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Detecting the phylogenetic position of *Bovista acuminata* (Agaricales, Basidiomycota) by an ITS-LSU combined analysis: the new genus *Bryoperdon* and revisitation of *Lycoperdon* subgen. *Apioperdon*

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Abstract

Bovista a cuminata, a rare, scantily reported species so far known from Europe and the Americas, occupies an isolated position in *Bovista* on account of its minutely verrucose spores, habitus and growth on moss cushions covering fallen trunks or rocks. Since it has not yet been studied from a molecular perspective, it was decided to sequence two recent collections from Italy. Based on morphological and molecular data, the genus *Bryoperdon* is established for it and placed in *Lycoperdaceae*, where it is close to *Apioperdon* comb. et stat. nov., which encompasses the *Lycoperdon pyriforme* clade. The delimitation of the genera *Bryoperdon* and *Apioperdon* from each other and from allied taxa is also discussed.

Key words: *Agaricomycetes*, *Agaricaceae*, gasteromycetoid fungi, ribosomal sequences, puffballs, Taxonomy

Introduction

Gasteromycetes are an artificial and polyphyletic assemblage of *Agaricomycetes* (*Basidiomycota*) characterized by spore formation within enclosed basidiomes (gasteroid basidiomes or gasterothecia according to Gube 2009) and statismosporic basidia (with passive spore release) (Reijnders 2000; Krüger *et al.* 2001; Bates 2004; Bates *et al.* 2009; Gube 2009). Most gasteroid basidiomes are thought to have evolved as an adaptation to animal dispersal and arid habitats (Savile 1955, 1968; Thiers 1984; Bruns *et al.* 1989). Gasteromycetation, the evolutionary process leading up to the production of gasteroid basidiomes (gasterothecia) instead of agaricoid basidiome (hymenothecia sensu Gube 2009) with exposed hymenium and active spore discharge (ballistoporic basidia), occurred many times and in many phyletic lines independently within *Agaricomycetes* (Heim 1951; Singer 1986; Matheny *et al.* 2006; Albee-Scott 2007; Wilson *et al.* 2011; Gube & Dörfelt 2012; Oberwinkler 2012). Among these evolutive lines, *Lycoperdaceae* Chevall. (1826: 348), a large family of true puffballs, is phylogenetically so close to the mainly agaricoid *Agaricaceae* Chevall. (1826: 121) (Moncalvo *et al.* 2002; Bates *et al.* 2009) as to be regarded as being included in it by some authors (*Agaricaceae* s.l., Vellinga 2004; Matheny *et al.* 2006; Gube 2009). *Lycoperdaceae* traditionally encompasses nine genera: *Bovista* Pers. (1794: 86), *Bovistella* Morgan (1892: 141), *Calvatia* Fr. (1849: 442), *Disciseda* Czern. (1845: 153), *Handkea* Kreisel (1989: 282), *Langermannia* Rostk. (1839: 23), *Lycoperdon* Pers. (1801: 140), *Morganella* Zeller (1948: 650) and *Vascellum* F. Šmarda (1958: 760) (Larsson & Jeppson 2008). The family includes species characterized by gasteroid basidiomes without a true stipe, and drying glebal structures. In addition, such basidiomes have a pure white gleba, at maturity turning into a brown powder of trillions of spores which are dispersed from the apical opening of the endoperidium, pigmented, often verrucolose spores and a branched, often pitted capillitium (Pegler *et al.* 1995; Calonge 1998; Poumarat 2003; Sarasini 2005). The species show a saprotrophic habit, either growing on the ground in forests or open grassland, or on dead wood. Intergeneric distinction is based on presence or absence of a true capillitium and/or of a paracapillitium, presence or absence of a diaphragm which separates the fertile portion of the gleba from the sterile basal portion, the structure of the capillitial threads, the opening pattern of the endoperidium, and the presence or absence of a subgleba (Kreisel 1962, 1969; Calonge 1998; Sarasini 2005; Larsson & Jeppson 2008). The type genus *Lycoperdon* includes small- to medium sized basidiomes (ca. 10–120 mm diam.), characterized

