Mycol Progress (2009) 8:289–299
DOI 10.1007/s11557-009-0599-z

ORIGINAL ARTICLE

Taxomomy, ecology and phylogenetic relationships of *Bovista pusilla* and *B. limosa* in North Europe

Ellen Larsson · Mikael Jeppson · Karl-Henrik Larsson

Received: 2 February 2009 / Revised: 15 April 2009 / Accepted: 30 April 2009 / Published online: 29 May 2009

Abstract *Bovista limosa*, described from Greenland, is a species characterised by a prominent, delimited peristome and a fimbriate stoma. Morphological observations have indicated the presence of a closely related species with different peristomal characters, observations that were further confirmed through a molecular phylogenetic study of the Lycoperdaceae where deviating sequences were tentatively named *Bovista cf. limosa*. Here, we show that *Bovista limosa* as presently understood consists of two species with slightly different morphology, different habitat preferences, and different albeit overlapping distribution ranges. The two species also have clearly distinct nuclear ribosomal sequences as evidenced by an analysis of the ITS and LSU regions. We demonstrate that *Bovista cf. limosa* is identical to *Lycoperdon pusillum* described by Batsch in 1789. Batsch's fungus has been variously interpreted and is sometimes treated as a nomen confusum. We resolve this confusion by selecting a lectotype from Batsch's original illustrations and an epitype from the material we have sequenced. A key to the small *Bovista* species discussed in the paper is provided.

Keywords *Bovista* · Morphology · Micro-morphology

E. Larsson (✉) · K.-H. Larsson
Plant and Environmental Sciences, University of Gothenburg,
Box 461, 405 30 Göteborg, Sweden
e-mail: ellen.larsson@dpes.gu.se

M. Jeppson
Lilla Håjumsgatan 4,
46135 Trollhättan, Sweden

Introduction

In a recent molecular phylogenetic study of Lycoperdaceae (Larsson & Jeppson 2008), a species closely related to but distinct from *Bovista limosa* Rostrup was identified and tentatively named *Bovista cf. limosa*. The unnamed taxon deviates morphologically from typical *B. limosa* by the absence of a delimited peristome. The limited selection of specimens studied indicated that the two taxa also differed in habitat preferences and distribution ranges.

Bovista limosa is typified by material collected in Greenland, and Rostrup (1894) described the stoma of his new species as fimbriate ("osculum fimbriatum"). Lloyd (1906) illustrated a similar fungus from Michigan (pl. 89; Figs. 1 and 2) that he identified as *B. echinella* Pat. and placed in *Bovistella*. A few years later, R. E. Fries (1910) reported and illustrated a similar species from Abisko in northernmost Sweden and, referring to Lloyd (1906), named it *Bovistella echinella* (Pat.) Lloyd. Typical features according to Fries (1910) were the minute size of the fruiting bodies and the presence of a well-delimited and protruding peristome. The unique structure of the peristome was later discussed by Lloyd (1910) and illustrated by a photo of the "*Geaster* mouth" of a Swedish specimen that Fries sent to Lloyd.

Lange (1948) maintained the traditional synonymy between *Bovista limosa* and *B. echinella* and concluded, after having compared Rostrup´s holotype with his own collections from Greenland, that the species had a well-defined mouth, "with an almost *Geaster*-like peristome of five to eight more or less distinct, fimbriate teeth". Lohwag (1933), on the other hand, described a collection of *B. echinella* from Austria where rather the absence of a characteristic stoma was indicated. Lohwag stated his specimens were characterised by a distinct ostiole with a

rugged, narrow margin ("deutliche Mundöffnung mit kerbig eingerissenem, schmalen Rande") and concluded that this kind of rupture must of course be looked upon as irregular when compared with the wonderfully shaped stoma in some species of Geaster ("Natürlich kann man diese Art des Aufreissens im Vergleich mit den wunderbar geformten Mundöffnungen mancher Geasterarten als unregelmässig ansehen…"). His illustration consequently gave no evidence of a delimited or a distinctly protruding stoma but shows a plain, somewhat irregular, opening with a slightly recurved edge.

Kreisel (1967) showed that *B. echinella* could be distinguished from *B. limosa* by the lack of a delimited, fimbriate stoma and by having a pitted capillitium. Hence Kreisel re-established the status of *B. limosa* and erected *Bovista* section *Geastrostoma* to accommodate it. Kreisel (1967) studied material of *B. limosa* from Michigan (USA), North Dakota (USA), Greenland (holotype), Norway, Sweden, UK, Belgium, and Austria and noted that only specimens from northerly areas were provided with a protruding and delimited, fimbriate peristome. Calonge and Demoulin (1975) reported *B. limosa* from Spain and observed that the specimens did not show the protruding and delimited stoma that others had reported from northern regions. Lange (1987) revised the collections of *B. limosa* from Greenland and concluded that the protruding ostiole could be observed in nearly all suitable specimens. He suggested that the specimens from Spain and from Central Europe, characterised by the absence of a delimited stoma, might belong to a separate taxon. Neither the line drawings of *B. limosa* from Switzerland (Monthoux and Röllin 1976) nor the photos of fruiting bodies of *B. limosa* from Wales (Pegler et al. 1995) and Italy (Sarasini 2005) show a delimited stoma. In these cases, the stoma appears to be slightly irregularly lobed, not fimbriate, and plain or somewhat protruding. Runge and Gröger (1990) reported *B. limosa* from Thüringen, Germany, with a "kegelförmig vorgezogene Peristom" reminiscent of the stoma of *Tulostoma brumale*. This strongly indicates the absence of a delimited and fimbriate stoma. It seems, however, that the variation with regard to the protrusion of the stoma might be rather wide.

We have restudied the morphology of a great number of specimens identified as *B. limosa* or *B. cf. limosa* and also sequenced two more specimens of each taxon. We conclude that *B. cf. limosa* is a distinct species that can be separated from *B. limosa* using morphological and ecological characteristics. We are also convinced that the unnamed species is identical to *Lycoperdon pusillum* Batsch described in 1789 from Jena in Thüringen, Germany. This species was transferred to *Bovista* by Persoon (1801) but unfortunately the name has been associated with great confusion. These disagreements are here resolved and the name *Bovista pusilla* unambiguously restored.

Materials and methods

Morphological studies

Macro-morphological characters (size, shape, colour, exoperidial ornamentation etc) were studied under a dissecting microscope. Micro-morphological characters of the mature gleba (capillitium and spores) and peridial features were observed using a light microscope with up to 1,250 × magnification. Capillitium and spores were mounted in Cotton Blue (in lactophenol) and measured after a short boiling. Peridial characters were studied using Melzer´s reagent. Glebal characters and spores were studied from completely mature, sectioned fruiting bodies. Peridial characters were studied in mature as well as in immature fruiting bodies.

Material studied

We examined numerous collections of *Bovista limosa* sensu lato originating from Greenland, Iceland, Fennoscandia, and Estonia together with a few collections from Belgium, Italy, and Russia. Sequenced collections are deposited in the herbarium at Plant and Environmental Sciences, University of Gothenburg (GB) if not otherwise indicated. Data on sequenced specimens, including Genbank accession numbers, and data on additional specimens studied are listed below. (MJ) designates the private herbarium of the second author. Herbarium acronyms follow Holmgren and Holmgren (1998).

Bovista limosa, sequenced specimens: Sweden, Härjedalen, Funäsdalen, Ljusnedal, on old soccer ground, M. Jeppson 5226, 23 July 1999. (GB) DQ112615. Härjedalen, Tännäs, N of lake Malmagen, grassy road track, I. Högström, 16 Aug. 2006. (GB) EU915075. Ångermanland, Ramsele, Vallen, on sandy road among herbs, E. Larsson 4-06, 18 July 2006. (GB) EU915074.

