

LEJEUNIA

REVUE DE BOTANIQUE

Nouvelle série N° 197

Décembre 2017

ON *CALVATIELLA*, *BOVISTELLA*, AND THE TAXONOMIC POSITION OF *CALVATIA UTRIFORMIS* (*LYCOPERDON UTRIFORME*, *HANDKEA UTRIFORMIS*)

par

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Abstract

A specimen of Lycoperdaceae collected in the Russian Far-East, in the same geographical area as the lost type of *Calvatiella lioui* C. H. Chow fits the original description, including the lectotype plate, and is designated as epitype. Its morphological characters and the identity of ITS sequences to those of *Calvatia utriformis* confirms the synonymy of the two names as already proposed by Kreisel and Calonge. Small morphological differences justify retention of the name at varietal rank. General considerations on the genera *Lycoperdon*, *Calvatia* and *Bovistella* justify the transfer of *Lycoperdon utriforme* (*Calvatia u.*, *Handkea u.*) to the genus *Bovistella*.

Résumé

Un spécimen de Lycoperdacées récolté dans l'Extrême Orient Russe, dans la même zone géographique que le type perdu de *Calvatiella lioui* C. H. Chow correspond à la description originale, y compris la planche lectotype,

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et est désigné comme épitype. Ses caractères morphologiques et l'identité de la séquence des ITS confirment la synonymie avec *Calvatia utriformis* proposée par Kreisel et Calonge. De petites différences morphologiques justifient de conserver le nom au rang de variété. Des considérations générales sur les genres *Lycoperdon*, *Calvatia* et *Bovistella* justifient le transfert de *Lycoperdon utriforme* (*Calvatia* u., *Handkea* u.) dans le genre *Bovistella*.

INTRODUCTION

The genus *Calvatiella* was described by C. H. Chow in 1936 (p.91) and included two species, both from China. Those were described as new: *Calvatiella sinensis* C. H. Chow (p. 92) and *C. lioui* C. H. Chow (p. 93). According to the author, the genus "... may be defined in short as having the peridium of *Calvatia* but the capillitium of *Bovista* and *Bovistella* " (p. 91). Unfortunately, if the descriptions and illustrations are good, some important features like the presence of a pseudodiaphragm are not mentioned and the type material of both species disappeared during the Second World War, as reported by Kreisel and Calonge (1993). Those authors nonetheless considered the descriptions sufficient enough for placing *C. sinensis* in synonymy of *Bovistella radicata* (Dur. et Mont.) Pat. and *C. lioui* in that of *Handkea utriformis* (Bull. : Pers.) Kreisel (= *Calvatia utriformis* (Bull. : Pers.) Jaap). They considered that *Calvatiella sinensis* is the type of *Calvatiella*, which thus became a synonym of *Bovistella*. It is not clear if they intended to lectotypify *Calvatiella* or were aware of a previous lectotypification, which we have not localized, but if this lectotypification does not exist, their statement "...the type species *Calvatiella sinensis* Chow..." can be accepted as a lectotypification.

During the study of specimens received from the herbarium (VLA) of the Institute of Biology and Soil Science, Far East Branch of the Russian Academy of Science one of us (Y. R.) noted a specimen that could have been referred to the genus *Bovistella*, but did not match any of its accepted species. It was close, however, to *Calvatiella lioui* through its macroscopic aspect and bovistoid capillitium with slit-like pores and hence, as noted by Kreisel and Calonge to *Calvatia utriformis*. The original

material of *Calvatiella lioui* having been lost, it was interesting to study this collection from the same part of Eastern Asia in detail.

METHODS

Light microscope photography and molecular work have been implemented with the equipment of the Centre for cellular and molecular technology of studying plants and fungi at the Komarov Botanical Institute RAS (St Petersburg). Scanning electron micrographs (SEM) were taken with the Carl Zeiss EVO-40 XPV of the South Science centre RAS. Microscopic measurements were made using the program Scandium 5.0.

DNA was extracted from the gleba following the manufacturer protocol of AxyPrep™ Multisource Genomic DNA Miniprep Kit (Axygen Biosciences (Calif., USA).