by spores usually released through an apical pore (stoma), frequent presence of a well-developed stem-like sterile base (subgleba), and spore-wall covered with spines, warts, or minute particles (Kreisel 1962; Demoulin 1971, 1972, 1983; Calonge 1998; Sarasini 2005; Jeppson 2006; Larsson & Jeppson 2008). Molecular studies have also indicated that *Lycoperdon*, as circumscribed in the current morphology-based classification, is polyphyletic (Bates 2004; Krüger *et al.* 2001; Krüger & Kreisel 2003). Recent phylogenetic analyses of *Lycoperdaceae* (Krüger & Gargas 2008; Larsson & Jeppson 2008; Bates *et al.* 2009) have shown that *Lycoperdon perlatum* Pers. (1796: 4), type species of the genus, clusters with *Bovistella*, *Handkea*, *Morganella*, *Vascellum*, and some other *Lycoperdon* species, with which it forms a large clade. Therefore, Larsson & Jeppson (2008) suggested to widen the concept of this genus to minimize name changes and combined *Bovistella*, *Morganella* and *Vascellum* as subgenera of *Lycoperdon*. Furthermore, *Handkea* was reduced to a later synonym of subgen. *Bovistella* (Morgan) Jeppson & E. Larss. (2008: 9) and *Langermannia* was combined as subgen. of *Calvatia* while subgenera *Bovista* and *Globaria* (Quél.) Kreisel (1964: 200) were confirmed in *Bovista*.

Based on the morphological and molecular data obtained from two recent Italian collections, the aim of the present paper is to shed light on the phylogenetic position of the rarely-reported *Lycoperdon acuminatum* Bosc (in Fries 1851: 134), a species later combined by Kreisel (1964) in *Bovista*, where it occupies an isolated position.

Material and methods

Morphology

The basidiomes were photographed fresh in habitat and in studio using a digital reflex camera Sony α300 and subsequently dried. Freehand sections of basidiomes were rehydrated in water and observed under a Leitz Diaplan light microscope in water, Melzer's reagent, ammoniacal Congo red, and Cotton blue. All measurements were made at a magnification of ×1000. The measurements of the spores do not include ornamentation.

The notation [n/m/p] indicates that measurements were made on 'n' randomly selected spores from 'm' basidiomes of 'p' collections. The following abbreviations are used: LM = Light microscopy; SEM = Scanning electron microscopy. Collections are deposited in TO. Herbarium acronyms follow Thiers [2016, continuously updated].

DNA extraction, PCR amplification and sequencing

Genomic DNA was isolated from 10 mg of two dried voucher specimens (Table 1), by using the DNeasy Plant Mini Kit (Qiagen, Milan) according to the manufacturer's instructions. The universal primers ITS1F/ITS4 were used for the ITS nrDNA region amplification (White *et al.* 1990; Gardes & Bruns 1993) and primers LR0R/LR6 (Vilgalys & Hester 1990, Vilgalys lab. <http://www.botany.duke.edu/fungi/mycolab>) for nrLSU amplification. Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems, Norwalk) in 25 mL reaction mixtures using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 mM of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega, Madison). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C for 35 cycles, 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1 % agarose gel and visualized by staining with ethidium bromide. The PCR products were purified with the AMPure XP kit (Beckman Coulter, Pasadena) and sequenced forward and reverse by MACROGEN (Seoul). The sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) and their accession numbers are reported in Suppl. Fig. 1, Fig. 1 and Table 1.

TABLE 1. Collections newly sequenced in this study.

Species	GenBank acc. number		Source, date and country
	nrITS	nrLSU	
<i>Bryoperdon acuminatum</i>	KY581201	KY581199	TO HG191016, 19/10/2016, Italy
<i>Bryoperdon acuminatum</i>	KY581202	KY581200	TO HG201016, 20/10/2016, Italy

Sequence alignment, dataset assembly and phylogenetic analysis

The sequences obtained in this study were checked and assembled using Geneious v. 5.3 (Drummond *et al.* 2010) and compared to those available in GenBank using the BLASTn algorithm. Based on the BLASTn results (sequences were selected based on the greatest similarity) and outcomes of recent phylogenetic studies focused on *Lycoperdaceae* (Larsson & Jeppson 2008; Bates *et al.* 2009; Jeppson *et al.* 2012; Kim *et al.* 2016), sequences were retrieved from

