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Determination of phylogenetic relationships of two species of *Phalangium* (Opiliones: Phalangiidae) by using 28S rRNA region

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Abstract

The species *Phalangium nalanae* Kurt, Erdek & Kurt 2023 and *Phalangium taylani* Kurt, Erdek & Kurt 2023 were previously described from Hakkari, Turkey based on morphological data and no molecular data are presented. In this study, the 28S rRNA gene region of these species belonging to the genus *Phalangium* was sequenced, and phylogenetic relationships of these species were revealed by performing maximum likelihood (ML) analysis and Bayesian inference (BI) analysis.

Keywords: 28S rRNA, Harvestmen, Phylogenetic analyses

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28S rRNA gen bölgesi kullanılarak iki *Phalangium* (Opiliones: Phalangiidae) türünün filogenetik ilişkilerinin belirlenmesi

Özet

Phalangium nalanae Kurt, Erdek & Kurt 2023 ve *Phalangium taylani* Kurt, Erdek & Kurt 2023 türleri morfolojik verilere dayanarak daha önceden, Türkiye (Hakkâri)'den tanımlanmış ve türlere ait moleküler veriler sunulmamıştır. Bu çalışmada; *Phalangium* cinsine ait bu türlerin 28S rRNA gen bölgesi dizilenmiş, maksimum olabilirlik (ML) analizi ve Bayesian çıkarım (BI) analizi yapılarak bu türlerin filogenetik ilişkileri ortaya konulmuştur.

Anahtar kelimeler: 28S rRNA, filogenetik analiz, otbiçen

1. Introduction

Opiliones, also known as harvestmen, are an order of arachnids that includes 6,740 known species worldwide. So far, more than 100 species have been reported from Türkiye [1, 2, 3].

Phalangium is a genus belonging to the order Opiliones (harvestmen) and approximately 42 species have been described worldwide [3]. Only 8 of these species are known from Türkiye [4, 5, 6, 7]. *Phalangium nalanae* and *P. taylani*, which belong to the genus *Phalangium*, were collected from Hakkari, Türkiye, and described based on their morphological characteristics. However, the study did not provide any information on the molecular data or phylogenetic relationships of the newly described species [6].

In recent years, morphological data as well as molecular techniques have been successfully used to identify and reclassify the species. The 28S rRNA gene region is commonly utilized to determine the phylogenetic relationships arachnids and other invertebrates [8]. At the same time, the 28S rRNA gene region is often used in conjunction with other gene regions for the determination of phylogenetic relationships among harvestmen [9, 10, 11, 12, 13, 14].

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This study aimed to analyze the phylogenetic positions and evolutionary relationships of *Phalangium nalanae* and *P. taylani* using 28S rRNA sequence data.

2. Materials and Methods

2.1.Sample collection and identification

All specimens of *Phalangium nalanae* and *P. taylani* collected from Hakkari, Türkiye were identified by Dr. Kemal KURT (Gümüşhane University, Türkiye) and preserved in 70% ethyl alcohol in the GUSAL (The Arachnology Laboratory at Şiran Vocational School, Gümüşhane University, Türkiye) [6]. The specimens stored in GUSAL were used for molecular studies.

2.2. DNA extraction, PCR amplification and sequencing

One adult individual from each species was washed with distilled water and the whole body and legs were smashed and genomic DNA was extracted using GeneAll & Exgene Tissue SV (Korea), following the manufacturer's instructions. The 28S rRNA gene was amplified with the primers ZX1: 5'-ACCCGCTGAATTTAAGCATAT-3' and ZR2: 5'-GCTATCCTGAGGGAAACTTCGG-3' [15].

PCR was performed using a total volume of 20 µl, which included 2 µl of DNA template, 10 µl of Mastermix (2x), 0.5 µl of each primer, and 7 µl of sterile distilled H2O. The amplification conditions consisted of an initial denaturation step at 95°C for 5 min, followed by denaturation at 95°C for 30 s, annealing at 52°C for 30 s, elongation at 72°C for 30 s, and a final elongation step at 72°C for 5 min (GeneAll, Seoul, Korea). The PCR products underwent evaluation for successful amplification through gel electrophoresis in 1% agarose. Subsequently, they were purified using a DNA gel extraction kit (WizPure™; Cat no: W1401).