PCR amplification of the ITS1-5.8S-ITS2 region was performed using the fungal specific primers ITS1F and ITS4B (Gardes and Bruns, 1993). The PCR products were purified with the GeneJet PCR Purification kit of Thermo Scientific.

Sequencing was performed with the ABI model 3130 Genetic Analyzer of Applied Biosystems and the BigDye™ Terminator Cycle Sequencing Ready reaction Kit, using the same primers as for amplification.

Data were processed with Sequencing Analysis 5.3.1 (Applied Biosystems) and treated with MEGA 6 (Tamura et al. 2013).

DESCRIPTION OF THE COLLECTION

Collection's details: Russian Federation, Primorsky krai, near the village of Fadeevka, 44°04'N, 131°24'E, comm. A. E. Kozhevnikov (VLA 20697).

Macroscopic characters (FIG. 1). Fruitbody turbinate, 11 cm high and 12 cm in diam., with soil agglomerated at the base. The specimen is mature, with the upper part destroyed. Exoperidium light brownish-grey, with thin spines up to 3 mm long, connivent in

clusters. Endoperidium olive-brown, cracking and disappearing in the upper part. Gleba powdery, rich brown, separated from the subgleba by a pseudodiaphragm. Subgleba prominent, cellular, brown.

Microscopic characters. Spores globose to subglobose, brown, almost smooth under the light microscope (FIG. 2) but verruculose under the scanning electron microscope (FIG. 4 and 5), uniguttulate, 3.7-4.3 μm , usually with hyaline straight or curved pedicels up to 4.5 μm . Capillitium (FIG. 2, 4 and 5) up to 13.0 μm in diam., subelastical, thin-walled, widened at the places of the bipartite ramifications, septate, light-brown to brown, with slit-like and occasionally roundish pores. Exoperidium with subspherical spherocysts (FIG. 3), moderately thick-walled up to 40 μm in diameter.

Sequencing of the ITS 1-5.8S-ITS 2 region gives a sequence identical to those of *Calvatia utriformis* GenBank EU833659 and DQ112607 and thus belongs to a clade that also includes *Bovistella radicata* as previously reported by Bates, Roberson and Desjardin (2004) and Larsson and Jeppson (2008). It should be noted that the sequence AJ237624 entered in GenBank under the name *Bovistella radicata* is also identical. We suspect this is a misidentification and that it is also *Calvatia utriformis*. DQ112608 is probably the sequence of a true *Bovistella radicata* and differs in four positions from that of *Calvatia utriformis* (see FIG. 2 in Larsson and Jeppson, 2008). It unfortunately does not cover the whole ITS 1.

TAXONOMIC CONCLUSION

The study of a recent collection from the same geographic area that fits the original description of *Calvatiella lioui* refines our knowledge of that taxon and confirms the opinion of Kreisel and Calonge (1993) that it is a synonym of *Calvatia utriformis*. A slight difference in the microscopical characters, that is a less fragile capillitium and spores frequently provided with a small pedicel, justifies, in our opinion, to give a varietal status to the taxon. It should be noted that those characters are those that

point toward classical *Bovistella* rather than *Calvatia* s. l. It was already noted by Moyersoen and Demoulin (1996, pp.39-40) that what was probably an immature *Calvatia utriformis* had been described by Reid (1969, p. 342) as a new species of *Bovistella*, *B. alpina*. It was then noted that the great fragility of the ripe capillitium of *Calvatia utriformis* must have led to its bovistoid character being overlooked and thus the fact it might be more related to *Bovistella radicata* than to other Lycoperdaceae.

Through the help of Dr Begoña Aguirre-Hudson, we could obtain from the Kew herbarium K(M) a piece of the type of *Bovistella alpina*. DNA extraction unfortunately proved unsuccessful. Intensive collections of puff-balls in Corsica, before and after the publication by Moyersoen and Demoulin however does not leave us doubts that the immature collection made by Reid is referable to *Calvatia utriformis*.

The loss of *Calvatiella lioui*'s type led Coetzee and Van Wyk (2012, p. 34) to designate plate III of the original publication as lectotype. The usefulness of this action is not apparent to us, since the name is based on the single specimen pictured there. Nonetheless the action seems legalistically correct and we must accept it. In the absence of the original specimen it thus seems useful to designate as epitype the collection on which we have based our interpretation.