GenBank and UNITE (<http://unite.ut.ee/>) databases for the comparative phylogenetic analysis. Two phylogenetic analyses were performed: the first, based on a large combined ITS and LSU sequences dataset, to detect the position of *Bovista acuminata* in the *Lycoperdaceae* (Suppl. Fig. 1); the second to focus only on the relationships among *Bovista acuminata* and its closer taxa (selected according to the first analysis) (Fig. 1). Alignments were generated for each single ITS and LSU dataset using MAFFT (Kato *et al.* 2002) with default conditions for gap openings and gap extension penalties. The alignments were then imported into MEGA v. 6.0 (Tamura *et al.* 2013) for manual adjustment. The best-fit models were estimated by the Bayesian Information Criterion (BIC) using jModelTest v. 2.1.7 (Darriba *et al.* 2012) to provide a substitution model for each single alignment. GTR+G models were chosen for both the ITS and LSU alignments. Phylogenetic analyses was performed using the Bayesian Inference (BI) approach, with combined ITS and LSU alignments and partitioned model. *Tulostoma kotlabae* (DQ112629) and *Tulostoma squamosum* (DQ415732) were chosen as outgroup taxa in the first analysis, following Larsson & Jeppson (2008); *Mycenastrum corium* (DQ112628) was chosen as outgroup taxon in the second analysis. Bayesian Inference was performed online using the CIPRES Science Gateway website (Miller *et al.* 2010). BI phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes v. 3.2.2 (Ronquist *et al.* 2012). Four incrementally heated simultaneous MCMC were run over 10 M generations. Trees were sampled every 1000 generations resulting in an overall sampling of 10,001 trees. The first 2500 trees were discarded as burn-in (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only BPP values over 0.70 are reported in the resulting trees (Fig. 1 and Suppl. Fig. 1).