2.3. Phylogenetic analyses

28S rRNA sequences obtained were blasted with NCBI BLAST to confirm species identification and to find sequence data of similar species, and the results were downloaded from GenBank [16]. Phylogenetic analyses were performed based on 28S rRNA gene sequences obtained from *Phalangium nalanae* and *P. taylani* and additional sequences of other species were retrieved from GenBank. The accession numbers for all sequences used in the phylogenetic analysis are listed in Table 1. The 28S rRNA sequences were aligned using Bioedit 7.2.5 version Software program [17]. The best-fit substitution model was determined using JModelTest v.2.1.8 [18]. The model with the lowest AIC (Akaike's information criteria) degree was selected [19]. The phylogenetic tree was reconstructed using Bayesian Inference (BI) analysis (MrBayes v.3.2.6) [20] and Maximum Likelihood (ML) analysis (MEGA X) [21]. Bootstrap analyses with 1000 replicates were used to evaluate the ML trees. The statistical support of the resulting BI trees was determined based on Bayesian posterior probability (BPP). Nodes with a BPP of 95% or greater were considered significant [22]. Bayesian Posterior Probability (BPP) was used to determine the BI tree topology. Uncorrected pairwise sequence divergence among 28S rRNA gene were calculated using MEGA version X [21].

Table 1. GenBank accession numbers for the samples used in the phylogenetic analysis

Species name	Sequence accession numbers	References
Paroligolophus agrestis	JQ437106	[12]
Rhampsinitus sp.	GQ912757	[10]
Odiellus pictus	JQ437107	[12]
Mitopus morio	KP276371	[23]

3. Results

Taxonomy

***Phalangium nalanae* Kurt, Erdek & Kurt 2023**

Description: Description of the species see Kurt et al., 2023[6].

Specimens examined: Türkiye, Hakkari Province, Ceyhanlı Village Road, 37°28'57.5"N; 43°33'49.8"E, 03.06.2020, leg. M. Erdek.

Distribution: Up to now only known from type locality in the Hakkari Province, Türkiye.

***Phalangium taylani* Kurt, Erdek & Kurt 2023**

Description: Description of the species see Kurt et al., 2023[6].

Specimens examined: Türkiye, Hakkari Province, Yüksekova District, Gürdere Village Road, 37°29'20.50"N 44°12'40.60"E, 03.06.2020, leg. M. Erdek.

Distribution: Up to now only known from type locality in the Hakkari Province, Türkiye.

Phylogenetic analyses.

A total of 506 base pairs of the 28S rRNA gene was obtained for two *Phalangium* species. According to model test results, the best-fit substitution model was chosen as HKY + G [24] for the 28S rRNA gene. Bayesian tree topology built on the basis of the 28S rRNA gene shows that the this two *Phalangium* species are quite different from *Phalangium opilio* species (Figure 1). The rooted tree is divided into two well-supported clades with 0.95 posterior probabilities. The two species are phylogenetically included in the genus *Phalangium* based on the 506-bp 28S rRNA. These two species diverged from each other with well supported 1.0 posterior probabilities. However, the genetic distance between *Phalangium* and other species was much lower (Table 2). This distance is about 0.04 % for the 28S rRNA gene of 506 bases between *Phalangium nalanae* and *P. taylani*.

