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Original Scientific Paper

Geastrum juliae (Geastrales, Agaricomycetes), a new species from the Russian Far East

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ABSTRACT:
A new species, *Geastrum juliae*, is described from Primorsky Krai in the Russian Far East. Based on the analysis of sequences retrieved from GenBank, this taxon is also found in China. The closest species is *G. rubellum* based on morphology and phylogenetics. We present the new species with illustrated descriptions and molecular data.

Keywords:
earthstars, gasteroid fungi, Gasteromycetes, ITS nrDNA, molecular identification, taxonomy

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INTRODUCTION

The mycobiota of the Far East of the Russian Federation is characterised by high species diversity. Many fungal species known in Russia are reported in this region only, and more than 60 new species have been described in the course of the studies of fungi in the Russian Far East (Noordeloos & Morozova 2010; Malysheva *et al.* 2013; Justo *et al.* 2014; Rebriev & Bulakh 2015; Rebriev & Zvyagina 2022). The species diversity of the genus *Geastrum* Persoon in the Russian Far East is estimated at 10 species (Rebriev 2007; Rebriev *et al.* 2020). Most of them were found in the coniferous and deciduous forests of Amur Oblast, the Jewish Autonomous Oblast, Khabarovsk Krai, and Primorsky Krai.

During a field trip to Primorsky Krai in 2018, a *Geastrum* specimen was collected (1 open fruitbody and 2 primordia), which could not be identified by solely morphological methods. The most similar species in terms of morphology, but not identical, was *Geastrum rubellum* P.-A. Moreau & C. Lécuru (Accioly *et al.* 2019). The finding was made in a deciduous forest dominated by *Quercus mongolica*, on litter mixed with soil. An attempt to find additional specimens in the same location in 2021 was unsuccessful. At the same time, when studying the materials from the personal collection of E.M. Bulakh, an additional specimen was found, consisting of three open fruitbodies. It was collected in a broadleaf forest in the Udegeyskaya Legenda National Park, Primorsky Krai.

Molecular genetic techniques showed that the ITS sequences of the discussed specimens from the Far East are conspecific and cluster in the same clade with those obtained from specimens from China which have been deposited in GenBank under the name *G.* aff. *rubellum*. This well-supported clade is sister to *G. rubellum*. The combination of morphological and molecular data indicates that the studied samples belong to a new species for science.

Table 1. The taxa, vouchers, locations, and GenBank accession numbers of the ITS sequences used in this study. The new sequences are in bold; the type specimens are marked with an asterisk (*).

Taxon	Voucher	GenBank accession Nos.	Locality
Geastrum juliae	**LE-F 335197* (holotype)**	**OM935687**	**Russian Federation**
G. juliae	**LE-F 335198* (paratype)**	**OM935688**	**Russian Federation**
G. fuscogleba	NY Trappe 9500	KF988377	USA
G. hirsutum	MA:Fungi:67886* (paratype)	MH538295	Brazil
G. minutisporum	CORD14*(holotype)	KM260664	Argentina
G. mirabile	TNS:KH-JPN10-714	JN845109	Japan
G. neoamericanum	INPA:259949* (paratype)	MH635025	Brazil
G. pseudolimbatum	UPS F-560804	KF988420	Sweden
G. pusillipilosum	UFRN:Fungos 2315* (holotype)	KX761175	Brazil
G. rubellum	UFRN:Fungos:2844* (paratype)	MH634999	Brazil
G. aff. *rubellum*	130822MFBPZH1075	MW554089	China
G. aff. *rubellum*	110116MFBPL0057	MW554312	China
G. aff. *rubellum*	130822MFBPL0255	MW554444	China
G. brunneocapillatum	UFRN Fungos 2286* (holotype)	MH634996	Brazil
G. baculicrystallum	UFRN:Fungos:1857	MH635018	Brazil
G. striatum	MJ8807	KC581960	Sweden
G. hungaricum	MJ8915	KC581963	Hungary

MATERIALS AND METHODS

Morphology. The examination of the microstructures under a Mikmed-6 light microscope (LM) was conducted using a 5% KOH solution. Scanning electronic microscopy microphotographs (SEM) were taken with a Carl Zeiss EVO-40 XVP scanning electron microscope in the South Science Center RAS Center of Collective Use (No. 501994). The microscopic measurements were made using the Scandium 5.0 programme. The spore dimensions are given with the ornamentation. The specimens examined were deposited in the herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences LE-F.

