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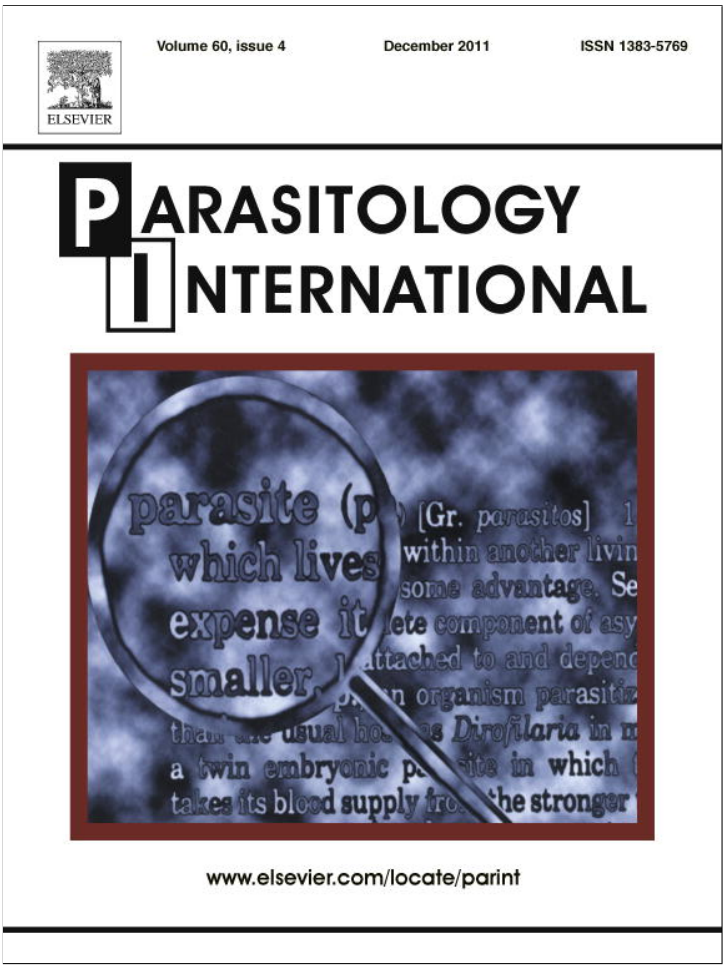

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Parasitology International 60 (2011) 541–543

Contents lists available at SciVerse ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Short communication

Genetic characterization of the *Psilotrema* (Digenea: Psilostomatidae) genus by partial 28S ribosomal DNA sequences

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ARTICLE INFO

Article history:
Received 10 February 2011
Received in revised form 8 August 2011
Accepted 12 September 2011
Available online 17 September 2011

Keywords:
Digenea
Psilostomatidae
Psilotrema
28S ribosomal DNA
Russian Far East

ABSTRACT

In the present study, we successfully amplified the 28S ribosomal DNA of 3 species of the *Psilotrema* genus (*Psilotrema oschmarini* Besprozvannykh, 2007, *P. acutirostris* Oschmarin, 1963 and *P. simillimum* Muhling, 1989) from the Russian Far East. The genetic divergence between these species was estimated, and the phylogenetic relationships of the *Psilotrema* genus and the Psilostomatidae family were inferred. The resulting data support the independent taxonomical status of *P. oschmarini* as a valid species in the Russian Far East. In spite of the considerable morphological differences between *P. simillimum* and *P. acutirostris*, these species are genetically very close to each other. Genetic distances, calculated from the pairwise comparison of two obtained clusters using transversions, show that Psilostomatidae is the most divergent family. Phylogenetic analysis revealed two clusters, which corresponds to family Psilostomatidae (basal cluster) and other Echinostomatoidea species.

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1. Introduction

The family Psilostomatidae consists of 13 genera of gastro-intestinal parasites of birds and mammals that, apart from the absence of a circumoral head-collar armed with spines (the presence of collar spines and their number constitute a very important parameter for the species determination), closely resemble the Echinostomatidae in their general morphology [1]. The genus *Psilotrema* of the family Psilostomatidae described in 1913, contains twelve species, distributed throughout Europe and Asia. The *Psilotrema* species used in the present study have a two-host life cycle involving one aquatic intermediate host and predacious definitive hosts. The intermediate host is a gastropod species of *Boreolona* or *Parafossaruls*, and the definitive hosts are piscivorous birds [2,3]. Species of the Psilostomatidae family are known to infect amphibians, waterfowl and mammals [4–5].

Aside from a few reports of Psilostomatidae species from different locations, there are few molecular and genetic studies contributing to the knowledge of molecular taxonomy and phylogeny in this group.

The aim of this study was to evaluate the phylogenetic relationships of the genus *Psilotrema* using ribosomal DNA sequence data generated from three species from South of Eurasian Far East.