Bovista limosa, additional specimens studied: Finland, Kuusamo, Sirkapuro, mossy road verge, A. Bohlin, J. and M. Jeppson 1146, 24 Aug. 1978 (MJ). – Greenland, W. Greenland, Søndre Strømfjord, west of the airport, naked sandy soil with *Salix*, H. Dissing, 21 July 1983 (C). S. Greenland, Narssarssuaq, along a little stream towards the Hospitalsee, moist sand, H. Dissing, 22 July 1981 (C). – Iceland, N. Múlasysla, Egilsstaðir, Egilsstaðaskógurinn, road verge in subalpine *Betula* woodland, M. Jeppson 4093, 7 Aug. 1993. (MJ). Jökuldalur, Skjöldólfsstaðir, road verge in *Kobresia*-grassland, M. Jeppson 4042, 31 July 1993. (MJ). – Norway, Nordland, Bjerkvik, NE side of Rombaken, sand-pit at the main road, with small *Salix* and *Betula*, J. Vauras 15172, 16 Aug. 1999 (MJ, dupl. ex TURA 8618). Oppland, Dovre, Grimsdalen, Buåi, on calcareous soil along road, 900 m asl, T. Schumacher and

K. Østmoe 473/82, 11 Sep. 1982 (O). Ibid., Grimsdalen, Storberget, calcareous soil, 930 m asl, T. Schumacher C12/84, 26 July 1984 (O). Ibid., Grimsdalen, Verkensæter, Gygergeitene, road bank, 900 m asl, J. and M. Jeppson 3742, 9 Aug. 1995 (MJ). Ibid., on road verge in alpine vegetation, J. and M. Jeppson 4262, 29 Aug. 1997 (MJ). Ibid., J. and M. Jeppson 5184, 4 Sep 1999 (MJ). Oppland, Lom, Bøverdalen, Bøvertun, subalpine *Dryas* heath, J. and M. Jeppson 4296, 30 Aug. 1997 (MJ). Sør-Trøndelag, Røros, Brekken, Sølendet/Torvollsveien, sandy road verge in subalpine grassland, S., J., and M. Jeppson 5424, 23 July 1999 (MJ). – Russia, Kamtchatka, Iazyrevsk, sandy road verge in coniferous forest (*Picea* and *Larix*), S.-Å. Hanson, 8 Aug 1997 (MJ). – Sweden, Dalarna, St. Tuna, Idkerberget, 200 mW of Idtjärn, on path, I. Andersson. 14 Sep. 1989 (S). Härjedalen, Funäsdalen, Fjällnäs, S., J., and M. Jeppson 5221, 23 July 1999 (MJ). Härjedalen, Ljusnedal, Sörmon, on sandy road verge and on abandoned soccer ground in subalpine *Betula* woodland, S. Jeppson, 16 Sep. 1999 (MJ). Ibid., Aug 2001 (MJ). Härjedalen, Tännäs, near Tännån, L-E. Kers 4945, 27 Aug 1978 (S). Härjedalen, Anderssjöåfallet, sandy-gravelly soil on car park, S. and J. Jeppson, 26 July 2001 (MJ). Härjedalen, Hamrafjället, 925 m asl, L-E. Kers, 28 Aug. 1976 (S). Ibid, L-E. Kers 4633, 28 Aug 1978 (S). Jämtland, Aspås, Gråmäraforsen, open grassland with scattered *Alnus incana* and *Betula*, B. Pettersson BEPLST 0225, 8 Sep 2002 (MJ). Jämtland, Åre, Handöl, the soapstone quarry, K. and A. Bohlin, 26 July 1998 (MJ). Medelpad, Selånger, Granloholm, C. Eriksson and J.-O. Wimo, 9 June 1987 (MJ). Ibid., 4 Aug 1990 (MJ). Torne Lappmark, Jukkasjärvi, road between Abisko and Björkliden, R. E. Fries, 24 July 1909 (S). Torne Lappmark, Abisko, at the dried out stream SW of Nuoljabäcken, R. Rydberg and O. Persson, 6 Sep. 1954 (S). Västergötland, Dalum, 200 m E of Silarpskullen, calcareous sandy soil in abandoned sand pit, M. Jeppson 5434, 6 Aug. 2000 (MJ); Västergötland, Karleby, Djupadalen, R-G. Carlsson, 17 April 2000 (MJ). Västergötland, Knätte, Knätte kullar, sandy soil on car park, J. and M. Jeppson 4784, 3 Aug. 1998 (MJ). Västergötland, Amnehärad, Gullspång, ruderal soil close to concrete factory, E. Grundel, 16 Sep. 2001 (MJ).

Bovista pusilla, sequenced specimens: Norway, Akershus, Bærum, Ostøya, among mosses on calcareous rock, 14 Oct. 2006, M. Jeppson 8305. (GB) EU915072. – Sweden, Öland, Dödevi strandängar, 500 m south of Tornholmsudde, on sandfield near the sea shore, 4 Oct. 1996, M. Jeppson 3971 (selected as epitype). (GB) DQ112614.Västergötland, Dala, Stenåsen, among mosses on calcareous flat rock, 28 Oct. 2006, M. Jeppson 8365. (GB) EU915073.

Bovista pusilla, additional specimens studied: Belgium, Antwerpen, Blokkerdijk, on calcareous sand, M. Jeppson, 5 Aug. 1987 (MJ). – Estonia, Saaremaa, Kipi, dry sandy heath, V. Liiv and M. Jeppson 2961, 2962, 25 Sep. 1990 (MJ). Saaremaa, Mändjala, sandy road verge, V. Liiv and M. Jeppson 2934, 2935, 2936, 2937, 2938, 25 Sep. 1990 (MJ). – Italy, Trento, Andalo, Pensione St. Moritz, 1050 m asl, among mosses and grasses, S. Jeppson, 18 Sep. 1975 (MJ). – Norway, Akershus, Bærum, Kjeholmen, among mosses on calcareous rock, M. Jeppson et al. 8292, 14 Oct. 2006 (MJ). Ibid., Ostøya, among mosses on calcareous rock, M. Jeppson et al. 8308, 14 Oct. 2006 (MJ). Akershus, Nesodden, Persteilene, among mosses on calcareous cliffs, A.-E. Torkelsen et al., 13 Oct. 2006 (MJ). Akershus, Oslo, Bygdøy, Hukodden, among mosses on calcareous rocks near the sea shore, A. Bohlin and M. Jeppson 2079, 19 Aug. 1985 (MJ). Ibid., Gressholmen, among mosses on calcareous rock, M. Jeppson et al. 8255, 13 Oct. 2006 (MJ) Ibid., Malmøykalven, among mosses on calcareous rock, A.-E. Torkelsen et al., 13 Oct. 2006 (MJ). Oppland, Nordre Land, Aust-Torpa, Røste, among mosses on calcareous rock, J. Nitare, 12 Sep. 1984 (MJ). – Sweden, Bohuslän, Askum, Ramsvikslandet, Haby, among mosses in exposed sandy heath-land near the sea shore, T. Schultz and M. Jeppson, 19 Sep. 2001 (MJ). Bohuslän, Tanum, Lindö, N of Vadkilen, among mosses between juniper bushes, J. Nitare, 28 Sep. 1978 (S). Bohuslän, Otterön, N of Flaskevik, dry meadow vegetation on shell-rich sand, J. Nitare, 17 Sep. 1978 (S). Dalsland, Skållerud, Ryr Nature Reserve, Kasen, among mosses on thin soil on cliffs of calcareous clay shale, M. Jeppson 3035, 11 Sep. 1990 (MJ). Gotland, Ardre, Vitvär NW, calcareous flat rock, J. and M. Jeppson 7293, 1 Oct. 2004 (MJ). Gotland, Burgsvik, Hundlausar, calcareous flat rock in alvar vegetation, 27 Sep. 2001, J. and M. Jeppson 5617 (MJ). Gotland, Gammelgarn, Grogarnsberget, on calcareous flat rocks, J. and M. Jeppson 5599, 5600, 26 Sep. 2001 (MJ). Ibid., Sandviken, sandy heathland near sea shore, J. and M. Jeppson 5584, 5585, 26 Sep. 2001 (MJ). Ibid., Skogbo, among mosses on calcareous flat rock, M. Jeppson 8242, 4 Nov. 2006 (MJ). Ibid., Sjaustre, in tortuletum on sandy sea shore, M. Jeppson 8250, 4 Nov. 2006 (MJ). Gotland, Hangvar, Ireviken, sandy heathland near the sea shore, J. and M. Jeppson 5644, 28 Sep. 2001 (MJ). Gotland, Sundre, Hallbjäns, on calcareous cliffs in alvar vegetation, J. and M. Jeppson 5627, 27 Sep. 2001 (MJ). Ibid., Hoburgen, calcareous flat rock, J. and M. Jeppson 5632, 27 Sep. 2001 (MJ). Gotland, Vamlingbo, Holmhällar, sandy heathland near the sea shore, J. and M. Jeppson 5637, 27 Sep. 2001 (MJ). Ibid., Nackshajd, among mosses on calcareous flat rock, J. and M. Jeppson 5618, 27 Sep. 2001 (MJ). Gotland, Visby, Brucebo nature reserve, among mosses on calcareous rock, J. and M. Jeppson 5528, 24 Sep. 2001 (MJ). Ibid., Ölbäcks hällmarksområde, alvar vegetation, J. and M. Jeppson 7260, 30 Sep. 2004 (MJ). Gotland, Väskinde, Själsö Hamn, among mosses in dry meadow vegetation close to the sea shore, J. and M.

