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New localities of *Gastropila fragilis* (*Lycoperdaceae*) in Europe and Asia

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The paper presents two new European localities (in Bulgaria and Russia) and a new record from Asia (Turkmenistan) of the rare gasteroid fungus *Gastropila fragilis* (= *Calvatia pachyderma*). A description and illustrations are provided based upon the Russian, Bulgarian and Turkmenian specimens. Differences from similar species are briefly discussed.

Key words: *Agaricaceae*, *Agaricales*, *Calvatia pachyderma*, gasteroid fungi, *Lycoperdaceae*.

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V článku jsou zveřejněny nové lokality vzácné břichatkovité houby *Gastropila fragilis* (= *Calvatia pachyderma*), dvě z Evropy (Bulharsko, Rusko) a jedna z Asie (Turkmenistán). Je připojen popis a ilustrace založené na těchto nálezech a krátce jsou diskutovány rozdíly od podobných druhů.

INTRODUCTION

Gastropila fragilis Homrich et J.E. Wright (*Lycoperdaceae*) is a rarely recorded fungus in Europe, known to date from a few scattered localities in the Mediterranean area (Demoulin et al. 1988, Martín & Sierra 1994, Calonge 1998, García Blanco 2006). Recently the authors discovered three new localities of this noteworthy species, extending its known distribution further east in Eurasia.

MATERIAL AND METHODS

The description below is based on a study of specimens of the fungus from the three localities. The specimens are kept in the V.L. Komarov Botanical Institute of

the Russian Academy of Sciences (LE), in the personal herbarium of Yu. Rebriev (noted as YR) and in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences (SOMF). Examination under a light microscope was carried out using a 5% KOH solution. Scanning electron microphotographs (SEM) of the Russian specimens were taken using a Carl Zeiss EVO-40 XVP microscope at the Southern Scientific Centre of the Russian Academy of Sciences, and the Bulgarian specimen was examined under a JEOL JSM-6390.

RESULTS

Gastropila fragilis (Lév.) Homrich et J.E. Wright, Mycologia 65: 781 (1973). Figs. 1, 2.

≡ *Mycenastrum fragile* Lév., Ann. Sci. Nat., Bot., Sér. 3, 2: 221 (1844).
≡ *Pila fragilis* (Lév.) Speg., Revista Chilena Hist. Nat. 25: 77 (1923) [nom. illeg.].
≡ *Scleroderma fragile* (Lév.) De Toni, in Berlese, De Toni et Fischer, Syll. Fung. 7: 140 (1888).
= *Calvatia pilula* Kreisel, Persoonia 14: 435 (1992).
= *Lycoperdon pachydermum* Peck, Bot. Gazette 7: 54 (1882).
≡ *Calvatia pachyderma* (Peck) Morgan, Journ. Cinn. Soc. Nat. Hist., 12: 167 (1890).

Selected descriptions and illustrations. Homrich & Wright (1973: 781–783, Fig. 1), Demoulin et al. (1988: 85–87, Figs. 1–4), Calonge (1998: 83–85, Fig. 21), Bates et al. (2009: 175–176, Fig. 5e, f), Sarasini (2005: 167–169).

Macrocharacters. Gasterocarp dehiscent, subglobose to turbinate, ca. 3 cm in diameter, with mycelial cord when immature. Exoperidium white, smooth, separable, cracking and gradually falling apart (absent in the Bulgarian specimen). Endoperidium more than 1 mm thick, shiny, rigid, persistent, cracking and sloughing in plates, brown. Gleba brownish-yellow when slightly immature, at maturity brownish, pulverulent. Subgleba absent.

Microcharacters. Basidia not seen. Basidiospores 4.4–6.2 × 3.6–4.9 (mean 5.0 × 3.6) µm (n = 50), length/width ratio 1.0–1.4 (mean 1.2), subglobose to ovoid, smooth (both under LM and SEM), yellowish to light yellow-brownish (water), with pedicel less than 1 µm long, sterigmal remnants very rarely present in mounts. Eucapillitium of '*Calvatia*' type, subhyaline to light brownish, 4–9(–14) (mean 5.9) µm in diameter, often branched, straight to subundulate, septate, in mounts mostly disarticulating at septa, with numerous small pores; wall 0.7–1.2 µm thick. Peridium three-layered.