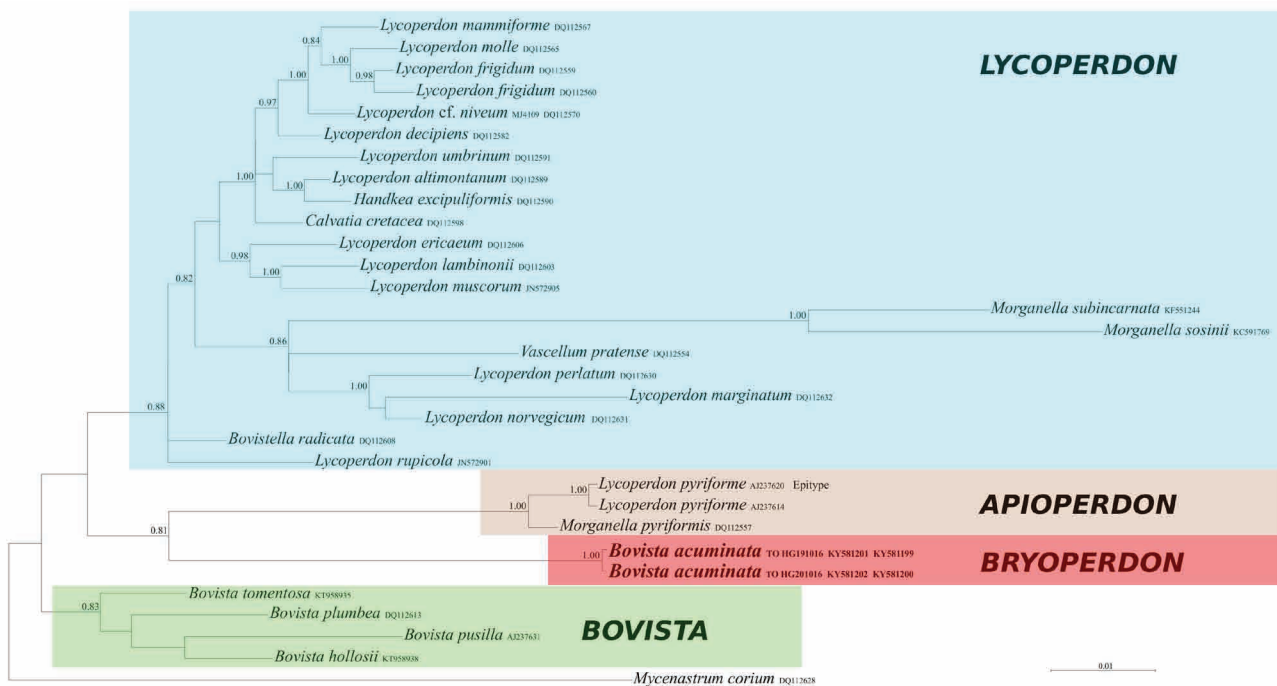


FIGURE 1. Bayesian phylogenetic analysis based on the combined nrITS/nrLSU dataset of selected *Lycoperdaceae*. BPP values ≥ 0.7 are shown on the branches. For each sequenced taxon the Genbank number is given. The newly sequenced collections are in bold.

Results

Molecular results

A fast Neighbor Joining (NJ) analysis in MEGA 6.0 (2000 bootstrap iterations) generated one NJ tree for nrITS and nrLSU, respectively (trees not shown). As the two generated trees were congruent, the two datasets were concatenated and analyzed. The large combined nrITS and nrLSU aligned dataset contained 128 sequences (including 123 from GenBank and 3 from UNITE) and was 1653 bp long (1–712 bp for nrITS and 713–1653 bp for nrLSU). For 41 sequences (38 from GenBank and 3 from UNITE) were present only the nrITS. The minor combined nrITS and nrLSU aligned dataset contained 31 sequences (including 29 from GenBank) and was 1620 bp long (1–710 bp for nrITS and 711–1620 bp for nrLSU). For 6 sequences (from GenBank) were present only the nrITS.

Most of the phylogenetic subgenera that were recognized by Larsson & Jeppson (2008) in *Lycoperdon* s.l., are also recovered in our large analysis (Suppl. Fig. 1), viz. subgenera *Lycoperdon*, *Bovistella* (Morgan) Jeppson & E. Larss. (2008: 12), *Morganella* (Zeller) Jeppson & E. Larss. (2008: 12), *Utraria* (Quél.) Jeppson & E. Larss. (2008: 12) and *Vascellum* (F. Šmarda) Jeppson & E. Larss. (2008: 12), with the exception of *Apioperdon* which clustered outside the genus. *Lycoperdon rupicola* seems to occupy a marginal position within *Lycoperdon*.

Lycoperdon pusillum (AB067724) from Japan [correct name = *Bovista pusilla* (Batsch) Pers.] represents a misinterpreted collection because it clusters neither with *Lycoperdon* nor with *Bovista pusilla* in *Bovista*.

In both our analyses (Suppl. Fig. 1 and Fig. 1) the two *Bovista acuminata* collections clustered in a clade (BPP = 1.00), outside *Lycoperdon* and sister (BPP = 0.91 and 0.81) to *Apioperdon* (the *Lycoperdon pyriforme* clade, BPP = 1.00).

Taxonomy

Bryoperdon Vizzini, *gen. nov.*

MycoBank MB 819911

≡ *Bovista* section *Xyloperdon* Kreisel, Beih. Nova Hedwigia 25: 222 (1967)

Diagnosis:—Basidiome small, ovoid, with mycelial cords; subgleba absent; capillitium of the *Lycoperdon* -type; spores smooth to minutely pustulose-verrucose, without debris of broken sterigmata; on moss covering tree trunks, logs and rocks.

Type:—*Lycoperdon acuminatum* Bosc

Etymology:—From the Greek words βρύον (bryon, referring to the moss) and περδεσθαι (perdesthai, meaning to break wind, and referring to *Lycoperdon*), and thus referring to the association of this puffball with moss.