As previously described [2,3], *P. oschmarini*, *P. acutirostris* and *P. simillimum* adults (a total of 10 specimens) were obtained from infected chickens during experimental work and fixed in 70% ethanol. DNA extraction was performed using proteinase K lysis followed by boiling with Chelex-100 [6]. The 28S rDNA fragments were amplified using the polymerase chain reaction (PCR) with the DIG12 (5′-AAG CAT ATC ACT AAG CGG-3′) and 1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′) primers following a standard procedure [7]. The PCR products were directly sequenced using the ABI Big Dye Terminator v.3.1 Cycle Sequencing kit (according to the manufacturer's instructions) and the ABI 3130 genetic analyzer at the Institute of Biology and Soil Science FEB RAS. The resulting sequences were submitted to the EMBL nucleotide database with the following accession numbers: *P. oschmarini*, FR819768–FR819774; *P. simillimum*, FR819775–FR819776 and *P. acutirostris*, FR819777. The 28S rDNA sequences were assembled with SeqScape v.2.6 software and aligned using MEGA 4.0 alignment explorer with default options [8]. The distance matrix was constructed using the general time reversible model (GTR) with gamma distribution [9]. This model showed the best fit to the data using Modeltest v. 3.07 software [10], with the Akaike information criterion [11]. Phylogenetic analysis of the nucleotide sequences, including neighbor-joining, minimum evolution, maximum parsimony, maximum likelihood and Bayesian methods, was performed using MEGA 4.0, PAUP v. 4b10 [12] and MrBayes v. 2.01 software [13]. A nonparametric bootstrap with 1000 replicates was used to evaluate the robustness of the clusters through nodal support [14].

The amplified 28S rDNA fragment of the *Psilotrema* species was approximately 900 bp, and the aligned sequences of this fragment were 759 bp in length. The values of the variable and parsimony-informative sites between the *Psilotrema* species were 13 and 12 for both *P. oschmarini*/*P. simillimum* and *P. oschmarini*/*P. acutirostris*. There was only one variable site between the 28S rDNA sequences

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doi:10.1016/j.parint.2011.09.005

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of the *P. acutirostris* and *P. simillimum* species. There were no variable sites among the 28S rDNA sequences of the different specimens of *P. oschmarini*. The *P. acutirostris* and *P. simillimum* species were closely related to each other (d = 0.01%) and with *P. oschmarini* (d = 1.7%) by the genetic divergence estimation. Divergence between the two genera of Psilostomatidae (*Psilotrema* and *Psilochasmus*) was 9.9%, which was almost equal to the minimal values of divergence between echinostomid families. The genetic divergence between the different families ranged between 10.3% and 22.9%, and the Psilostomatidae family was closely related to the Echinostomatidae family (d = 11.7%) and was more distantly related to the Cyclocoelidae family (p = 22.9%). Phylogenetic analysis revealed two different clusters of the Echinostomatidae superfamily (Fig. 1). The first cluster contained the Psilostomatidae family: it was monophyletic and had a high statistical support (bootstrap = 84–100%). Within this cluster, *P. acutirostris* and *P simillimum* were sister species (bootstrap = 99%), whereas *P. oschmarini* was a distinct group (bootstrap = 99–100%) and *P. oxyurus* was a basal species. The second cluster included the other echinostomids with low bootstrapping (<50%). The genetic divergence between the two obtained clusters, calculated using all substitution types (d), was 14.2%, and the divergence, calculated using only transversions (v), was 4.2%. This value pointed to a higher divergence between the two clusters in comparison with the divergence between the four different families that grouped in the second cluster (v = 3.0–3.7%).

Our data support the independent taxonomical status of *P. oschmarini* as a valid species in the Russian Far East [3]. In spite of the considerable morphological differences between *P. simillimum*