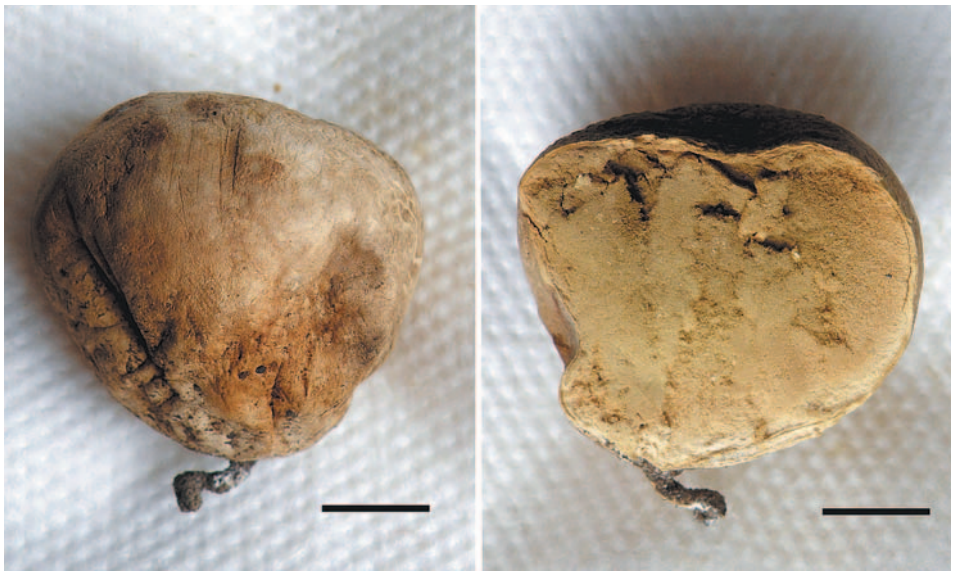

Fig. 1. *Gastropila fragilis*. Basidiomata (YR 0882). Scale bar = 1 cm.