Jeppson 8103, 13 Sep. 2006 (MJ). Närke, Glanshammar, 450 m NE of the church, L-E. Kers 4209, 11 Nov. 1973 (S). Ibid., J. and M. Jeppson 7688, 11 Oct. 2005 (MJ). Närke, Lerbäck, Örberga, among low mosses on calcareous cliffs, K-G. Nilsson, 22 Sep. 1987 (MJ). Södermanland, Mörkö, Egelsvik, Kalkberget, among mosses on calcareous cliffs, M. Jeppson 7218, 16 Sep. 2004 (MJ). Ibid., Oaxen, L-E. Kers 4322, 11 Sep. 1972 (S). Södermanland, Nämdö, Mörtö, among mosses or on open soil around moist depressions, also among mosses on occasionally wet zones on calcareous rock, L-E. Kers 4838, 6 Aug. 1977 (S). Ibid., L-E. Kers 4738, 12 Aug 1973 (S). Södermanland, Nynäshamn, Rassa vikar, among mosses on calcareous cliffs, A. Arnell, M. Gothnier amd M. Jeppson 7202, 16 Sep. 2004 (MJ). Södermanland, Tunaberg, Tunaberg, L-E. Kers 4154, 22 Oct. 1973 (S). Södermanland, Trosa-Vagnhärad, Jättarsberget, 500 m S of Furholmen, L-E. Kers 4615, 8 Aug. 1976 (S). Ibid., Lånestaåsen, L-E. Kers 4970, 16 Sep. 1978 (S). Södermanland, Vårdinge, Nynäs, on calcareous rock, M. Jeppson 7243, 19 Sep. 2004 (MJ). Södermanland, Ösmo, Malhuvud, L-E. Kers, 15 Nov. 1972 (S). Ibid., among mosses on calcareous rock, M. Jeppson et al. 7210, 16 Sep. 2004 (MJ). Uppland, Dalarö, Utö, Kroka, among mosses on calcareous cliffs near the sea shore, M. Jeppson 6910, 4 Nov. 2004 (MJ). Ibid., N of Gruvsamhället, L-E. Kers 4753, 29 Oct. 1972 (S). Ibid., Gruvbryggan, L-E. Kers 4751, 11 Nov. 1972 (S). Uppland, Djurö, Runmarö, Lerkila, among mosses on calcareous flat rock, Å. Strid 17037, 15 Nov. 1982 (S). Ibid., Nore, on moss tussocks on calcareous cliffs near the sea shore, M. Jeppson 6935, 5 Nov. 2004 (MJ. Iibid., Vitträsk W, among mosses on calcareous cliffs, S. Thoresdotter and M. Jeppson 7233, 17 Sep. 2004 (MJ). Ibid., Uppebyträsk, on calcareous flat rock, M. Jeppson 6937, 5 Nov. 2004 (MJ). Uppland, Älvkarleby, R. E. Fries, 11 Sep. 1910 (S). Ibid., L-E. Kers 4519, 24 Sep. 1975 (S). Västergötland, Dala, Djupadalen nature reserve, calcareous flat rocks in alvar vegetation, M. Jeppson 612b, 29 Sep. 1974 (MJ). Ibid., J. and M. Jeppson 3628, 4 Nov. 1994 (MJ). Ibid., Stenåsen, among mosses on exposed calcareous flat rock in alvar vegetation, M. Jeppson 8363, 28 Oct. 2006 (MJ). Ibid., Nya Dala, among mosses on exposed calcareous flat rock, M. Jeppson 8364, 28 Oct. 2006 (MJ). Västergötland, Högstena, Öja Hed nature reserve, among mosses on calcareous flat rock in alvar vegetation, M. Jeppson 7665, 2 Nov. 2005 (MJ). Västergötland, Österplana, Österplana Hed och Vall nature reserve, among mosses on calcareous flat rock in alvar vegetation, S., J., and M. Jeppson 2562, 28 Aug. 1988 (MJ). Öland, Algutsrum, Höge ås, E. Hultqvist, 2 Nov. 2003 (MJ). Öland, Böda, Getterum, sandy-gravelly road verge, S., J., and M. Jeppson 2634, 9 Sep. 1988 (MJ). Öland, Gärdslösa, Lindby, dry meadow vegetation in abandoned sand pit, J. and M. Jeppson 5170, 9 Oct. 1999 (MJ). Öland, Högby: 2 km S of Hornsjöns Pensionat, alvar vegetation, among mosses, K-G. Bringer, 14 June 1973 (S). Ibid., Dödevi strandängar, on sandy grass heath near sea shore, H. Kreisel, J. and M. Jeppson 3970, 4 Oct. 1996 (MJ). Ibid., Högby fyrområde, among mosses on calcareous sand, S., J., and M. Jeppson 2300, 3 Oct. 1987 (MJ). Öland, Högsrum, Höghäll, among mosses on calcareous flat rock in alvar vegetation, J. and M. Jeppson 7160, 26 Sep. 2004 (MJ). Ibid., Karums alvar, among mosses on calcareous flat rock in alvar vegetation, M. Jeppson 8066, 11 Nov. 2006 (MJ). Ibid., Vedby, alvar vegetation, J. and M. Jeppson 3961, 3 Oct. 1990 (MJ). Öland, Resmo, Resmo alvar, among mosses on calcareous flat rock in alvar vegetation, M. Jeppson 7711, 11 Nov. 2005 (MJ). Ibid., Stora Alvaret, W. of Möckelmossen, J. and M. Jeppson 985, 29 Oct. 1976 (MJ). Ibid., Gynge alvar, alvar vegetation, M. Jeppson 7707, 11 Nov. 2005 (MJ). Öland, Räpplinge, Borgholms slottsalvar, alvar vegetation, J. and M. Jeppson 2333, 3 Oct. 1987 (MJ). Öland, Stenåsa, Stora Alvaret, SE of Möckelmossen, calcareous rock in alvar vegetation, J. and M. Jeppson 7119, 24 Sep. 2004 (MJ). Öland, Södra Möckleby, Gettlinge, alvar vegetation, J. and M. Jeppson 2926, 21 Oct. 1990 (MJ). Ibid., E of Gettlinge, alvar vegetetation, J. and M. Jeppson 5124, 7 Oct. 1999 (MJ). Öland, Vickleby, L. Vickleby alvar, on calcareous rock in alvar vegetation, M. Jeppson 4612, 9 Oct. 1998 (MJ). Östergötland, Krokek, Marmorbruket, L-E. Kers 3836, 18 Nov. 1972 (S).