Bryoperdon acuminatum (Bosc) Vizzini, *comb. nov.* (Fig. 2)

MycoBank MB 819912

Basionym: *Lycoperdon acuminatum* Bosc, in Fries, Nov. Symb. Myc.: 134 (1851)

≡ *Lycoperdon acuminatum* var. *seurati* Pat., Bull. Soc. mycol. Fr. 20: 135 (1904)

≡ *Lycoperdon pyriforme* var. *acuminatum* (Bosc) F. Šmarda, Fl. ČSR, B-1, Gasteromycetes: 341 (1958)

≡ *Bovista acuminata* (Bosc) Kreisel, Feddes Repert. 69: 201 (1964)

= *Lycoperdon calyptriforme* Berk., Grevillea 2(no. 16): 50 (1873)

= *Lycoperdon leprosum* Berk. & Ravenel, Trans. Albany Inst. 9: 313 (1879)

Selected descriptions:—Kreisel (1967: 84–87, as *B. acuminata*); Kreisel & Karasch (2005: 10–12, as *B. acuminata*); Sarasini (2005: 126–128, as *B. acuminata*); Poumarat (2007: 54–59, as *B. acuminata*).

Selected iconography:—Coker & Couch (1928, Pl. 49); Kreisel & Karasch (2005: Abb. 2); Sarasini (2005: 127, 128); Poumarat (2007: 56); Pérez-De-Gregorio (2014: 1608).

Description (based on the two Italian collections)

basidiomes 6–15 mm high and 4–10 mm broad, ovoid, obovoid, typically pointed at apex and rounded at base (Fig. 2a, b), with 1–2 white rhizomorphic cords rooting into the moss cushion, at first yellowish-white, pale ochraceous, hazelnut, then pinkish-brown. *Exoperidium* consisting of small, up to 0.2 mm high, quite persistent pyramidal spines, singly or more frequently arranged in groups of 2–3(–4), with convergent, connected apices (Fig. 2c, d). *Endoperidium* very thin, papery, at first whitish, then ochre-brown to dark brown, sometimes with violaceous hues. *Stoma* apical, irregularly elliptic, up to 20–30 µm long. *Gleba* yellowish-green, tobacco-brown when mature. *Pseudocolumella* indistinct. *Subgleba* absent. *Spores* globose, (3.0–) 3.7–4.0 (–4.3) µm in diam. [2/2/60], yellowish-brown in LM, almost completely smooth to only finely warted (A–B in the sense of Demoulin 1971, 1972); no debris of broken pedicels observed. *Paracapillitium* absent. *Capillitium* of the *Lycoperdon* -type (in the sense of Kreisel 1962, 1967), yellowish-pale brown in LM, 3–6 µm broad, with very rare, scattered septa and abundant small pits. *Spines* of the exoperidium consisting of chains of short, inflated, up to 20 µm in diam., clavate to subglobose elements.

Habit and habitat:—gregarious on moss cushions covering logs and rocks, in autumn.

Material examined:—ITALY. Piedmont, Torino, Valle di Susa, Loc. Mattie, *Castanea sativa* wood, in a cushion of *Hypnum cupressiforme* Hedwig mixed with *Hedwigia stellata* Hedenäs on a siliceous boulder on the ground, 730 m a.s.l., 19 October 2016, A. Vizzini and Mattie Elementary School pupils, TO HG191016 (TO!); ibidem, in a cushion of *Hypnum cupressiforme* covering a chestnut log, 20 October 2016, A. Vizzini, TO HG201016 (TO!).