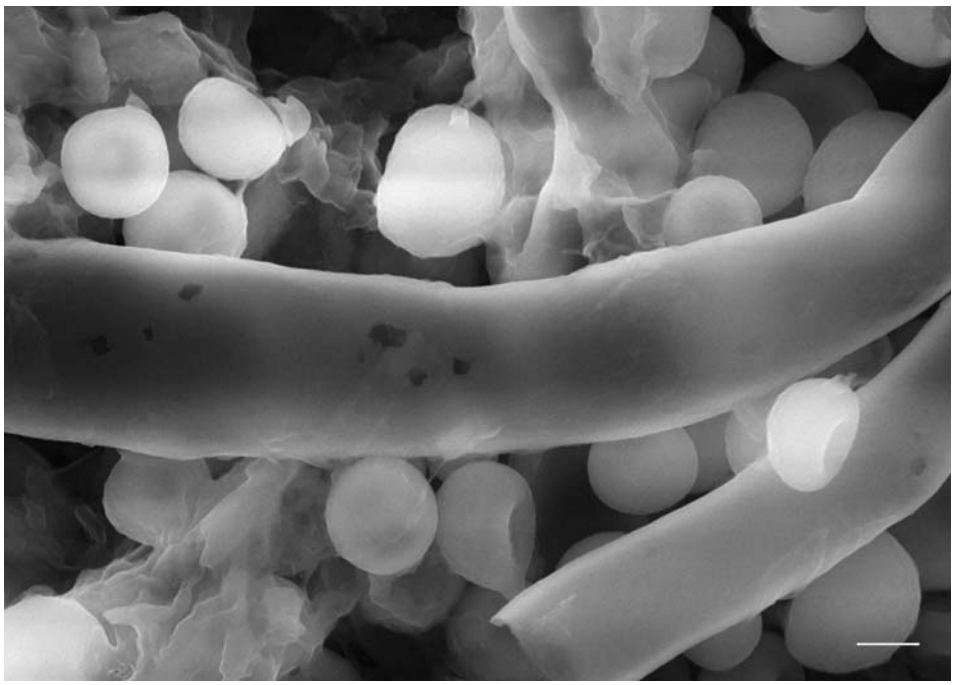

Fig. 2. *Gastropila fragilis*. Basidiospores and capillitium with pores (LE 287400). Scale bar = 2 µm.

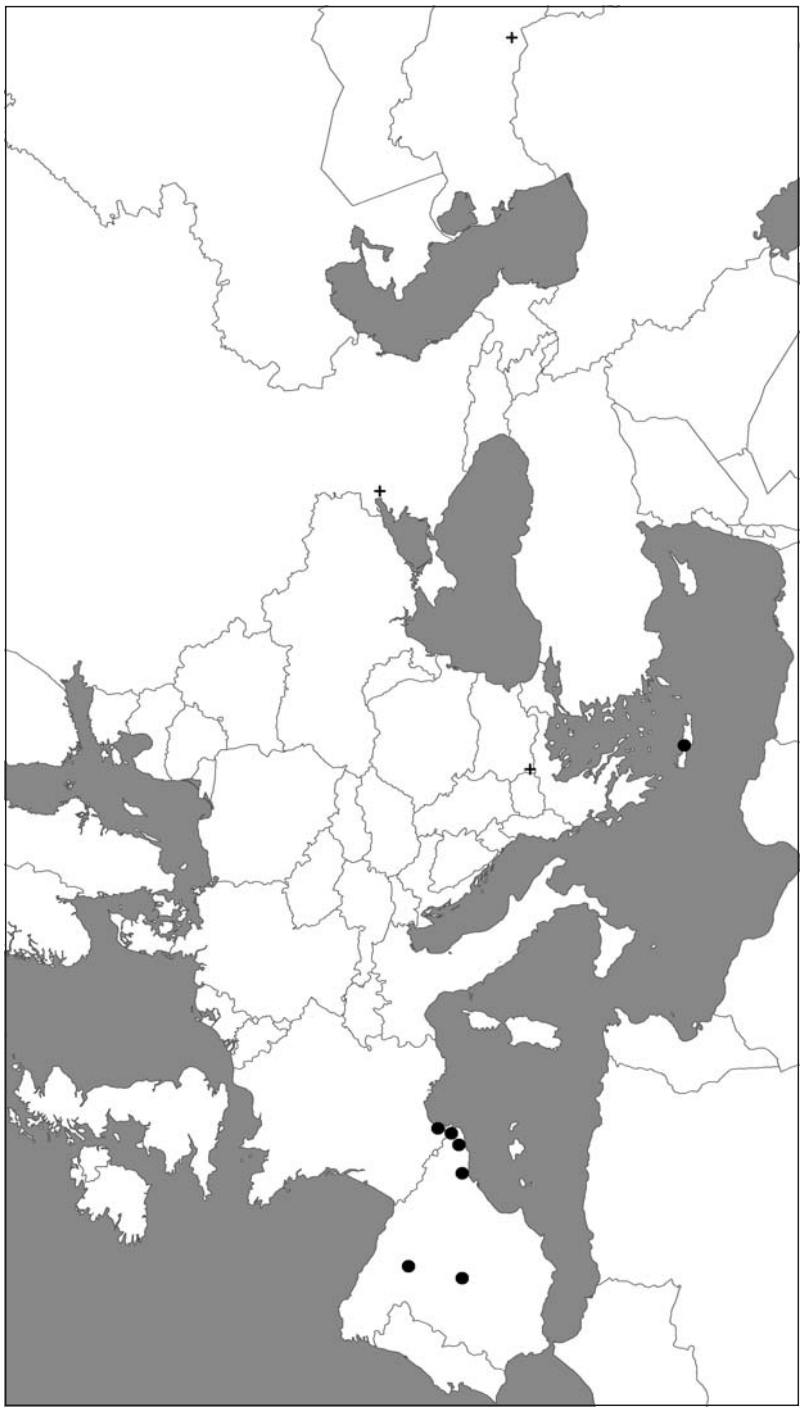

Fig. 3. Distribution of *Gastropila fragilis* in Eurasia. Localities known from the literature are marked with dots, the new localities are indicated with crosses.