Phylogenetic analyses. The genomic DNA for all the samples was extracted using the NucleoSpin™ Plant II kit. The DNA was then amplified with the primers ITS1-F and ITS4-B (Gardes & Bruns 1993). Purification of PCR products and sequencing were done by LGC Genomics GmbH company (Berlin, Germany). The sequences were assembled in Sequencher 5.4.6 (https://www.genecodes.com/). Seventeen ITS sequences, including 2 newly generated ones (marked in bold in Table 1) and 15 retrieved from the GenBank database, were used in the phylogenetic analysis. The sequences were first aligned in MAFFT (Katoh *et al.* 2019), then the alignment was manually optimised.

The trees generated by Maximum Likelihood (ML) and Bayesian Interference (BI) analyses were similar in topology. They were performed using RAxML v. 8.2 (Stamatakis 2014) implemented in raxmlGUI v. 2.0 (Edler *et al.* 2020) and MrBayes v. 3.2 (Ronquist *et al.* 2012), respectively. The ML tree with both bootstrap support (BS) and posterior probability values (PP) is shown in Fig. 1. PP values ≥ 0.7 and BS support values ≥ 50% are shown at the nodes. The newly sequenced collections of *G. juliae* are indicated in bold; the type specimens are indicated with an asterisk (*). Two-letter country codes (ISO 3166-1 alpha-2) reflecting the origin of the specimens are also provided.

Pairwise percent identity values (P%I) of the ITS sequences were calculated using Geneious v. 8.1.9 (https://www.geneious.com/).

RESULTS

Geastrum juliae **Rebriev, E.M. Bulakh & Biketova, *sp. nov.*** (Figs. 2–6)
Mycobank No: MB843279.

Diagnosis: Pseudoparenchymatous layer pale-cream with a pinkish tint when fresh, light brown to brown when dry. Basidiospores globose, 2.6–3.7 µm diam; densely verrucose in SEM, warts single or occasionally fused into small aggregations. Capillitium hyphae up to 4.0–4.5 µm wide.