Epitypification of *Calvatiella lioui* C. H. Chow, Bull. Fan Mem. Inst. Biol., Bot. VII (2): 93, 1936. We designate as epitype the following collection: Russian federation, Primorsky krai, near the village of Fadeevka, 44°04'N, 131°24'E, comm. A. E. Kozhevnikov (VLA 20697).
Registration identifier (Index Fungorum): 554055.

This study also provided the opportunity to give more attention to the taxonomic position of *Calvatia utriformis*. In the phylogeny chapter of his thesis devoted to the genus *Lycoperdon*, one of us (V. D., 1971) considered the genus *Calvatia* highly heterogeneous, being derived at least three times from *Lycoperdon* through enlargement to the whole upper peridium of the fragility

of the pore area of *Lycoperdon* (p. 266 and fig. 25). This phylogenetic reconstruction was largely inspired from Hennigian principles, analysing characters as primitive or derived (in 1973, V. D. must have been one of the first mycologist to cite Hennig, 1966). If the ideas of Hennig were found valuable for elaborating phylogenetic trees, his concept of a classification being a strict transcription of a phylogenetic tree was not accepted. This was the same position as that elaborated in details by Mayr, one of the greatest biologists of the twentieth century (1981).

We maintain this philosophy, which most importantly acknowledges the need to have so called paraphyletic taxa, as advocated by the lamented Dick Brummitt and the numerous signatories of the manifesto published in *Taxon* in 2005 (Nordal and Stedje, 2005). A paraphyletic *Lycoperdon* (see FIG. 2 in Larsson and Jeppson, 2008, for a general account of the phylogeny suggested by the ribosomal locus) is important to describe the evolution of the Lycoperdaceae and a useful link to all the literature since the end of the nineteenth century which used morphologically well defined genera. This does not mean that when enough evidence is available, clearly polyphyletic genera like *Calvatia* could not be dismembered. This must however be done carefully and the use of a single character such as the slit-like pores in the capillitium, used to define the genus *Handkea* Kreisel (1989), leads to a smaller but still artificial genus, as can be ascertained from the morphology and supported by sequencing since Bates, Roberson and Desjardin (2004) and Larsson and Jeppson (2008). With those principles in mind we consider *Bovistella radicata* and *Calvatia utriformis* so closely related both from the standpoint of morphology and ribosomal locus sequence that they should belong to the same genus, which would then be *Bovistella*, a genus distinct enough from *Lycoperdon* s. str. to be worth recognising. We thus introduce the following new combination.

New combination: *Bovistella utriformis* (Bull. : Pers.) Demoulin et Rebriev,

Basionym: *Lycoperdon utriforme* Bull., *Herbier France* 10 (109-120): pl. 450, fig. I, 1790 : Pers., *Syn. Meth. Fung.*: 143, 1801.

Registration identifier (Index Fungorum): 554053.

Since we consider *Calvatiella lioui* is adequately treated as a variety of the former we also introduce the

New combination (stat. nov.): *Bovistella utrififormis* (Bull. : Pers.) var. *lioui* (C. H. Chow) Demoulin et Rebriev

Basionym: *Calvatiella lioui* C. H. Chow, Bull. Fan Mem. Inst. Biol., Bot. VII (2): 93, 1936.

Registration identifier (Index Fungorum): 554054.

ACKNOWLEDGMENTS

We thank Dr E. M. Bulakh for the opportunity to study specimens from the VLA herbarium and Dr Begoña Aguirre-Hudson (K) for the opportunity to study a part of the type of *Bovistella alpina*. We wish to express our gratitude to Drs E. F. and V. F. Malysheva for their invaluable help with the molecular study, as well as L. Cornet for information treatment. We also thank Dr K. V. Dvadnenko for his help with SEM.