Molecular methods

Ingroup and outgroup taxa for the phylogenetic analysis were selected in accordance with the results from our earlier published study of *Lycoperdaceae* (Larsson and Jeppson 2008). The dataset contained 21 ingroup sequences representing four species from *Bovista* subg. *Globaria*, eight species from *Bovista* subg. *Bovista*, three *Calvatia* species, and *Disciseda bovista*. *Mycenastrum corium* was selected as outgroup.

Two sequences of *Bovista limosa* and two representing *Bovista cf. limosa* were generated specifically for this study. These sequences were all obtained from herbarium specimens. DNA extraction, PCR conditions and sequencing followed Larsson and Jeppson (2008). Primers used to amplify the complete ITS region and the 5′end of the LSU region were ITS1F (Gardes and Bruns 1993) and LR21 (Hopple and Vilgalys 1999). Primers used for sequencing were ITS1, ITS3, ITS4 (White et al. 1990).

Sequences were edited and assembled using Sequencher 3.1 (Gene Codes, Ann Arbor). Aligning was done with MAFFT (Katoh et al 2002) followed by manual adjustment using the data editor in PAUP* (Swofford 2003).

Heuristic searches for most parsimonious trees were performed using PAUP*. All transformations were considered unordered and equally weighted. Variable regions with ambiguous alignment were excluded and gaps treated as missing data. Searches used 1,000 random-addition sequence replicates and TBR branch swapping. Relative robustness of clades was assessed by the bootstrap method using 1,000 heuristic search replicates with 100 random taxon addition sequence replicates and TBR branch swapping, saving 100 trees from each replicate.

Results

Molecular data

The aligned dataset had 1,625 characters. After exclusion of ambiguous areas, 1,454 characters remained for the analyses. Of these, 1,253 were constant, 96 variable but parsimony uninformative, and 105 parsimony informative. Maximum parsimony analysis yielded 6 equally most parsimonious trees (length=313, CI=0.7125, RI=0.7376). Here, one of the trees is presented as a phylogram with bootstrap frequencies (BS) above 50% indicated on branches (Fig. 1).

The analysis recovered *Bovista* as a monophyletic clade with 82% BS, *Bovista* subg. *Globaria* with 84% BS, and *Bovista* subg. *Bovista* with 94% BS. *Bovista limosa* (100% BS) and *B. cf. limosa* (100% BS) are recovered as distinct clades within subg. *Bovista*. Sequences from these two clades differ by 12 substitutions and a 2-bp insertion/deletion event in the ITS1 region and 7 substitutions in the ITS2 region. Within each clade, sequences are identical.

Morphological and ecological data

The collections originating from northern localities (south to ca. 58°N in Fennoscandia) coincide with the morphological concept of *Bovista limosa* as described by Rostrup (1894), Eckblad (1971), and Lange (1948, 1987). The holotype from Greenland deposited at C was requested but could unfortunately not be located (H. Knudsen, personal communication). Collections originating from southern localities (north to ca. 60°N in Fennoscandia) coincide morphologically with the descriptions of *Bovista limosa* by. e.g.. Monthoux and Röllin (1976), Lohwag (1933), and

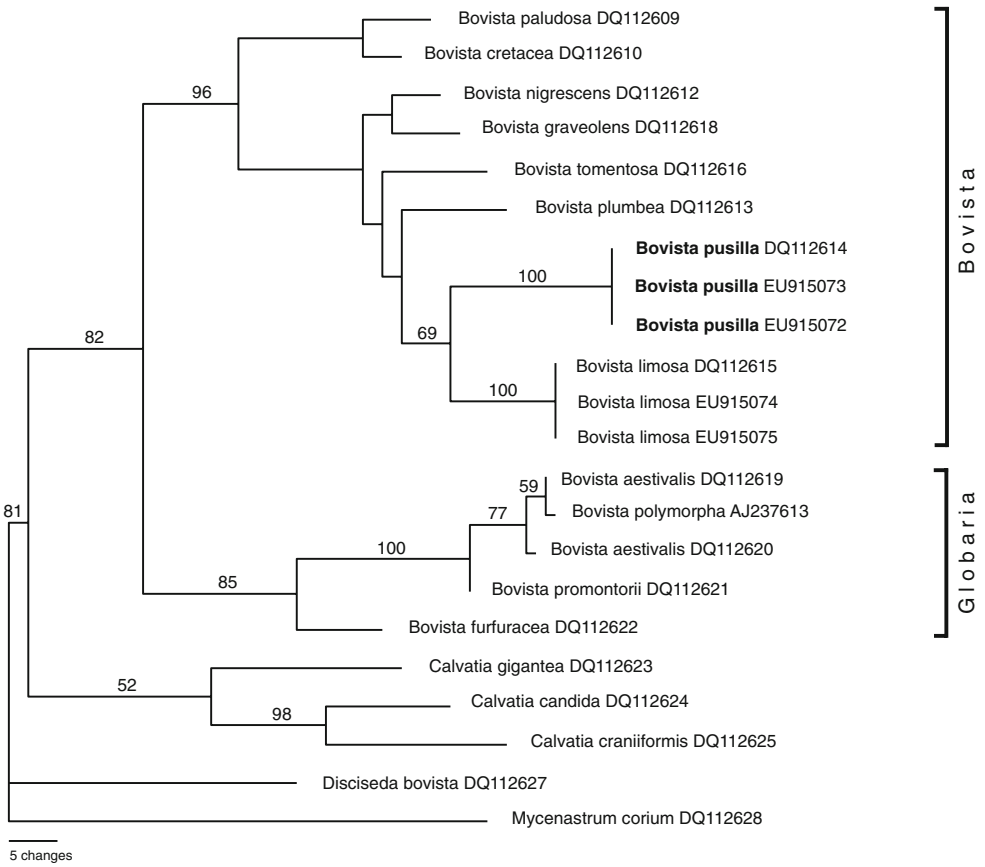

Fig. 1 One of six equally most parsimonious trees presented as a phylogram showing relative branch lengths. Bootstrap values are noted on branches

Pegler et al. (1995). This is the taxon that was named *B. cf. limosa* by Larsson and Jeppson (2008). The two taxa occupy different, but slightly overlapping, distribution ranges (Figs. 2 and 3).

The main and most apparent morphologial difference is the presence of a delimited peristome, a peristomal depression, and a fimbriate, protruding stoma in *B. limosa* (Fig. 4), whereas *B. cf. limosa* has a plain, flattened or protruding, slightly lobed (but not fimbriate) stoma which is not surrounded by a depression. The fimbriate stoma of *B. limosa* is characterised by an abundance of up to 10 µm wide, somewhat tortuous, hyphae with tapering ends. These hyphae seem to originate from the peripheral capillitium, connected to the interior wall of the endoperidium. In the non-fimbriate mouth zone of *B. cf. limosa* such hyphae are rare or lacking. With regard to the exoperidial ornamentation in mature specimens, *B. limosa* is usually characterised by rather large, separate, whitish-yellowish flocculae strongly contrasting to the darker endoperidium whereas in *B. cf. limosa* the exoperidial features are more membraneous with less conspicuous and ±coalescent flocculae being on the average more greyish in colour. There is further a slight difference in the average size of the fruiting bodies, where *B. limosa* is the smaller of the two.

