in a well-supported clade with *Ottia meiospora* with high interspecific divergences. Additionally, there are obvious morphological differences between *Ottia meiospora* and the new taxon. Due to low species diversity with few molecular sequences in this lineage, we decided to propose a new species and genus *Pauciramus yunnanensis*, and provisionally classified *Pauciramus* into the family Ottiaceae with necessary revisions to the description of this family for the newly added taxon. The revised Ottiaceae is composed of small freshwater red alga and characterized by uniseriate branched filaments, cells with parietal cup or ribbon-shaped chloroplasts,

without pyrenoids, including two taxa, one is epiphytic/endophytic (*Ottia*) and the other is epilithic (*Pauciramus*).

Pauciramus K. P. Fang, F. R. Nan, & S. L. Xie gen. nov.

Type species: *Pauciramus yunnanensis* K. P. Fang, F. R. Nan, & S. L. Xie sp. nov.

Description: Small freshwater red alga, caespitose and densely pulvinate, consisting of gracile and uniseriate filaments with well-developed rhizoids, rarely branched; cylindrical vegetative cells with a single, ribbon-shaped and parietal chloroplast, without pyrenoids; reproduction by tetraspores, tetrasporangia single, obovoidal, cruciately divided sporangial branchlet only at the upper portion of filaments. Sexual reproduction unknown.

Etymology: From Latin for the “sparse branch”.

Pauciramus yunnanensis K. P. Fang, F. R. Nan, & S. L. Xie sp. nov. (Fig.4a–i)

Description: Plants caespitose, woolly, densely pulvinate, purplish red, about 0.1–0.2-cm high, attached to the substratum by well-developed rhizoids, consisting of uniseriate, gracile filaments. Rhizoids forming from basal cells of filaments, comprising 1–3 cells, 30–80- μ m long, 3–5 μ m in diameter. Main filaments 50–200 cell-storeys, rarely branched at the base and middle, usually have dense sporangial short lateral branchlet at the upper portion. Vegetative cells cylindrical, 6–8 μ m in diameter and 5–14- μ m long, 1–2 times longer than diameter gradually increases from the base to the upper of main filaments, more or less constricted at the cross walls, walls 1.5–2.5- μ m thick, terminal hairs absent; each cell with a single, ribbon-shaped, parietal chloroplast, without pyrenoids. Reproduction by means of tetrasporangia, single, obovoidal, cruciately divided, 5–13 μ m in diameter and 7–15- μ m long, terminating short lateral branchlets consisting of 1–3 cells, unequally distributed only at the upper portion of thallus. Sexual reproduction unknown.

Diagnosis: Diagnostic DNA sequence: *rbcL*, SSU, and COI-5P (accession number: MW874643 for *rbcL*, MW879164 for SSU, and MW874644 for COI-5P).

Type locality: China—Yunnan, Ailao Mountain (23°58'22"N, 101°31'25"E, ASL: 1 987.7 m). On rocks hit by drops of mountain spring water in Shimenxia, a canyon of Ailao Mountain.

Holotype: Liquid immersion specimen (holotype: SXU-YN19053) and herbarium vouchers (isotype: SXU-YN19054, SXU-YN19055).

Etymology: The species epithet refers to the type locality (Yunnan, China).

4 DISCUSSION

Molecular phylogenetic analyses have had a significant impact on subclass Nemaliophycidae with considerable taxonomic changes at the family and order levels: Balbianiales from Acrochaetiales (Sheath and Müller, 1999), Balliales from Ceramiales (Choi et al., 2000), Colaconematales from Acrochaetiales (Harper and Saunders, 2002), and Thoreaales from Batrachospermales (Müller et al., 2002). The taxa of order Acrochaetiales have a simple vegetative structure consisting of monosiphonous, branched, heterotrichous, filaments with apical growth, reproduction by monosporangia or tetrasporangia, sexuality by simple carpogonium (Kumano, 2002), such that it has had the most confusing taxonomic histories in red algae, with about 800 species names and twenty generic names described and used (Harper and Saunders, 2002; Guiry and Guiry, 2021). The introduction of molecular phylogenetic analyses has significantly improved the complicated and chaotic situation. However, the relationship among genera and their familial assignments are still uncertain (Saunders et al., 1995; Volovsek et al., 2000; Harper and Saunders, 2002; Clayden and Saunders, 2008, 2014). In this study, we describe a newly collected uniseriate freshwater red algae (SXU-YN19053) from Ailao Mountain, Yunnan, China, based on morphological observations and phylogenetic analysis.

The phylogenetic trees revealed that Acrochaetiales is polyphyletic. The Ailao Mountain specimen (SXU-YN19053) was in a well-supported clade with *Ottia meiospora* (from Hawaii, USA) and sister to order Palmariales and other families of the Acrochaetiales. And the genetic distance levels between *Ottia-Pauciramus* complex and the other taxa of Acrochaetiales-Palmariales complex (including order Acrochaetiales, order Palmariales, and the only freshwater group *Audouinella*) are significantly higher than the genetic distance between these taxa. This indicates that *Ottia-Pauciramus* complex may have the same status as Acrochaetiales and Palmariales at the order level, and *Ottia-Pauciramus* complex can be regarded as an order, so that it can solve the polyphyly of Acrochaetiales.

Molecular phylogenetic analyses based on *rbcL*, SSU, and COI-5P show that the *rbcL* sequence has better distinguishability at different taxonomic levels

of order, family, and genus, which may be used as a basis for classification of genus, family, and order in the future.

The molecular sequences of the freshwater taxa are very rare in Acrochaetiales-Ottiales-Palmariales at present. *Audouinella* is the only genus with both marine and freshwater representatives. As molecular analyses have shown that many of these freshwater taxa are sporophyte stages of other freshwater orders, (Necchi and Zucchi, 1997; Pueschel et al., 2000; Chiasson et al., 2005, 2007; Necchi and Oliveira, 2011; Chen et al., 2014), there are now comparatively few species of true *Audouinella* with only one species *A. hermannii* supported by molecular data. Many scholars believe that the freshwater groups of *Audouinella* should be classified into a family Audouinellaceae that needs to be resurrected (Harper and Saunders, 2002; Saunders et al., 2018). Similarly, *Ottia* has only one species supported by molecular sequence. Therefore, there are only three freshwater species supported by molecular evidence including the newly proposed taxa in this study. For a monospecific genus seems impractical for the establishment of a new family, only a new genus *Pauciramus* was proposed in a new order Ottiales to accommodate the uniseriate red algal described in this study, and provisionally classified this taxon to the family Ottiaceae.

5 DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S3 and Supplementary Figs.S1–S3) is available in the online version of this article at <https://doi.org/10.1007/s00343-021-1154-2>.