REFERENCES

- BATES, S. T., ROBERSON, R. W. & DESJARDIN, D. E., 2009. - Arizona gasteroid fungi I: *Lycoperdaceae* (Agaricales, Basidiomycota). *Fungal Diversity* **37**: 153-207.
- CHOW, C. H., 1936. - Notes on the genus *Calvatiella*. *Bull. Fan Mem. Inst. Biol., Bot.* **VII** (2): 91-94.
- COETZEE, J. C. & VAN WYK, A. E., 2012. - Nomenclatural and taxonomic notes on *Calvatia* (*Lycoperdaceae*) and associated genera. *Mycotaxon* **121**: 29-36.
- DEMOULIN, V., 1971. - Le genre *Lycoperdon* en Europe et en Amérique du Nord. Thesis, University of Liège (Belgium): 284pp.
- DEMOULIN, V., 1973. - Phytogeography of the Fungal Genus *Lycoperdon* in Relation to the Opening of the Atlantic. *Nature* **242** (5393): 123-125.
- HENNIG, W., 1966. - Phylogenetic systematics. Urbana, University of Illinois Press: 263 pp.
- KREISEL, H., 1989. - Studies in the *Calvatia* complex (Basidiomycetes). *Nova Hedwigia* **48** (3-4): 281-296.
- KREISEL, H. & CALONGE, F. D., 1993. - *Calvatiella* Chow, a synonym for *Bovistella* Morgan. *Mycotaxon* **XLVIII**: 13-25.
- LARSSON, E. & JEPSON, M., 2008. - Phylogenetic relationships among species and genera of *Lycoperdaceae* based on ITS and LSU sequence data from north European taxa. *Mycol. Res.* **112** (1): 4-22.

- MAYR, E., 1981. - Biological Classification: Toward a Synthesis of Opposing Methodologies. *Science* **214** (4520): 510-516.
- MOYERSON, B. & DEMOULIN, V., 1996. - Les Gastéromycètes de Corse: taxonomie, écologie, chorologie. *Lejeunia* **152**: 128 pp.
- NORDAL, I. & STEDJE, B., 2005. - Paraphyletic taxa should be accepted. *Taxon* **54** (1): 5-8
- REID, D. A., 1969 ("1968"). - Spring Fungi in Corsica. *Rev. Mycol.* **XXXIII** (5): 341-368.
- TAMURA, K. et al., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **30** (12): 2725-2729.

FIGURES

All the figures are from the epitype of *Calvatiella lioui* (VLA 20697).



FIG. 1. – General view of the fruitbody.