The micro-morphological characters of the mature gleba are basically similar in the two species. However, *B. cf. limosa* shows a tendency to form more abundant septa in the capillitial hyphae of the peripheral, lycoperdoid parts of the gleba. The central part of the gleba is in both species composed of an intermediate *Bovista-Lycoperdon*-type of capillitium, devoid of septa. The pits in the capillitial walls that characterise several *Bovista* species are lacking in both *B. limosa* and *B. cf. limosa*. The spore morphology of the two species is also similar as to size and ornamentation, although the pedicel often seems to be somewhat better preserved in *B. cf. limosa.* Some important discriminating morphological characters are compared in Table 1.

Bovista limosa is a species occurring in boreal and arctic-alpine habitats. The type specimen was collected by Nils Hartz (Rostrup 1894), who stated the type to grow "mellem mos i kær" (between mosses in swamp). Lange (1948; as *Bovista echinella*) described the collecting sites in

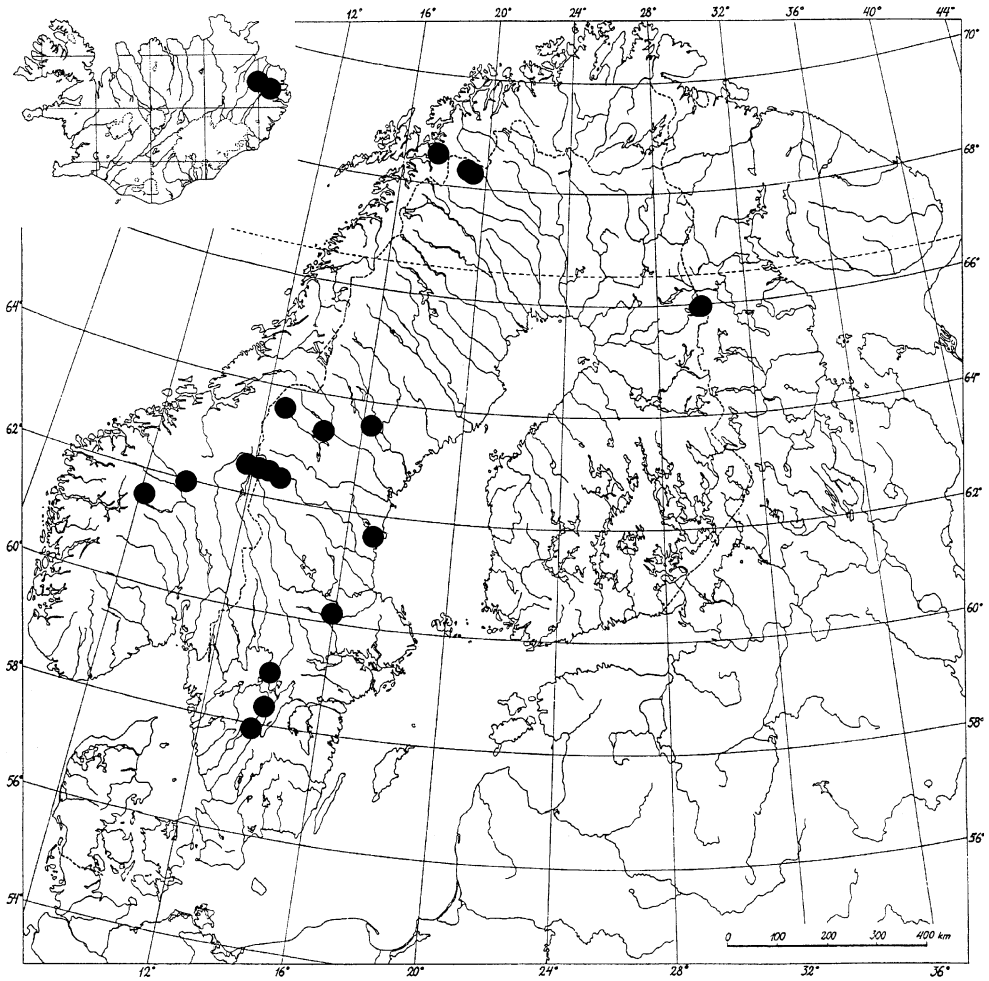

Fig. 2 Distribution of *Bovista limosa* in Fennoscandia and Iceland

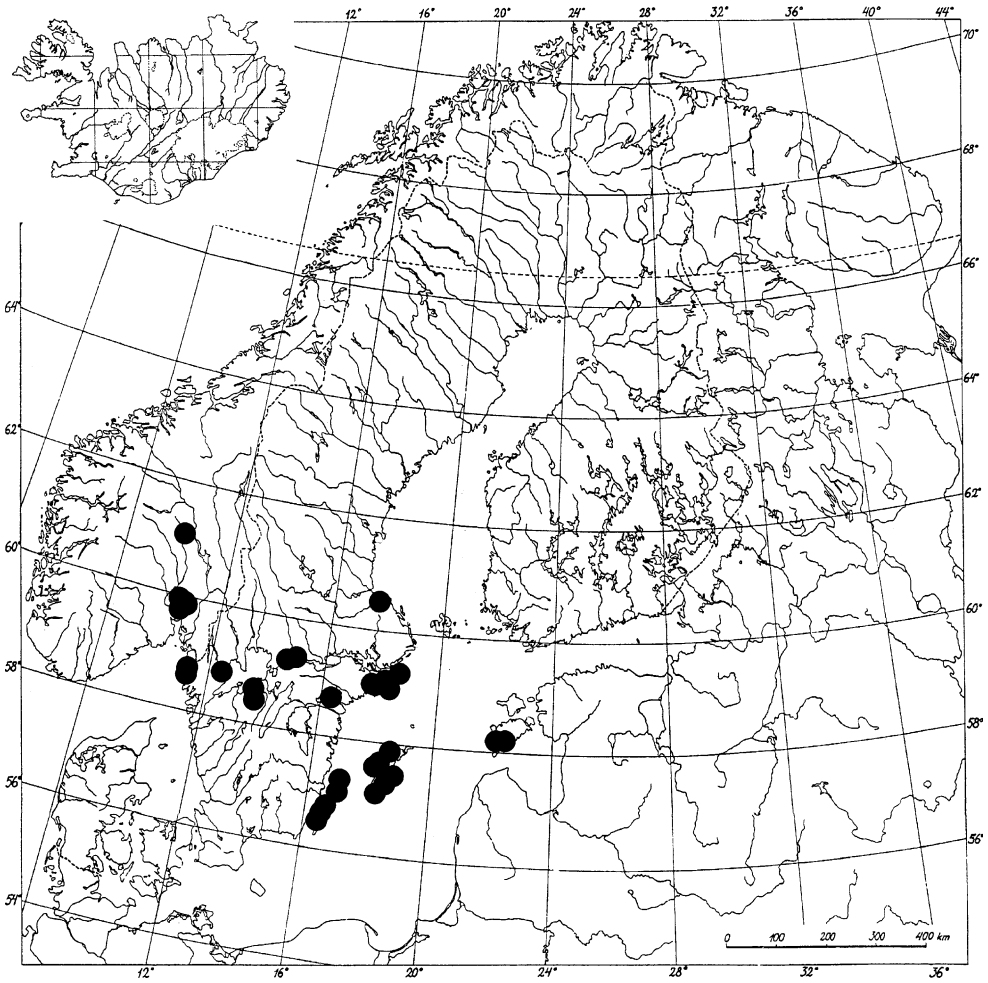

Fig. 3 Distribution of *Bovista pusilla* in Fennoscandia and the Baltic countries

the Søndre Strømfjord area in west Greenland as "somewhat humid localities on the lower part of south slopes". In our experience, *B. limosa* is a species growing in a sparse vegetation of low mosses and herbs, often with some human disturbance, as on verges, car parks and abandoned soccer grounds. However, it is also found in natural habitats in temporarily wet sites close to streams and swamps, particularly in alpine, calciphilous *Dryas* vegetation.