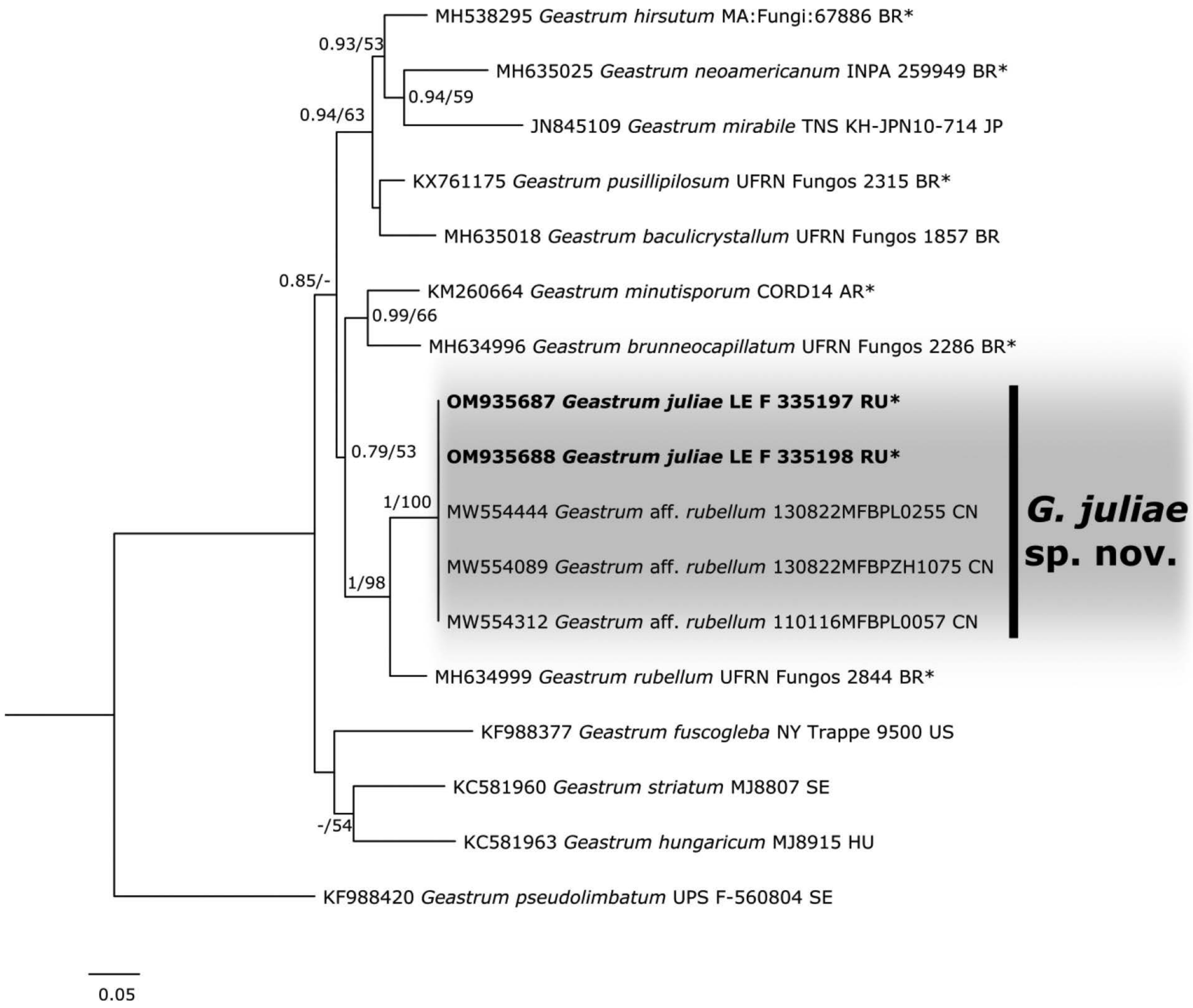

Fig. 1. The ITS rDNA phylogenetic tree of *Geastrum pro parte* obtained using Maximum Likelihood (ML). The bootstrap support (BS ≥ 50%) and posterior probability values (PP ≥ 0.7) are shown at the nodes. The newly sequenced collections of *G. juliae* are indicated in bold; the type specimens are indicated with an asterisk (*). The GenBank accession numbers of the sequences are indicated before the species names and the voucher numbers are given after. Two-letter country codes (ISO 3166-1 alpha-2) reflecting the origin of the specimens are provided.

Description: *Unexpanded basidiomata* 9–20 mm diam, egg-shaped when young and becoming subglobose to depressed-globose. Subiculum whitish to pale cream coloured, loose and poorly developed. *Expanded basidiomata* 11–26 mm high × 16–35 mm wide. *Exoperidium* saccate, non-hygrometric, splitting in 6–8 revolute or straight, more or less equal broad rays. *Mycelial layer* flesh-pink to pinkish-brown when fresh and brown when dried, tomentose and persistent. *Fibrous layer* loose papyraceous, white. *Pseudoparenchymatous layer* pale-cream with a pinkish tint when fresh, light brown to brown when dry, normally not cracked, persistent. *Endoperidial body* sessile, globose to subglobose, 7–18 mm diam, pale pinkish-grey, grey to brownish-grey; endoperidial surface glabrous. *Peristome* fibrillose, slightly lighter than or of the same colour as the endoperidial body, mammiform, inconspicuously delimited. *Stalk* absent. *Apophysis* absent. *Columella* well developed, intruding into more than half of the gleba. *Mature gleba* dark brown to blackish. *Basidiospores* brown, globose, 2.6–3.7 µm diam; ornamentation conspicuous in LM, densely verrucose in SEM and consisting of 0.25–0.4 µm in height, low, truncate warts, single or occasionally fused into small aggregations. *Capillitium hyphae* up to 4.0–4.5 µm wide, rarely septate, pale brown to brown and concolourous with the spores, not branched, thick-walled, with well visible lumen, tips acute.

Holotype: Russian Federation, Primorsky Krai, Krasnoarmeysky District, the Udegeyskaya Legenda National Park, N 45.75°, E 135.47°, on litter in a broadleaf forest, 20.08.2015, E. M. Bulakh (Holotype LE-F 335197; ITS sequence GenBank OM935687).

Fig. 2. Russia, in the vicinity of the village of Gorno-Tayezhnoe, a broadleaf forest dominated by *Quercus mongolica* (Photo Yu. Rebriev).

Additional specimens examined: Russian Federation, Primorsky Krai, Ussuriysky District, the village of Gorno-Tayezhnoe, N 43°42', E 132°08', a deciduous forest dominated by *Quercus mongolica*, on litter mixed with soil, 10.08.2018, Ju. V. Belova (LE-F 335198; ITS sequence GenBank OM935688).

Ecology and distribution: This species grows in broadleaf forests, on decaying litter mixed with soil. It is known from Primorsky Krai in the Russian Federation and China (based on data from GenBank). It is likely to grow in other regions of the Russian Far East, as well as in Japan and North Korea with a moderate monsoon climate.

Etymology: Named after Julia V. Belova, the collector of one of the specimens.