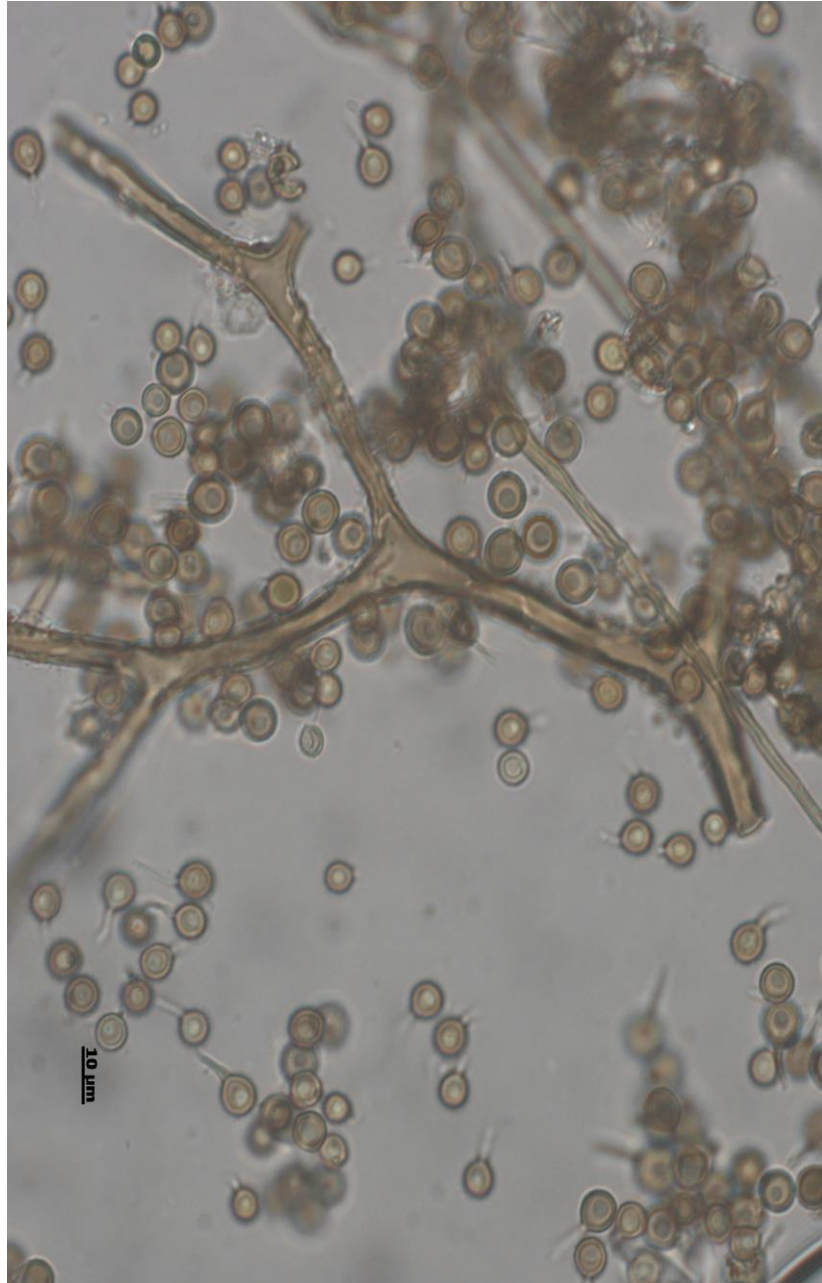


FIG.2. – Capillitium and spores as seen in light microscopy.



FIG. 3. – Spherocysts from the exoperidium as seen in light microscopy.