Bovista cf. limosa occurs on dry, sandy soil and on calcareous cliffs and is regularly found in the temperate and hemiboreal vegetation zones of North Europe. See further description below.

Discussion

Based on morphological characters and phylogenetic analyses of ITS and LSU sequence data, two distinct species were identified within the current concept of *Bovista limosa* (Larsson and Jeppson 2008). In this study, additional sequences of the two species were generated and more studies of morphology, ecology and distribution patterns were performed. The results confirm the occurrence of two closely related but distinct species (Fig. 1). Despite the differences in morphology, distribution patterns and ecology the presence of two species was for a long time overlooked.

Kreisel (1967) reported several small *Bovista*-species lacking a delimited peristome but none of them seem to match the characters of *B. cf. limosa*. *Bovista echinella* can be distinguished on microscopical characteristics (pitted capillitium). This also seems to be the case with *B. albosquamosa* Kreisel (fragile, septate capillitium in central part of gleba) and the rarely collected and incompletely known *B. trachyspora* (strongly ornamented spores). However, a name to be considered for *B. cf. limosa* is *Lycoperdon pusillum* Batsch, transferred to *Bovista* by Persoon (1801) and adopted by Kreisel (1962, 1967). However, Kreisel (1967) thought that the description and plate of *Lycoperdon pusillum* (Batsch 1789) was ambiguous and preferred to base his concept on an emended description of *Lycoperdon pusillum* by Schumacher (1803). Kreisel´s concept of *Bovista pusilla* thus refers to a small subglobose puffball with a lycoperdoid capillitium

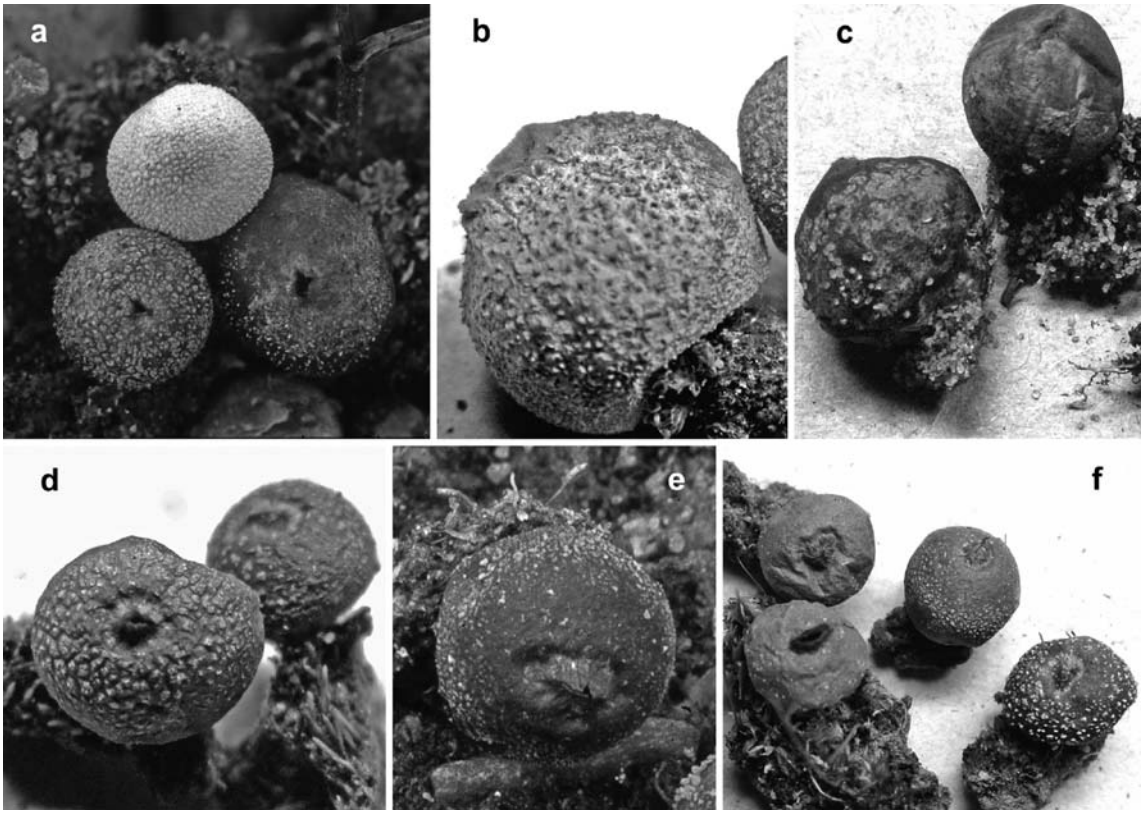

Fig. 4 Fruiting bodies of *Bovista pusilla* (**a**–**c**) and *Bovista limosa* (**d**–**f**): **a** collection number MJ 5170, photo J. Jeppson; **b**, **c** details of the ostiole, collection number MJ 7210; **d**, **e** details of the ostiole, collection number MJ 5184, photo J. Jeppson; **f** collection number MJ 1146

throughout the gleba combined with apedicellate spores, characters that are clearly different from those present in *B. limosa* and *B. cf. limosa*. *Lycoperdon ericetorum* Pers., *L. dermoxanthum* Vitt., *Bovista obovata* Massee, *B. monticola* Spegazz., and *Lycoperdon hungaricum* Hollós were among the synonyms mentioned by Kreisel. Following Kreisel (1967), *Bovista pusilla* (Batsch:Pers.) Pers. became a widely accepted name used by several authors, e.g. Ulvinen in *Nordic Macromycetes* (Hansen and Knudsen 1997).

Ortega and Buendía (1989) pointed out that Kreisel's concept of *Bovista pusilla* did not match the original description. Accordingly, they replaced *B. pusilla* sensu Kreisel (1967) with *B. dermoxantha* (Vittad.) De Toni. This interpretation was followed by Pegler et al. (1995) and Calonge (1998) who concluded that *B. pusilla* should be considered a *nomen ambiguum* despite the disadvantageous loss of a well-known name. Pegler et al. (1995) emphasised that a comparison of illustrations and herbarium collections of *B. pusilla* showed "widespread confusion in application of the name" and stressed the need for a careful revision of all records of *B. pusilla*. Moyersoen and Demoulin (1996) showed that *B. pusilla* in the sense of Kreisel (1967) in fact covered two independent species, *B. dermoxantha* and *B. furfuracea* (J. F. Gmelin) Pers, both having lycoperdoid

Table 1 Morphological characters discriminating between *Bovista limosa* and *B. pusilla*

	Bovista limosa	*Bovista pusilla*
Fruiting body size	(3–)5–6(–10) mm in diam.	(5–)9–15(–20) mm in diam.
Exoperidium in mature specimens	Whitish-yellowish, often conspicuous, isolated warts or flocculae	Whitish-greyish, less conspicuous, ±coalescent warts and flocculae
Peristome	Conspicuous, delimited, with peristomal depression, protruding, fimbriate	Inconspicuous, non-delimited, plain, flat to somewhat protruding, not fimbriate
Pedicel	3–8 µm; often short and badly preserved	3–9 µm; usually well preserved
Peripheral capillitium	±*Lycoperdon*-type with rare septa	±*Lycoperdon*-type with scattered to abundant septa

characters of the capillitium and apedicellate spores. The former species is characterised by the presence of a minute subgleba and rather coarsely warted spores whereas the latter always lacks a subgleba and has weakly ornamented spores. This division and renaming of *B. pusilla* sensu Kreisel (1967) was widely accepted (e.g. Jeppson 1998; Hallingbäck and Aronsson 1998; Poumarat 2003) although Kreisel (2001) still preferred to maintain the name *B. pusilla* for what Moyersoen and Demoulin (1996) called *B. furfuracea*.