Material examined

Russia. Rostov region: Azov, on dry basic soil, 47°06' N, 39°25' E, 16 Jul. 1999, leg. Ermolova (LE 287400); Rostov-on-Don, on soil in a lawn in the city, 47°15' N, 39°43' E, collector and date unknown (YR 0882). – Bulgaria. Southwest Bulgaria, Petrich District, on dry, eroded, basic, sandy soil (chromic cambrisols) nearby the cemetery of the village of Kulata, 41°23'21.9" N, 23°22'39.9" E, alt. ca. 100 m, 21 Apr. 2009, leg. B. Assyov (SOMF 29355). – Turkmenistan. Ahal Province: Tejen railway station, in oasis, 37°22'00" N, 60°30'00" E, alt. ca. 161 m, 24 Apr. 1951, leg. Kalumbetov (LE 262982).

DISCUSSION

All newly found specimens fit well the current concept of *Gastropila fragilis* (see Demoulin et al. 1988, Calonge 1998, Bates et al. 2009, Sarasini 2005). However, the basidiocarps of this species usually exceed 6 cm in diameter according to the literature available.

Gastropila fragilis is morphologically similar to *Calvatia complutensis* G. Moreno, Kreisel & Altés, and *C. booniana* A.H. Sm., which also have a tough and rupturing endoperidium and are so far known in Europe only from Spain (Moreno et al. 1996). Superficially similar is also *C. lepidophora* (Ellis & Everh.) Coker & Couch, a species not yet recorded in most countries of Europe, but known from Armenia (Melik-Khachatrjan & Martirosjan 1971) and also in Asia from Kazakhstan (Schwartzman & Filimonova 1970, specimens in LE), and the Russian Far East (Sosin 1973, doubtful information). A comparison of the four species is presented in Tab. 1.

The nomenclature and the placement of this fungus have been much debated and are the subject of controversy. Spegazzini (1923) erected the monotypic genus *Pila* Speg. for *Mycenastrum fragile* Lév. This name is however unavailable, being a later homonym of *Pila* C.E. Bertrand & Renault (fossil *Chlorophyceae*). In attempt to solve this problem, Homrich & Wright (1973) proposed *Gastropila* Homrich et J.E. Wright, a decision supported by Ponce de Leon (1976), Calonge & Martín (1990), Lange (1993), and Calonge (1998). Other authors refrained from recognising a separate genus (Zeller & Smith 1964, Kreisel 1992), but retain the species in different subgeneric entities of the genus *Calvatia*. Demoulin (1993) admitted the peculiar features of *G. fragilis*, but nonetheless preferred not to separate the genus *Gastropila* at that point and placed the species in *Calvatia*, a genus considered artificial by him. Bates et al. (2009) have shown that *C. pachyderma* (i.e. *G. fragilis*) falls within a well supported clade corresponding to the genus *Langermannia* s.l. Nonetheless, the exact phylogenetic affiliation of *G. fragilis* still remains unresolved and it has been suggested by Larsson & Jeppson (2008) that a detailed subdivision of *Lycoperdaceae* is yet to be achieved. Further molecular studies will hopefully unequivocally establish its appropriate generic position. In addition there has been much confusion concerning the type material of

Lycoperdon pachydermum Peck, as Calonge & Martín (1990) found verrucose spores, thus contradicting the previous studies of Ponce de Leon (1976) and Wright (1990), who reported a specimen with smooth spores. This prompted Kreisel (1992) to propose a nomen novum, *Calvatia pilula*. The problem was finally solved by Demoulin (1993), who after re-examination concluded that the verrucose spores observed by Calonge and Martín are due to contamination, also confirming the conspecificity of *Lycoperon pachydermum* and *Mycenastrum fragile*.