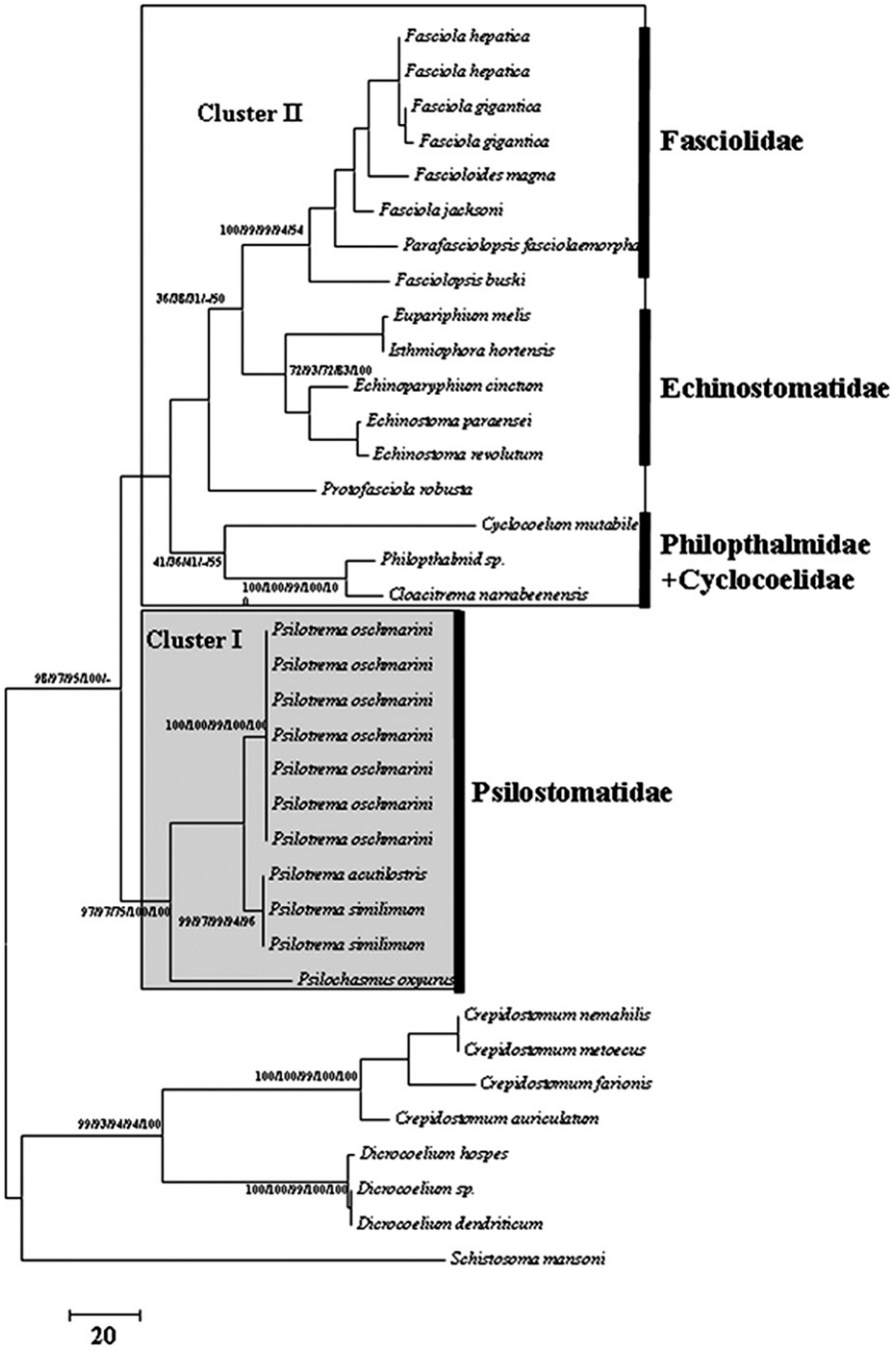

Fig. 1. Maximum parsimony tree of Echinostomatidae employing a GTR + G substitution model for a partial 28S rDNA sequence dataset. The nodal numbers are bootstrap statistical values (%) as determined by NJ/ME/MP/ML/Bayesian methods.

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and *P. acutirostris* [2,3], these species are genetically very close to each other. Our results are in agreement with the majority of taxonomical concepts and indicate the evident differentiation between Psilostomatidae and the other species of Echinostomatidae [1,15–20] and the close relationships between the Psilostomatidae and Echinostomatidae species [4,18]. Genetic distances, calculated from the pairwise comparison of two obtained clusters using transversions, showed that Psilostomatidae is the most divergent family (v = 4.2%), suggesting that base transversions are the main factor for the differentiation of Psilostomatidae from the other Echinostomatidae species, despite that fact that base transitions prevail. The accumulation of transversion-type substitutions is known to be often found at high taxonomical levels. The molecular data obtained demonstrated an initial step in the accumulation of transversional substitutions between the 28S rDNA sequences of Psilostomatidae and the other species of Echinostomatidae, which can be regarded as possible evidence of the earliest differentiation of the Psilostomatidae from the ancestral trematodes of the Echinostomatidae. Further studies are needed to uncover the phylogeny of the *Psilotrema* genus and the Psilostomatidae family.

Acknowledgments

This study was supported by a research grant of Far Eastern Branch of Russian Academy of Sciences (No. 11-III-B-06-068). I would like to thank Vladimir V. Basprozvannykh Ph.D. (IBSS FEB RAS) for providing the material and important notes and comments, Samer Alasaad Ph. D., Alexandra Dubrovina Ph.D., and Elena Sundukova Ph. D. for helpful comments.

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