Moyersoen and Demoulin (1996) concluded that the species described and depicted by Batsch was the same as *B. limosa*. Referring to the confusion surrounding the name *Lycoperdon pusillum* and the rules in the International Code of Botanical Nomenclature, Moyersoen and Demoulin (1996) suggested that Batsch's name, in spite of being older, should be rejected as the valid name for *B. limosa*. However, no such proposal has, to our knowledge, been put forward to the nomenclatural committee for fungi (McNeill et al 2006).

We have shown that *Bovista limosa* consists of two species with different ITS sequences, slightly different morphology, clearly different ecology, and different, albeit overlapping, distribution ranges.

From Batsch's (1789) original description of *Lycoperdon pusillum* it is evident that it refers to a species identical to or closely related to *Bovista limosa*. This observation was already made and commented upon by Calonge and Demoulin (1975), Ortega and Buendía (1989), Moyersoen and Demoulin (1996) and Calonge (1998). Batsch mentioned the small sessile, globose fruitbodies with blackish-brown to violet brownish colours. The accompanying illustrations (Batsch 1789, Fig. 228a–c) agree well with his description. With regard to the stoma, that appears to be a crucial macro-morphological feature, Batsch wrote: "*Orificium plerumque minutum angulatum, vel majus subrotundum, marginibus reflexis, semper solum in acumine occurrens*." This does not imply a delimited, fimbriate stoma with a peristomal depression and is in agreement with his plate, where one specimen (228b) shows a protruding and recurvedly lobed (not fimbriate) stoma without a delimited peristomal depression, whereas another depicted specimen (228a) seems to be provided with a plain, not protruding and slightly irregular stoma. Figure 228c shows a sectioned, probably immature fruitbody with a slight tendency to protrude at the apex. Batsch's specimens were found "*in plano sicco montoso*" on the northern side of a forest, in the vicinity of Jena in Germany. Although also recorded on calcareous sand (Pegler et al 1995; Jeppson 1993), the occurrence on dry, flat rocks is typical for *B. cf. limosa*. Furthermore, Runge and Gröger (1990) recorded *B. limosa* with a protruding but not delimited nor fimbriate stoma on calcareous loamy sand in the vicinity of Gotha in Thüringen, i.e. not very distant from Jena where Batsch's *Lycoperdon pusillum* was collected some 200 years earlier.

We conclude that morphological characters, ecology and distribution pattern indicate that the unnamed species that we tentatively have called *B. cf. limosa* is the same fungus that Batsch described as *Lycoperdon pusillum*. This leaves us with two alternatives. Either, we can follow Moyersoen and Demoulin (1996) and propose rejection of *L. pusillum*. This solution involves the creation of a new name for our unnamed species. Or, we can accept *L. pusillum,* typify it by Batsch's illustration but also add an epitype from the material we have studied. That would re-establish *L. pusillum* as an unambiguous taxon clearly separated from the other three taxa with which the name has been associated.

The suggestion to reject the name *Lycoperdon pusillum* (Moyersoen and Demoulin 1996) was a reasonable and logical step as long as the species was identified as identical to *Bovista limosa*. Now we have shown that the concept of *Bovista limosa* applied by Moyersoen and Demoulin (1996) and many others covers two distinct species, the main argument for rejecting *L. pusillum* has disappeared. In fact, the argument for rejecting *Lycoperdon pusillum* as a convoluted name now applies also to the name *Bovista limosa*.

The name *Lycoperdon pusillum* does not compete with any other known name and its reintroduction does not entail any nomenclatural changes. As we understand the rules of the International Code for Botanical Nomenclature (Articles 56 and 57) strict requirements for a rejection of *Lycoperdon pusillum* are lacking although one could still argue that the many misapplications of the name makes its revival less attractive. However, one can also argue that, after many years of confusion, the matter can now be resolved without any nomenclatural changes, thus both increasing stability and preserving the priority principle.

Taxonomy

Bovista pusilla (Batsch) Pers., Synopsis methodica fungorum (Göttingen) 1:138 (1801)

Lycoperdon pusillum Batsch, Elenchus fungorum, cont. sec, (Halle): 123 & tab. 41, Fig. 228 (1789).

Lectotypus (hic designatus): Batsch, AJGC, Elenchus fungorum, cont. sec. (Halle) tab. 41, Fig. 228a.

Epitypus (hic designatus): Sweden, Öland, Dödevi strandängar, 500 m south of Tornholmsudde, on sand-field near the sea shore, 4 Oct 1996, leg. M. Jeppson 3971 (GB).

Fruiting body subglobose, (0.5–)0.9–1.5(–2.0)cm in diameter with a basal mycelial tuft. Exoperidium initially white, smooth to finely warted or tufted. At maturity, the

exoperidium disappears in the lower portion of the fruiting body but usually remains as a greyish-yellowish brown membrane or small granules in the upper part. Endoperidium at maturity usually exposed towards the base, dark brownish sometimes with slightly violet tinges. In old and weathered specimens, the endoperidium is fully exposed and dark brown.

Apical stoma flat to somewhat protruding, sometimes lobed with recurving edges, not surrounded by a delimited peristome or a "peristomal depression". Mature gleba dark brown, subgleba lacking.

Spores subglobose to globose, 4.5–5.5 µm in diameter, finely warted and provided with a pedicel, 3–9 µm long.

Capillitium heteromorph. In centre of gleba of intermediate *Bovista-Lycoperdon*-type with dichotomous branching and tapering ends; main stems up to 10 µm in diameter. Walls ca. 1 µm, without pores. No true septa. Peripheral portions of gleba of *Lycoperdon*-type with long, scarcely branched threads. Walls thin (<1 µm), without pores. Septa scattered to abundant.

Exoperidium consisting of an inner, pseudoparenchymatous layer and an outer hyphal layer with septate, thin-walled hyphae (2–4 µm in diameter) with club-shaped to spherical terminal cells (7–13 µm in diameter). The exoperidial warts are built up of congregations of ±spherical terminal elements.

Endoperidium constructed of interwoven hyphae 2–4 µm in diameter, ±parallel to the surface. Mouth zone of mature specimens composed of interwoven endoperidial hyphae.

Habitat and distribution: *Bovista pusilla* is found among mosses and small herbs forming a patchy vegetation on calcareous sand, cliffs and flat rock in dry and exposed situations. It is a characteristic species in the thin and dry moss cover on calcareous rock in the alvar vegetation. There are additional records from coastal sand dunes in the Baltic region and along the Swedish west coast. All our records are from calcareous sites in the temperate and hemiboreal vegetation zones. A number of the localities of *B. pusilla* are characterised by an abundance of more or less xerophilous gasteromycetes like *B. tomentosa*, *Geastrum schmidelii*, *G. minimum*, *G. elegans*, and *Tulostoma brumale*.

