Available online at www.sciencedirect.com

ScienceDirect

European Journal of Protistology 65 (2018) 16–30

European Journal of PROTISTOLOGY

www.elsevier.com/locate/ejop

New contributions to *Gruberia lanceolata* (Gruber, 1884) Kahl, 1932 based on analyses of multiple populations and genes (Ciliophora, Heterotrichea, Gruberiidae)

Xiangrui Chen[a,1], Shahed Uddin Ahmed Shazib[b,1], Ji Hye Kim[b,c], Min Seok Kim[b,d], Mann Kyoon Shin[b,*]

[a] *School of Marine Sciences, Ningbo University, Ningbo 315211, China*
[b] *Department of Biological Sciences, University of Ulsan, Ulsan 44610, South Korea*
[c] *Natural Science Research Institute, Gangneung-Wonju National University, Gangneung 25457, South Korea*
[d] *Nakdong River Environment Research Center, Goryeong-gun, Gyeongbuk 40103, South Korea*

Received 10 December 2017; received in revised form 23 April 2018; accepted 2 May 2018
Available online 9 May 2018

Abstract

Gruberia Kahl, 1932 is a species-poor genus comprising only seven named species. Most of these species have not been reinvestigated since the original reports. In the present work, we investigated the taxonomy and phylogeny of *Gruberia lanceolata* (Gruber, 1884) Kahl, 1932 based on analyses of morphology and multiple gene sequences from four South Korean populations. This species is mainly characterized by a well-developed peristome region, segmented paroral membrane, and moniliform macronucleus. Some morphological features were not stable among the four populations investigated, such as body shape and size, cell color, and the ratio of oral length to body length. However, our molecular analyses of four different genetic markers – three nuclear DNA markers (18S rDNA, ITS1-5.8S-ITS2 region, D1D2 of 28S rDNA) and one mitochondrial (mt) marker (CO1 gene) – indicated that all Korean populations examined were the same species. Based on our present findings and historic works, we propose that *G. calkinsi*, *G. aculeata*, and *G. beninensis* are junior synonyms of *G. lanceolata*.

Keywords: *Gruberia lanceolata*; Heterotrichea; Morphology; Multiple genes; Phylogeny

Introduction

Gruberia Kahl, 1932 and *Spirostomum* Ehrenberg, 1834 were the two main members of the family Spirostomidae Stein, 1867 in the classification systems of Corliss (1979) and Lynn (2008). Molecular studies conducted over the past 10 years, however, do not support this grouping (Boscaro et al. 2014; Gong et al. 2007; Miao et al. 2009; Schmidt et al. 2007; Thamm et al. 2010). Based on morphological characters and further evidence from molecular analyses, Shazib et al. (2014) established the family Gruberiidae comprising only the name-bearing type-genus *Gruberia*. Indeed, Gruberiidae species have a segmented paroral membrane and meridional somatic ciliature during body contraction, which are the main differences to the Spirostomidae.

Gruberia Kahl, 1932 is a species-poor genus comprising only seven species ever described: *G. uninucleata* Kahl, 1932 (type species); *G. lanceolata* (Gruber, 1884) Kahl, 1932; *G. calkinsi* Beltran, 1933; *G. aculeata* Ozaki and Yagiu, 1941; *G. binucleata* Dragesco, 1954; *G. nematodomorpha* Lepsi,

*Corresponding author.
E-mail address: mkshin@ulsan.ac.kr (M.K. Shin).
[1] These authors contributed equally.

https://doi.org/10.1016/j.ejop.2018.05.001
0932-4739/

1965, and *G. beninensis* Dragesco and Dragesco-Kernéis, 1986. Only *G. calkinsi* and *G. beninensis* were studied using silver staining method. *Gruberia* is therefore considered a rare and poorly investigated genus.

We collected four populations of *Gruberia lanceolata* from brackish or saline waters of South Korea, and described them using the basic criteria recently proposed by Warren et al. (2017). We present and discuss morphological and molecular data on these populations obtained by live observations, protargol-impregnation techniques, and sequencing of multiple genes. Based on our newly collected morphological and phylogenetic data, we clarify contradictions and errors in the taxonomy of four species of *Gruberia*. This study represents a new contribution to the recently published literature on heterotrichid ciliates (Chen et al. 2017; Fernandes et al. 2015, 2016; Pan and Stoeck 2017; Shazib et al. 2014, 2016; Yan et al. 2015, 2016).