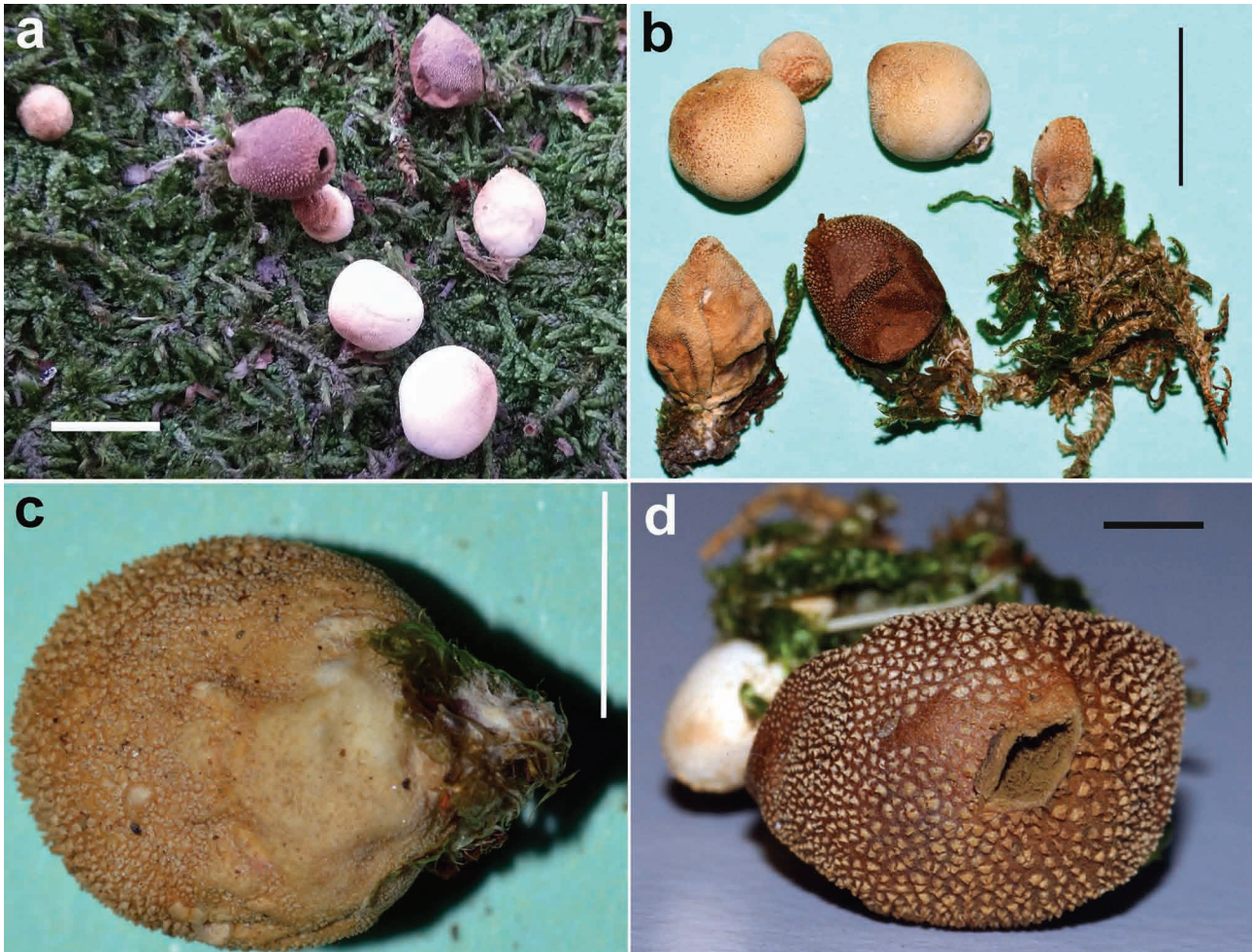


FIGURE 2. *Bryoperdon acuminatum*. Fresh basidiomes (TO HG191016). **a** in field; **b** in studio; **c** particular of the exoperidium; **d** particular of the exoperidium and ellipsoid-lacerate stoma. Scale bars: a, b = 10 mm; c = 5 mm; d = 2 mm. Pictures by A. Vizzini.

Apioperdon (Kreisel & D. Krüger) Vizzini, *comb. nov. et stat. nov.*

Mycobank MB 819913

Basionym: *Morganella* subgenus *Apioperdon* Kreisel & D. Krüger, *Mycotaxon* 86: 174 (2003)

≡ *Lycoperdon* subgenus *Apioperdon* (Kreisel & D. Krüger) Jeppson & E. Larss., *Mycol. Res.* 112: 11 (2008)

Type:—*Lycoperdon pyriforme* Schaeff.