DISCUSSION

Geastrum juliae belongs to the *Geastrum* sect. *Myceliostroma*. Its morphology is similar to that of *G. rubellum* which is distinguished by a purplish-pink pseudoparenchymatous layer in fresh condition and bigger spores with longer and not aggregated warts (Accioly *et al.* 2019). *Geastrum hirsutum* Baseia & Calonge is distinguished by a yellowish-brown exoperidium with long dark brown hairs and thin capillitium hyphae up to 1.5 µm wide (Baseia & Calonge 2006). *Geastrum pusillipilosum* J.O. Sousa, Alfredo, R.J. Ferreira, M.P. Martín & Baseia clearly differs by its brown exoperidium without pink tints and bigger spores with non-aggregated warts (Crous *et al.* 2016).

All the mentioned species have clear phylogenetic differences. Based on our phylogenetic analysis, *G. juliae* represents a well-supported (PP = 1, BS = 100%) and distinct clade from the neighbouring *G. rubellum*. The ITS sequences in the "juliae" clade share a P%I value of 99.3%. Therefore, *G. juliae* is a well-defined species based on both morphological and genetic criteria.

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Fig. 3. A mature basidiome from the holotype collection LE-F 335197, the coin diam. = 18.75 mm. (Photo Yu. Rebriev)

Fig. 4. A mature basidiome from the collection LE-F 335198 *in situ* (Photo Yu. Rebriev).

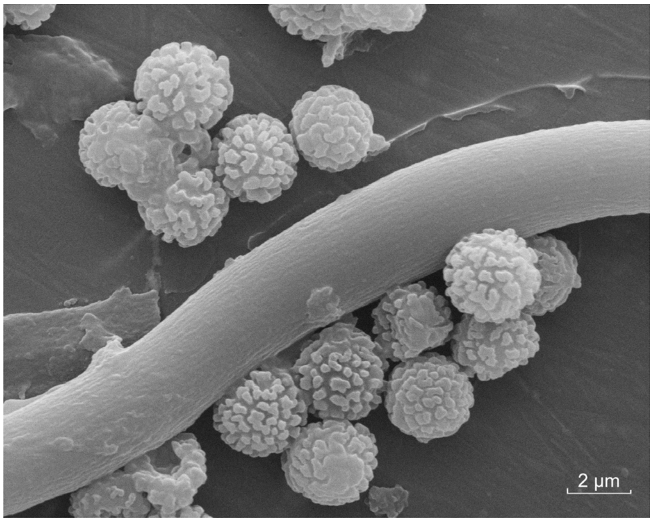

Fig. 5. Basidiospores and capillitium under SEM (Photo Yu. Rebriev and A. Nazarenko).

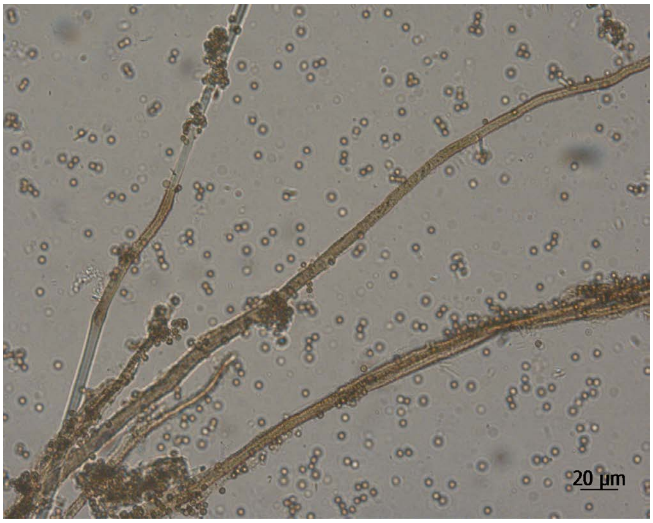

Fig. 6. Basidiospores and capillitium under LM (Photo Yu. Rebriev).

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REZIME

Geastrum juliae (Geastrales, Agaricomycetes), nova vrsta sa krajnjeg istoka Rusije

Yury A. Rebriev, Eugeniya M. Bulakh i Alona Yu. Biketova

Nova vrsta, *Geastrum juliae*, opisana je iz Primorske pokrajine sa krajnjeg istoka Rusije. Prema analizi sekvenci dostupnih u GenBank, ovaj takson se takođe nalazi i u Kini. Na osnovu morfoloških i filogenetskih karakteristika, najsrodnija joj je vrsta *G. rubellum*. Nova vrsta je predstavljena ilustrovanim opisima i molekularnim podacima.

Ključne reči: zemljana zvezda**,** gasteroidne gljive, Gasteromycetes, ITS nuklearne ribozomske DNK, molekularna identifikacija, taksonomija