Material and Methods

Sample collection

Four populations of *Gruberia lanceolata* were collected from 2012 to 2014 in South Korea. All samples were collected from brackish or saline waters. The Ulsan population was collected from a drainage outlet in the Taehwagang area (salinity 19‰) (N35°32′59″, E129°21′01″), Ulsan, South Korea on 18 June 2014; Jeju population-1 was collected from brackish water (salinity 10‰) with decaying algae (*Ulva lactuca*) in a salt wetland of Ojo-ri (N33°27′50″, E126°54′53″), Jeju Island, South Korea on 23 June 2014; Jeju population-2 was collected from saline water (salinity 39‰) with red debris in a salt wetland of Ojo-ri (N33°27′54″, E126°55′04″), Jeju Island, South Korea on 14 June 2012; and the Jindo population (N34°21′50″, E126°09′50″) was collected from saline water (salinity 39‰) with debris in a salt wetland, Jindo Island, South Korea on 7 July 2016.

Morphological investigations

The raw cultures were kept in the laboratory for about two weeks. Living cells were observed using bright field and differential interference contrast microscopy (Axio Imager A1; Carl Zeiss). The protargol silver preparation method described by Wilbert (1975) was used to reveal the infraciliature. The protargol reagent was synthesized following the protocol of Pan et al. (2013). Counts, measurements, and drawings of stained specimens were carried out with the help of a camera lucida. Terminology and systematics mainly follow Lynn (2008) and Shazib et al. (2014).

DNA extraction, amplification, and sequencing

Single cells from each population were picked, washed and transferred into 1.5 ml microtubes for molecular analyses. Genomic DNA was isolated using the RED Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, MO, USA), as described by Shazib et al. (2014). Ribosomal DNA sequences (18S rDNA, ITS1-5.8S-ITS2 region, and D1D2 region of 28S rDNA) and mitochondrial CO1 gene sequences were amplified by PCR reactions using the TaKaRa high fidelity Ex Taq DNA polymerase Kit (TaKaRa Bio-medicals, Otsu, Japan). PCR primers, reaction cycling parameters and sequencing details can be found in Shazib et al. (2016) and Strüder-Kypke and Lynn (2010).

Genetic distances and phylogenetic analyses

Sequences were checked and assembled into contig using Geneious ver. 8.1.7 (http://www.geneious.com, Kearse et al. 2012). For 18S rDNA gene phylogenetic analysis, all sequences, except those generated for the three new *Gruberia lanceolata* populations (Ulsan population, Jeju population-1, and Jindo population), were retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/). Alignments were constructed in Geneious using the MUSCLE algorithm (Edgar 2004). Unreliable and poorly aligned columns were masked and removed using Gblocks ver. 0.91b (Castresana 2000; Talavera and Castresana 2007). The final 18S rDNA sequence alignment contained 1523 nucleotide characters. GTR + G + I was the best fitting evolutionary models for the alignment were selected under the Akaike Information Criterion (AIC) in MrModeltest ver. 2.3 (Nylander 2008). Bayesian inference (BI) was performed in MrBayes ver. 3.2.6 (Ronquist et al. 2012) using the GTR + G + I nucleotide substitution model, with two parallel runs. MCMC chain was run for one million generations with a sampling frequency of 100 and the first 2500 trees were discarded as burn-in. Maximum likelihood (ML) analyses were carried out using RAxML-HPC2 ver. 8.2.10 (Stamatakis 2014) on the CIPRES Science Gateway ver. 3.3 (http://www.phylo.org/index.php/portal/v33) using the GTR + G + I evolutionary model. The best scoring ML tree was searched using a rapid bootstrap analysis (Stamatakis 2014). Reliability of the branching patterns in the best ML tree were calculated from the 1000 bootstrap replicates. Phylogenetic trees were visualized and edited using the software FigTree ver. 1.4 (A. Rambaut at http://tree.bio.ed.ac.uk/software/figtree/). Six karyorelictean species were considered as the outgroup taxa for rooting the trees. Pairwise uncorrected *p*-distances and numbers of nucleotide differences for all four DNA markers (18S rDNA, ITS1-5.8S-ITS2 region, D1D2 region of 28S rDNA, mt CO1) among five *Gruberia* taxa were calculated in Geneious.

Results and Discussion

Class Heterotrichea Stein, 1859

Order Heterotrichida Stein, 1859