Apioperdon pyriforme (Schaeff.) Vizzini, *comb. nov.*

Mycobank MB 819914

Basionym: *Lycoperdon pyriforme* Schaeff., *Fung. bavar. palat. nasc.* (Ratisbonae) 4: 128 (1774)

≡ *Utraria pyriformis* (Schaeff.) Quél., *Mém. Soc. Émul. Montbéliard, Sér. 2* 5: 369 (1873)

≡ *Morganella pyriformis* (Schaeff.) Kreisel & D. Krüger, *Mycotaxon* 86: 175 (2003)

= *Lycoperdon ovoideum* Bull., *Herb. Fr. (Paris)* 10: tab. 435:3 (1790)

= *Lycoperdon serotinum* Bonord., *Bot. Ztg.* 15: 631 (1857)

= *Lycoperdon desmazieri* Lloyd, *Mycol. Writ.* 2 (Letter 19): 213 (1905)

Type:—(selected by Krüger & Kreisel 2003) *Iconotype*: J.C. Schaeffer, *Fungorum Bavar. II*: 185 (1763); *Epitype*: Germany, Bavaria, collection Os97/91 (REG181091Os; GenBank ITS sequence AJ237620).

Selected descriptions:—Demoulin (1971: 187–192, as *L. pyriforme*); Pegler *et al.* (1995: 144–145, as *L. pyriforme*); Calonge (1998: 134–135, as *L. pyriforme*); Bates (2004: 244–248, as *Morganella pyriformis*); Sarasini (2005: 216–218, as *L. pyriforme*).

Discussion

Lycoperdon acuminatum was originally described from Costa Rica (Bosc in Fries 1851), but later it turned out to have a wide distribution in North and Central America (Morgan 1892; Lloyd 1905; Coker & Couch 1928; Swartz 1936; Bowerman 1961; Kreisel 1967; Esqueda-Valle *et al.* 1999). It has been rarely reported also from Europe (Sarasini 2005; Kreisel & Karasch 2005; Poumarat 2007; Pérez-De-Gregorio 2014; Rebriev 2015).

Lycoperdon acuminatum is well characterized by its small, oblong, conical basidiomes with white mycelial cords, the absence of a subgleba, the pitted *Lycoperdon*-type capillitium, the smooth to minutely ornamented spores, and the exoperidial spines made up of chains of inflated elements (Kreisel 1967; Kreisel & Karasch 2005; Sarasini 2005; Poumarat 2007). It grows typically on moss-covered bark of living trees, tree trunks (up to 7 meters above the ground) and logs (Cupressaceae and Angiosperms) (Coker & Couch 1928; Kreisel 1967; Pérez-De-Gregorio 2014), but some collections were made also between moss cushions covering rocks, boulders and cliffs (Sarasini 2005; Kreisel & Karasch 2005; Poumarat 2007), and one (Bowerman 1961) was reported to grow “among moss in an open field”. Bowerman’s record was considered doubtful by Kreisel (1967). The specimens of both Italian collections were found in a chestnut forest among cushions of *Hypnum cupressiforme* which covered a chestnut log and a boulder on the ground, respectively. *Hypnum cupressiforme* is very common on both acidic to slightly base-rich bark and siliceous rock (Cortini Pedrotti 2005; Frey *et al.* 2006). Therefore, according with our observations and what stated by Kreisel & Karasch (2005) and Sarasini (2005), it is the presence of, and the association with mosses and not the timber matrix that allows the growth of the fungus which, consequently, should be considered a bryophilous species.

Lycoperdon acuminatum was regarded as a variety of *L. pyriforme* by Šmarda (1958), due to the presence of fairly smooth spores and apparently similar habitat, but, based on the absence of the subgleba, and its “lignicolous habitat”, it was recombined in *Bovista* by Kreisel (1964), who included it in the subgen. *Globalaria* (Quél.) Kreisel (1964: 200). Later, in his worldwide monograph on *Bovista* (1967), Kreisel accommodated *Bovista acuminata* in the new section *Xyloperdon* Kreisel (1967: 222), established because of the unique features exhibited by Bosc’s species. Our molecular analyses (Suppl. Fig. 1 and Fig. 1) clearly indicate that *L. acuminatum* does not belong either to *Bovista* or to *Lycoperdon*, but that it occupies an independent position, sister to *L. pyriforme*. Consequently, it was taken the decision to describe this new evolutive line as the genus *Bryoperdon* (see above). The new genus differs morphologically from *Lycoperdon* s.l. (including *Bovistella*, *Morganella* and *Vascellum*) mainly in the absence of a subgleba, and from *Bovista* in the basidiome shape, the bryophilous association and the exoperidium consisting of chained, inflated elements.

