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European Journal of Protistology 50 (2014) 134–152

European Journal of PROTISTOLOGY

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Bozasella gracilis n. sp. (Ciliophora, Entodiniomorphida) from Asian elephant and phylogenetic analysis of entodiniomorphids and vestibuliferids

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Received 1 May 2013; received in revised form 17 January 2014; accepted 20 January 2014
Available online 25 January 2014

Abstract

Bozasella gracilis n. sp. in the order Entodiniomorphida was found in fecal samples of an Asian elephant kept in a zoo. The ciliate has general and infraciliary similarities to the families Ophryoscolecidae and Cycloposthiidae. Phylogenetic trees were inferred from 18S rRNA gene sequences of *B. gracilis*, 45 entodiniomorphids, 10 vestibuliferids, 5 macropodiniids, and an outgroup, using maximum likelihood, Bayesian inference, and neighbor joining analyses. Of them, there were 32 new sequences; 26 entodiniomorphid species in the genera, *Bozasella, Triplumaria, Gassovskiella, Ditoxum, Spirodinium, Triadinium, Tetratoxum, Pseudoentodinium, Ochoterenaia, Circodinium, Blepharocorys, Sulcoarcus, Didesmis, Alloiozona, Blepharoconus*, *Hemiprorodon*, and *Prorodonopsis*, and 6 vestibuliferid species in the genera, *Buxtonella, Balantidium, Helicozoster, Latteuria*, and *Paraisotricha*. Thirty additional sequences were retrieved from the GenBank database. Phylogenetic trees revealed non-monophylies of the orders Entodiniomorphida and Vestibuliferida, the suborders Entodiniomorphina and Blepharocorythina, and the families Cycloposthiidae and Paraisotrichidae. *Bozasella gracilis* was sister to *Triplumaria*. In addition, to avoid homonymy, we propose Gilchristinidae nom. nov., *Gilchristina* nom. nov. and *Gilchristina artemis* (Ito, Van Hoven, Miyazaki & Imai, 2006) comb. nov.

Keywords: *Bozasella gracilis* n. sp.; 18S rRNA gene sequence; Elephant; Entodiniomorphida; *Gilchristina artemis* (Ito, Van Hoven, Miyazaki & Imai, 2006) comb. nov.; Vestibuliferida

Introduction

Intestinal ciliates in the order Entodiniomorphida and the order Vestibuliferida have been described from African and Asian elephants (Buisson 1923; Eloff and Van Hoven 1980; Kofoid 1935; Ito et al. 2010, 2011; Latteur 1958, 1966, 1967; Latteur and Bousez 1970; Latteur and Dartevelle 1971; Latteur et al. 1970; Mandal and Choudhury 1983a,b, 1984; Timoshenko and Imai 1995, 1997; Wolska 1967, 1968, 1970, 1986). In our study on the phylogeny of entodiniomorphids and vestibuliferids, we found a unique new entodiniomorphid species from Asian elephants, which has morphological characters of both the family Ophryoscolecidae and the family Cycloposthiidae in its general appearance and infraciliature. In the following, we describe this new species, *Bozasella gracilis* n. sp. and its infraciliary bands. Further, we sequenced

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http://dx.doi.org/10.1016/j.ejop.2014.01.003

the 18S rRNA gene of *B. gracilis*, 25 entodiniomorphids, and six vestibuliferids and discuss the phylogenies of the orders Entodiniomorphida and Vestibuliferida.

Material and Methods

Sampling

Fecal samples containing *Bozasella gracilis* n. sp. for morphological study were collected from a female Asian elephant (*Elephas maximus maximus*) which was born in Sri Lanka and was kept in Tokushima zoo, Tokushima prefecture, Japan. The samples were immediately fixed in five times the volume of 10% formalin solution within five minutes after defecation and stored in a dark place after they were filtered through two layers of gauze into a bottle in order to remove plant and feed material.

We collected 12 samples for the 18S rRNA gene sequences and isolated 1–7 species from each sample. Cells of *B. gracilis*, 25 entodiniomorphid ciliates and six vestibuliferid ciliates were obtained from these samples of five elephants, four horses, and a cattle listed in Table 1. Fecal samples were fixed within five minutes after defecation. Intestinal contents of a horse were fixed immediately after euthanasia due to limb disorder. A rectal content of a cattle was fixed just after rectal examination. All samples were fixed in five times the volume of 80% ethanol and preserved in the refrigerator (4 °C) after they were filtered through two layers of gauzes; the supernatant was replaced with 100% ethanol. All samples were also fixed in five times the volume of 10% formalin solution to determine their species composition. Micrographs of 25 entodiniomorphid ciliates and six vestibuliferid ciliates fixed in formalin solution were shown in Figs 13–43.

Morphology and silver impregnation

The infraciliary bands of the new species were stained using the pyridinated silver carbonate impregnation method,

Table 1. List of entodiniomorphid and vestibuliferid ciliates analyzed in this study, including specimen, host animal, and location.

Species	Specimen	Host animal (birth place)	Location
Order Entodiniomorphida			
Bozasella gracilis n. sp.	Feces	Asian elephant (Sri Lanka)	Tokushima Zoo
Triplumaria solea	Feces	Asian elephant (Sri Lanka)	Tokushima Zoo
Triplumaria sukuna	Feces	Asian elephant (India)	Kobe Oji Zoo
Triplumaria dvoinosi	Feces	Asian elephant (Sri Lanka)	Tokushima Zoo
Triplumaria fulgora	Feces	Asian elephant (India)	Kobe Oji Zoo
Triplumaria harpagonis	Feces	Asian elephant (India)	Kobe Oji Zoo
Gassovskiella galea	Feces	Yonaguni horse	Yonaguni island
Ditoxum funinucleum	Feces	Yonaguni horse	Yonaguni island
Spirodinium equi	Feces	Yonaguni horse	Yonaguni island
Triadinium caudatum	Dorsal colon	Pony	Matsue
Tetratoxum parvum	Feces	Yonaguni horse	Yonaguni island
Tetratoxum unifasciculatum	Feces	Riding horse	Yasugi
Tetratoxum excavatum	Dorsal colon	Pony	Matsue
Pseudoentodinium elephantis	Feces	Asian elephant (India)	Kobe Oji Zoo
Ochoterenaia appendiculata	Feces	Riding horse	Yasugi
Circodinium minimum	Feces	Yonaguni horse	Yonaguni island
Blepharocorys microcorys	Feces	Riding horse	Yasugi
Blepharocorys angusta	Feces	Yonaguni horse	Yonaguni island
Blepharocorys jubata	Feces	Kiso horse	Matsue
Blepharocorys uncinata	Cecum	Pony	Matsue
Sulcoarcus pellucidulus	Feces	Yonaguni horse	Yonaguni island
Didesmis ovalis	Ventral colon	Pony	Matsue
Alloiozona trizona	Cecum	Pony	Matsue
Blepharoconus hemiciliatus	Feces	Riding horse	Yasugi
Hemiprorodon gymnoprosthium	Ventral colon	Pony	Matsue
Prorodonopsis coli	Dorsal colon	Pony	Matsue
Order Vestibuliferida			
Buxtonella sulcata	Rectum	Japanese black beef cattle	Matsue
Balantidium coli	Feces	Asian elephant (India)	Japan
Helicozoster indicus	Feces	Asian elephant (Thailand)	Miyazaki Phoenix Zoo
Latteuria polyfaria	Feces	African bush elephant (South Africa)	Himeji Central Park
Latteuria media	Feces	Asian elephant (India)	Kobe Oji Zoo
Paraisotricha minuta	Ventral colon	Pony	Matsue