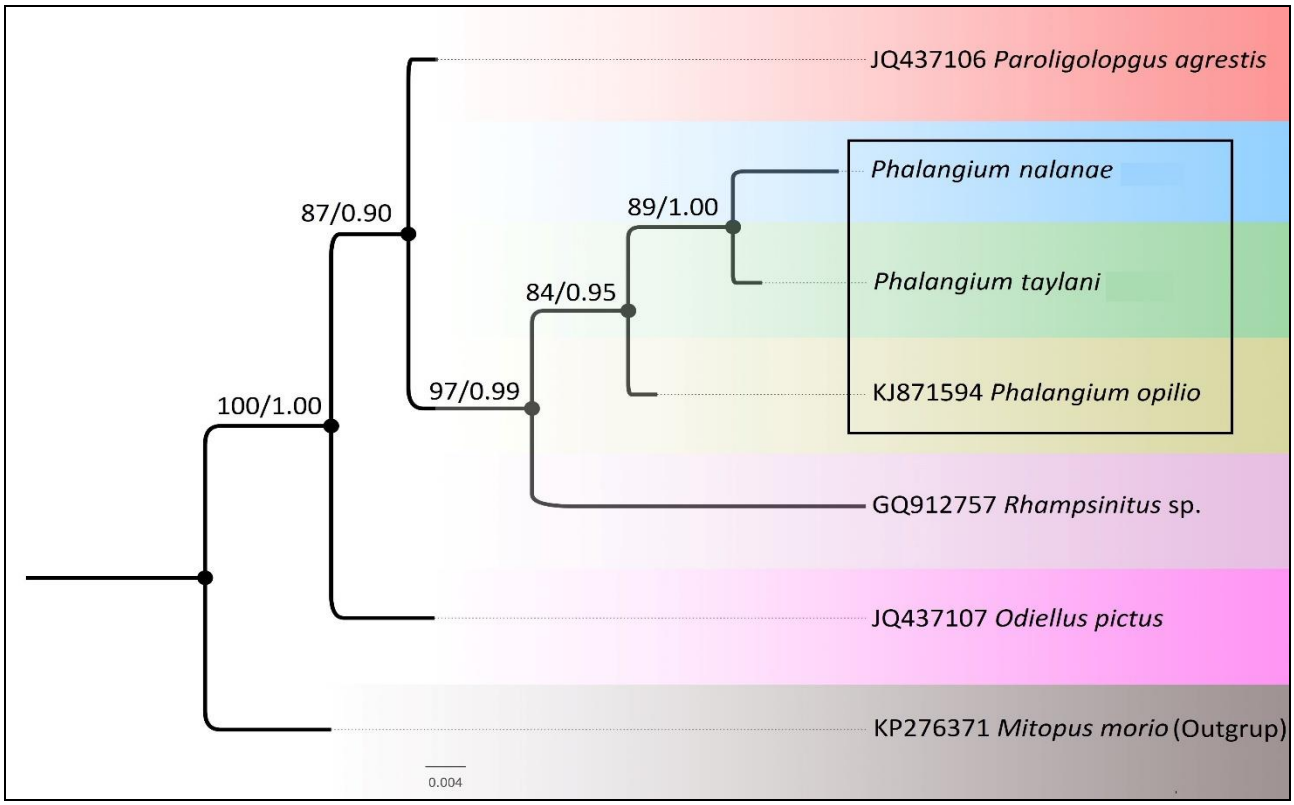

Figure 1. Phylogenetic tree based on combined 28S rRNA gene data. Bootstrap and posterior probability values are indicated above/below branches in order ML/BI

4.Conclusions and discussion

When the DNA sequences of the 28S rRNA gene obtained from GenBank and *Phalangium nalanae* and *P. taylani* species were analyzed, it was found that the genetic distances between the harvestmen species were very low. This did not lead to a situation where the species belonging to this group could not be separated from each other in the

phylogenetic tree. The phylogenetic analysis shows that the genus *Phalangium* is clearly separated from the other genera despite this low genetic distance, and the phylogenetic tree clearly shows that these two species are phylogenetically distinct from *Phalangium opilio*, a closely related species.

Since *Phalangium* species have been generally studied based on morphological data so far, this study is expected to make an important contribution to the literature. In addition, the lack of genetic data on *Phalangium* species in GenBank is very important in terms of adding new genetic data to the literature.

Table 2. Uncorrected genetic distances between some harvestmen species used in this study using a 506bp 28S rRNA gene fragment

Species	1	2	3	4	5	6	7
Phalangium nalanae	-						
Phalangium taylani	0.004						
Phalangium opilio	0.008	0.004					
Paroligolophus agrestis	0.016	0.012	0.008				
Odiellus pictus	0.022	0.018	0.014	0.006			
Rhampsinitus sp.	0.024	0.020	0.018	0.020	0.026		
Mitopus morio	0.030	0.026	0.022	0.014	0.014	0.034	-

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