Remarks: From the protologue (Batsch 1789), it cannot be established if the three fruiting bodies depicted as Fig. 228 emanate from one single mycelium. Therefore, we choose to select one of the figures as lectotype. The two figures showing complete fruiting bodies (228a, b) are seemingly quite different as to the morphology of the stoma. However, we have seen both these types in our own material and the important thing is that neither of them have a delimited peristome with a peristomal depression. A plain, slightly irregular stoma, as seen in Fig. 228a, is the most common one in our material which is why this illustration is selected as lectotype. Batsch's publication is available at www.biolib.de.

Bovista limosa Rostr., Meddel. Grønland 18:52 (1894).

For a description and illustration of *Bovista limosa* we refer to Fries (1910) and Lange (1948, 1987).

A key to the small Bovista species discussed in the paper

1 Subgleba present. *Lycoperdon dermoxanthum*
1* Subgleba lacking. 2
2 Capillitium of Lycoperdon-type throughout the gleba. 4
2* Capillitium in centre of the gleba of Bovista-type or intermediate Bovista-Lycoperdon-type. 3
3 Capillitium of Bovista-type, with numerous pores. *B. echinella*
3* Capilltium heteromorph; in centre of gleba of intermediate Bovista-Lycoperdon-type. 5
4 Capillitium without pores; spores coarsely warted, pedicellate. *B. trachyspora*
4* Capillitium with numerous pores; spores finely warted, apedicellate. *B. furfuracea*
5 With a delimited, fimbriate peristome. *B. limosa*
5* With a plain, somewhat lobed stoma; peristome neither delimited nor fimbriate. 6
6 Capillitium in central part of gleba with frequent septa. *B. albosquamosa*
6* Capillitium in central part of gleba without septa *B. pusilla*

Acknowledgements Henning Knudsen for support with material and literature as well as to Sven-Åke Hanson, Trond Schumacher, Signe and Jörgen Jeppson, Rolf-Göran Carlsson and the curator at S for providing material. Ron Petersen and anonymous reviewer for suggestions of improvements of earlier versions of the manuscript. Financial support for this study was received from The Swedish Taxonomy Initiative, ArtDatabanken SLU, to EL (Grant dha 7/04 and 146/05) and Kapten Carl Stenholms fund to M.J.

References

Batsch AJGC (1789) Elenchus fungorum. Continuatio secunda. Halle.
Calonge FD, Demoulin V (1975) Les Gastéromycètes d'Espagne. Bull Trimest Soc Mycol Fr 91:247–292
Calonge FD (1998) Flora Mycologica Iberica Vol. 3. Gasteromycetes, 1. Lycoperdales, Nidulariales, Phallales, Sclerodermatales, Tulostomatales. J. Cramer, Madrid, Berlin, Stuttgart.
Demoulin V (1968) Gastéromycètes de Belgique: *Sclerodermatales*, *Tulostomatales*, *Lycoperdales*. Bull Jard Bot Natl Belg 38:1–101. doi:10.2307/3667475
Eckblad FE (1971) The Gasteromycetes of Finnmark (Northernmost Norway). Astarte 4:7–21
Fries RE (1910) Ett märkligt Gasteromycet-fynd. Sven Bot Tidskr 3:176–177
Fries TM (1921) Sveriges Gasteromyceter. Ark Bot 17:1–63
Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizas

and rusts. Mol Ecol 2:113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Hallingbäck T, Aronsson G (1998) Ekologisk katalog över storsvampar och myxomyceter. 2nd revised and extended ed, ArtDatabanken, SLU, Uppsala.

Holmgren PK, Holmgren NH (1998, continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium, New York, http://sweetgum.nybg.org/ih/

Hansen L, Knudsen H (1997) Nordic Macromycetes vol 3. Heterobasidioid, Aphyllophoroid and Gasteromycetoid Basidiomycetes. Nordsvamp, Copenhagen

Hopple JS Jr, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Mol Phylogenet Evol 13:1–19. doi:10.1006/mpev.1999.0634

Jeppson M (1993) Svampexkursion till Saaremaa. Anteckningar från en resa i österled. Jordstjärnan 14(1):33–37

Jeppson M (1998) Sandäggsvampen, *Bovista pusilla*, egentligen två arter. Jordstjärnan 19(2):31–37

Jeppson M (1999) Gasteromyceter på Västergötlands kalktorrängar. Jordstjärnan 20(3):9–28

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. doi:10.1093/nar/gkf436

Kreisel H (1962) Die Lycoperdaceae der DDR. Bibl Mycol ▪▪▪:36. Reprint, J. Cramer, Lehre.

Kresiel H (1964) Vorläufige Übersicht der Gattung *Bovista* Dill. ex Pers. Feddes Repert 69:196–211

Kreisel H (1967) Taxonomisch-Pflanzengeographische Monographie der Gattung *Bovista*. Beih Nova Hedwigia 25:1–244

Kreisel H (2001) Checklist to the gasteral and secotioid Basidiomycetes of Europe, Africa and the Middle East. Österr Z Pilzk, N S 10:213–313

Larsson E, Jeppson M (2008) Phylogenetic relationships among species and genera of Lycoperdaceae based on ITS and LSU sequence data from North European taxa. Mycol Res 112:4–22. doi:10.1016/j.mycres.2007.10.018

Lange M (1948) Macromycetes IV. The Gasteromycetes of Greenland. Medd Gronl 147:1–32

Lange M (1987) Arctic Gasteromycetes. The genus *Bovista* in Greenland and Svalbard. In: Laursen GA, Ammirati JF, Redhead SA (eds) Arctic and Alpine Mycology II. Plenum Press, New York, pp 261–271

Lloyd CG (1906) The genus *Bovistella*. Mycol Notes Lloyd Libr Mus 23:277–287 + plate 89

Lloyd CG (1910) A *Bovistella* with a Geaster mouth. Mycol Notes Lloyd Libr Mus 34:452

Lohwag H (1933) Mykologische Studien VIII. Bovista echinella Pat. und Lycoperdon velatum Vitt. Beih Bot Centralbl Abt 2(51):269–286

McNeill J, Barrie FF, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersema J, Turland NJ (2006) International Code of Botanical Nomenclature (Vienna Code). Adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. Regnum Veg 146:I–XVI, 1–568.

Monthoux O, Röllin O (1976) La flore fongique des stations xériques de la région de Genève IV. Lycoperdaceae: genre Bovista Pers Candollea 31:247–256

Moyersoen B, Demoulin V (1996) Les gastéromycètes de Corse: Taxonomie, Écologie, Chorologie. Lejeunia, n s 152:1–128

Ortega A, Buendía GA (1989) Estudio del complejo Bovista aestivalis (Bon.) Demoulin–B. pusilla (Batsch) Pers. sensu Kreisel en España. Cryptogam Mycol 10:9–18

Pegler DN, Læssøe T, Spooner BM (1995) British Puffballs. Earthstars and Stinkhorns, Royal Botanic Gardens, Kew

Persoon CH (1801) Synopsis methodica fungorum. Gottingae.

Poumarat S (2003) Clé de Gasteromycetes épigés d'Europe. Fédérations de Associations Mycologiques Méditerranéennes, Nice

Rostrup E (1894) Øst-Grønlands Svampe. Medd Gronl 18:44–81

Runge A, Gröger F (1990) Neue Funde von *Bovista limosa* in Deutschland. Mykol Mitteilungsbl 33:91–94

Sarasini M (2005) Gasteromiceti epigei. Assoziazione Micologica Bresadola, Fondazione Centro Studi Micologici, Trento

Schumacher CF (1803) Enumeratio plantarum in partibus Saellandiae septentrionalis et orientalis. Vol. 2. Havniae.

Swofford DL (2003) PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sinauer Associates, Sunderland, MA

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sininsky JJ, White TJ (eds) PCR Protocols: A Guide to Method and Applications. Academic Press, New York, pp 315–322