The geographic distribution of *G. fragilis* appears to be meridional and boreo-subtropic. The species is well known from North America (Western USA, Mexico; Moreno et al. 1996, Bates et al. 2009), where it is not uncommon. In South America records exist from Argentina, Brazil, Chile, and Uruguay (Spegazzini 1920, 1923; Homrich & Wright 1973; Trielveiler-Pereira & Baseia 2009). The Asian records (Iran, Nepal) of the species are considered erroneous (Demoulin 1993), but its distribution in Asia is now confirmed. A single record exists from Africa (Bottomley 1948), but judging from the description it has globose, finely verrucose spores; therefore this record might also have to be considered as doubtful. In the southern hemisphere the fungus is present also in Australia (Ponce de Leon 1976). In Europe *G. fragilis* is so far known from very few scattered localities in the Mediterranean area – Spain and France (Demoulin et al. 1988, Martín & Sierra 1994, Calonge 1998, García Blanco 2006). There is one much earlier report of the species from Europe, namely Crete (Petrak 1943), described as a new form, *C. pachyderma* f. *tenuior* Lohwag & Swoboda, and said to differ by the somewhat thinner peridium. This was possibly not known to Demoulin et al. (1988), who considered their own French and Spanish collections as the first European records, but it is listed by Zervakis et al. (1998). The new collections described here extend the known distribution range of the species further east (Fig. 3). It is yet unclear whether *G. fragilis* is rare in Europe and Asia or merely under-recorded, especially considering that the endoperidium may entirely rupture in rather inconspicuous scales, as is the case with the Bulgarian specimen.

In Bulgaria *G. fragilis* was found on sandy soil in open sub-Mediterranean dry grasslands at the edge of thermophilous scrubland with e.g. *Quercus coccifera*, *Carpinus orientalis*, *Juniperus oxycedrus*, and *Pistacia terebinthus*. The Russian specimens were also collected in xerophytic ruderal places among grasses.

Tab. 1. Comparison of *G. fragilis* (personal observations supplemented with data from Demoulin et al. 1988, Calonge 1998), *C. complutensis* (Moreno et al. 1996), *C. booniana* and *C. lepidophora* (Zeller & Smith 1964, personal observations).

Morphological characteristics	***Gastropila fragilis***	***Calvatia complutensis***	***Calvatia booniana***	***Calvatia lepidophora***
Basidiocarp diam. (mm)	(20)60–170(250)	23–70	200–600	90–200
Base	abruptly tapered, frequently deeply plicate, without subgleba	rounded (globose or pyriform), scarcely plicate, without subgleba	more or less spherical, subgleba absent or rudimentary	more or less spherical, subgleba absent or rudimentary
Exoperidium	thin	thin	thin	ca. 2 mm thick
Endoperidium	1–4 mm thick	0.5–1.5 mm thick	2–4 mm thick	thin
Mature gleba	yellow brown to umber brown	dark olivaceous brown	olive-brown	dull olive-brown
Capillitium	with frequent pits	with less frequent pits	occasionally pitted	not pitted
Basidiospores	4.0–6.0 × 3.5–5.0 µm, subglobose to ovoid, smooth, with pedicel <1.0 µm	(3.2)4.8–5.6(6.4) µm, globose to ovoid, glabrous under LM but slightly verruculose under SEM, with a pedicel 0.5–1.2 µm	3.9–6.0(6.6) × 3.3–5.5 µm, globose to subglobose, smooth to very finely punctate, pedicel evident	(4.5)5.5–6.5 × (5.0)5.5–6.0 µm, subglobose to broadly ovate, densely verruculose, pedicel inconspicuous

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