Morphologically, *Bovista ochrotricha* Kreisel (1967: 87) is somewhat similar to *B. acuminatum*. The former is a rare species present in Asia and Europe (Kreisel 2001), where it grows on the mossy bark of living trees (in Europe mainly *Quercus* spp.); it differs in having more verrucose spores and exoperidium made up of non-enlarged elements with lanceolate apex, mixed with setiform ones (Kreisel 1967; Calonge *et al.* 1993; Calonge 1996, 1998). Future studies might indicate a phylogenetic relationship between *Bovista ochrotricha* (included by Kreisel 1967 in the same section *Xyloperdon* in *Bovista*) and *Bryoperdon acuminatum*.

According to Jeppson *et al.* (2012), the North European *Lycoperdon rupicola* Jeppson, E. Larss. & M.P. Martín (2012: 892), a species growing mainly in thin moss carpets (eg. *Racomitrium* spp. and *Dicranum* spp.) on more or less flat siliceous rocks and boulders, might be morphologically related to *L. acuminatum*. *Lycoperdon rupicola*, however, is distinguished by a well-developed subgleba and a prominent, stellately-lobed, protruding stoma and, phylogenetically, is a true *Lycoperdon*, despite its apparent unrelatedness to the described subgenera in *Lycoperdon* (Suppl. Fig. 1 and Fig. 1).

In the large combined ITS/LSU analysis, *Bryoperdon* is sister (BPP = 0.91) to a well-supported clade (BPP = 1.00) formed by sequences from collections named *L. pyriforme* (epitype collection included) or *Morganella pyriforme* and environmental sequences (Suppl. Fig. 1). This clade, also, is outside *Lycoperdon*. The same topology is recovered also in the second analysis (Fig. 1).

Lycoperdon pyriforme is characteristically delimited by clustered, obovoid to obpyriform basidiomes, its lignicolous habitat, the presence of a true non-pitted capillitium, abundant paracapillitium, a permanently white, cellular subgleba, conspicuous white mycelial strands connecting basidiomes to the substrate, smooth to minutely ornamented spores, and exoperidium covered with large sphaerocytes provided with spiny, finger-like protuberances (Kreisel 1962; Demoulin 1971; Pegler *et al.* 1995; Calonge 1998; Krüger & Kreisel 2003; Sarasini 2005). Kreisel (1962) and Demoulin (1971) already considered the species to be taxonomically isolated from the rest of the genus. Kreisel (1962) established the new series *Pyriformia* (1962: 124) for it. Krüger & Kreisel (2003), unfortunately based on a poor taxon sampling, recognized *L. pyriforme* in *Morganella* in the new subgenus *Apioperdon* Kreisel & Krüger

(2003: 174). Later, Bates (2004), Bates *et al.* (2009), Krüger & Gargas (2008) and Larsson & Jeppson (2008), based on a larger taxon-sampling, highlighted the isolated position of *L. pyriforme* in *Lycoperdon*; Larsson & Jeppson (2008), following a conservative approach, recognized *Apioperdon*, but as a subgenus of *Lycoperdon* s.l., viz. subgenus *Apioperdon* (Kreisel & D. Krüger) Jeppson & E. Larss. (2008: 14). In the ITS phylogeny of the Korean *Lycoperdon* species by Kim *et al.* (2016), *L. pyriforme* falls outside *Lycoperdon* and is sister (but with no statistical support) to *Bovista*.

L. pyriforme is phylogenetically distinct from *Lycoperdon* s.s., and exhibits a sufficient number of peculiar, morphological features, hence the decision to elevate *Apioperdon* to genus level (see above). With *Bryoperdon* it shares the minutely decorated spores (see SEM photographs in Krüger & Kreisel 2003 for *Apioperdon* and Kreisel & Karasch 2005 for *Bryoperdon*), partly the habitat (on decaying, rotting wood logs, stumps or debris, even though it is not strictly moss-associated), but it differs in the poreless capillitium, presence of paracapillitium and of subgleba, and the very striking, spinose to irregularly-shaped sphaerocytes of the exoperidium.

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