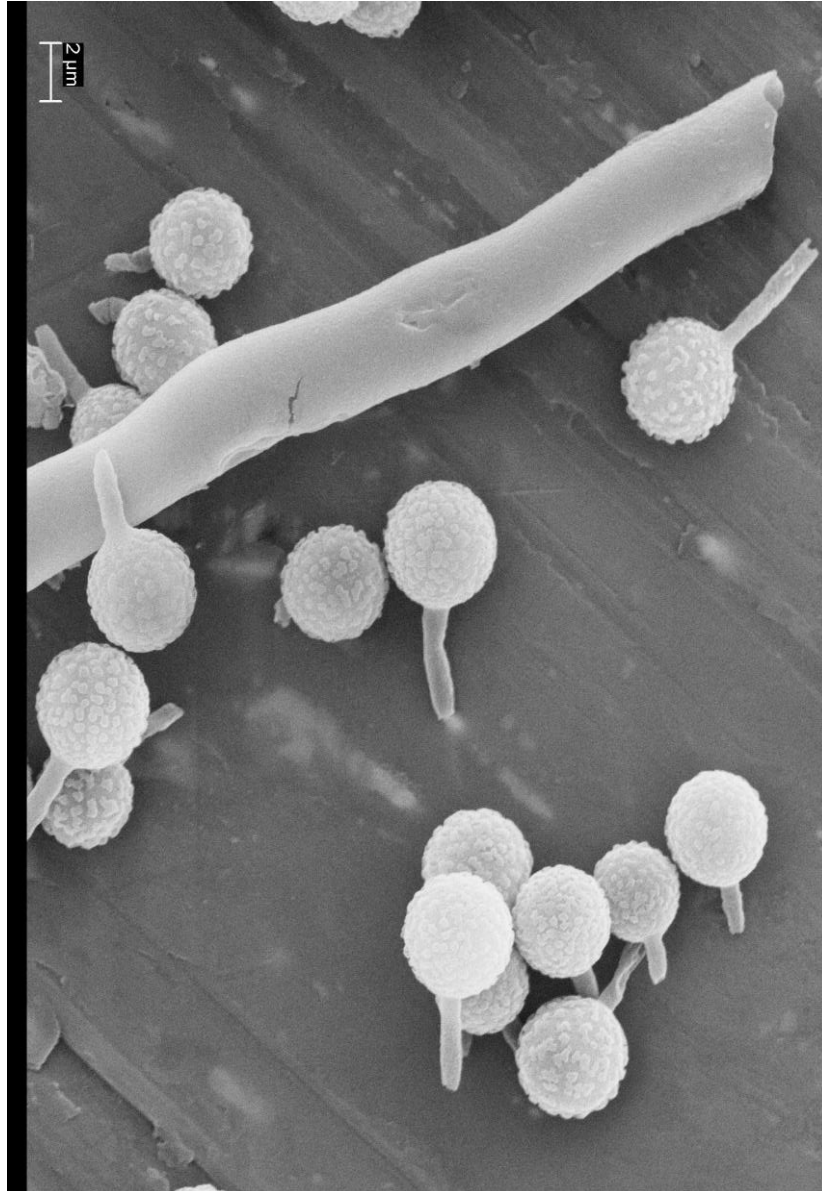


FIG. 4 – Capillitium and spores as seen under the SEM.

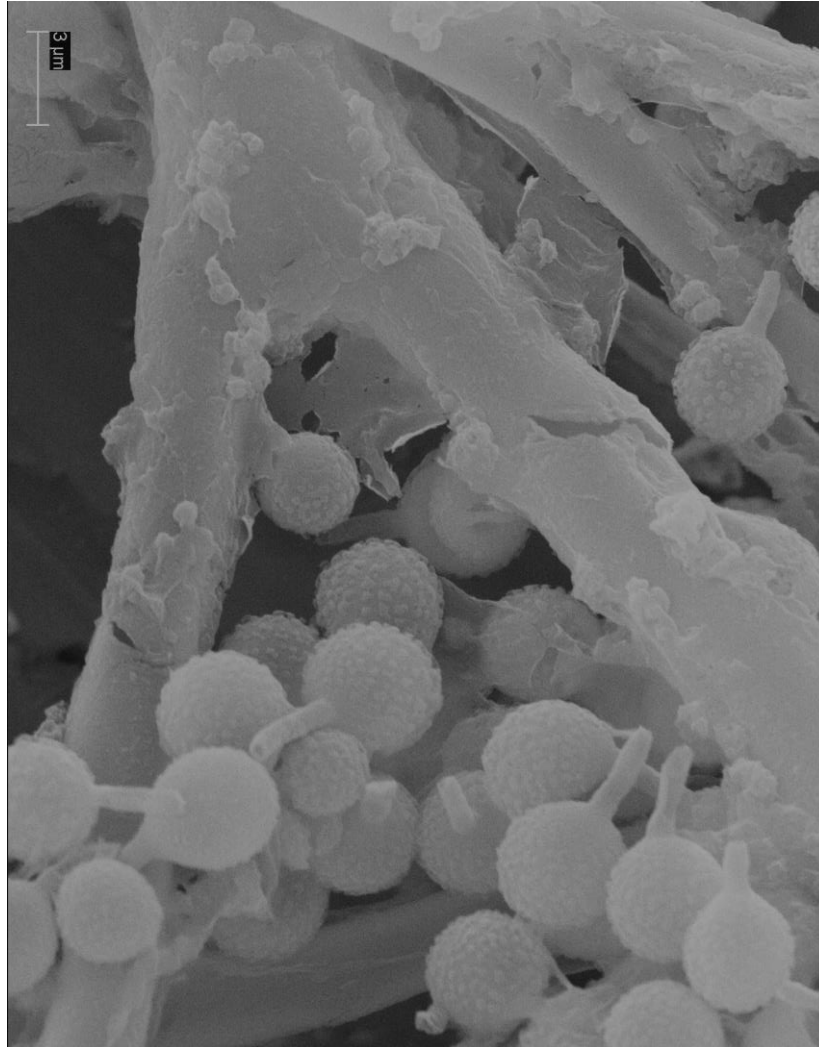


FIG. 5 – Capillitium and spores as seen under the SEM.



Manuscrit "camera ready" réalisé par le cadre APE de la Société Botanique de Liège (Ministère de l'Emploi de la Région Wallonne, réf. NM 2373).

FNRS

Publication supportée par le Fonds National de la Recherche Scientifique de Belgique.