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Revision of species previously reported from Brazil under *Morganella*

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Abstract

In our study, seventy-two specimens from Brazilian herbaria, New Zealand Fungal and Plant Disease Collection-PDD Herbarium, and the New York Botanical Garden Herbarium under *Morganella* were analyzed, including the paratype of *M. mexicana* and holotypes of *M. arenicola*, *M. albstipitata*, *M. compacta*, *M. nuda*, *M. rimosa*, and *M. velutina*. Specimens were studied morphologically following the literature for *Morganella* genus and for DNA extraction, amplification and molecular analyzes following literature such as Bruns and Gradens, Martín and Winka. New sequences (ITS and LSU nrDNA) were obtained and compared with homologous sequences of GenBank. As a result of these analyses, a new *Lycoperdon* subgenus, two new species and five new combinations are proposed. A key is provided to the species studied in subgenera *Arenicola* and *Morganella*.

Key Words gasteroid fungi, Lycoperdaceae, *Lycoperdon*, nrDNA sequences, phylogenetic analyses, puffballs, taxonomy.

Introduction

Brazil has the most extensive tropical forests in the world, a vast territorial extension, geographic and climatic diversity, and hosts a huge biological diversity, all of which makes it leading among mega-diverse countries, sheltering almost 10-20% of the world's species and 40% of the world's tropical forests (Lewinsohn and Prado 2005; Mittermeier et al. 2005; Peres 2005). In recent years, several new taxa of gasteroid fungi in the family Lycoperdaceae have been found in Brazilian biomes including five new *Morganella* species (Trierveiler-Pereira et al. 2010; Cortez et al. 2011; Alfredo et al. 2012; Alves and Cortez 2013a, b; Alfredo and Baseia 2014; Alfredo et al. 2014a, b).

The genus *Morganella* was first proposed by Zeller (1948), and afterwards emended by Kreisel and Dring (1967). Those authors segregated *Morganella* from *Lycoperdon* on the basis of the absence of capillitium, only paracapillitium accompanied by corrugated glebal membranes being present, and a diaphragm being absent. The type species is *Morganella mexicana* Zeller, a synonym of *Lycoperdon fuligineum* Berk. et Curt. The genus is recognized by its epigeous, depressed globose to pyriform basidiomata, composed of double peridium (exoperidium and endoperidium), a reduced subgleba, and a gleba with abundant paracapillitium, without any true capillitium, and asperulate to echinulate basidiospores (Kreisel and Dring 1967; Ponce de León 1971; Suárez and Wright 1996). Most of the species

in *Morganella* are lignicolous, except *M. stercoraria* P. Ponce de León that was found in cow dung (Ponce de León 1971), and *M. arenicola* Alfredo & Baseia often found in sandy soil (Alfredo et al. 2014a).

Larsson and Jeppson (2008) compared sequences of the internal transcribed spacer (ITS), and the large subunit (LSU) of nuclear ribosomal DNA sequences of a number of Lycoperdaceae, including two *Morganella* specimens of *M. subincarnata* (Peck) Kreisel & Dring from Europe (Germany), and *M. fuliginea* (Berk. & M.A. Curtis) Kreisel & Dring from South America (Paraguay); they showed that these *Morganella* species were part of a larger *Lycoperdon* clade, which made *Lycoperdon* paraphyletic. Larsson and Jeppson (2008) proposed *Morganella* as a subgenus of *Lycoperdon*; and the two *Morganella* species included in their analyses recovered their original names: *Lycoperdon fuligineum* Berk. & M.A. Curtis and *L. subincarnatum* Peck.

Currently, in the taxonomic databases, Species Fungorum (<http://www.speciesfungorum.org/>), bibliographic database, Index Fungorum (<http://www.indexfungorum.org>) and Mycobank (<http://www.mycobank.org>), 12 species are under *Morganella*; mainly described from tropical ecosystems (Kirk et al. 2008).

In the light of molecular analyses, based on sequences of the internal transcribed spacer regions (ITS) and large subunit (LSU) of nuclear ribosomal DNA of two *Morganella* species, the genus *Morganella* was proposed as a subgenus of *Lycoperdon*. (ITS and LSU nrDNA markers), the aim of this paper was to reevaluate the Brazilian species described under *Morganella*, and to review the morphological features to delimitate the species. As a result of these analyses, following Larsson and Jeppson (2008) a new subgenus is proposed in *Lycoperdon*, sister group of the subgenus *Morganella*; and two new *Lycoperdon* species and five new combinations are proposed.

Material and Methods

Material studied

Specimens were obtained from Brazilian herbaria (including types): Herbário UFRN (Rio Grande do Norte), Herbário Anchieta–PACA (Rio Grande do Sul), Herbário INPA (Amazônia) and Herbário–ICN (Rio Grande do Sul). Also, collections from Herbario del Real Jardín Botánico de Madrid (MA-Fungi) and the New York Botanical Garden (NY) were analyzed. Data of collections studied are included in Table 1.

Morphological analysis

Specimens were studied and identified following the specific literature for *Morganella*: Kreisel and Dring 1967; Ponce de León 1971; Morales et al. 1974; Suárez and Wright 1996. The specimens were studied, photographed and measures as in our previous papers: Alfredo et al. (2012); Alfredo and Baseia (2014); Alfredo et al. (2014a, b). Microstructures were mounted in 5% KOH and, also in lactophenol cotton blue to observe paracapillitium, capillitium and basidiospores and examined using light microscopy (Olympus BX41TF); also, the photographs and measures were made using a Nikon Eclipse Ni light microscope coupled with a Nikon DS-Ri camera, supported by NIS-Elements AR 4.00.03 software, to more accuracy. Following Demoulin (1972a), basidiospores pattern ornamentation was classified in: [A] smooth to punctate, [B] slightly verrucose, [C] verrucose and [D] strongly verrucose, the basidiospores measures were done including and excluding ornamentation (table 3), in accordance Demoulin (1972b) the density of basidiospores was found using the follow calculation: number of ornamentation divided by the diameter of the spore multiplied by 3.14, to give a number of warty by a reduced area of 10 μ ; spore ornamentation was also analyzed using scanning electron microscopy (SEM), the preparation being as described by Silva et al. (2011). The descriptive terminology of the names of shapes of sphaerocysts and basidiospores follow those adopted by Stearn (2010). The code colors follow Kornerup and Wanscher (1978).

DNA isolation, amplification and sequencing

Total genomic DNA was extracted using DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA) following the instructions of the manufacturers; lysis buffer incubation was done overnight at 55°C. PCR amplifications were done in a 25 μ l reaction mix using a master mix (1.0–2.5 units of Invitrogen Taq DNA Polymerase, 1 $\times$ PCR Rxn buffer minus MgCl₂, 1.5 mM MgCl₂, 0.2 mM of each dNTPs, 0.5 mM of each primer) or using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK) as described in Winka et al. (1998). The internal transcribed spacer of ribosomal nrDNA (ITS) was amplified with the primer pair ITS5/ITS4 (White et al. 1990), following thermal cycling conditions in Martín and Winka (2000), to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and flanking parts of the small subunit (SSU) and large subunit (LSU) nuclear ribosomal genes; when these primers failed, the reverse primer ITS4B (Gardes and Bruns 1993) was used with ITS5. The partial 5' end of nuclear ribosomal large subunit RNA gene sequences (nrLSU) including D1-D2 domains was also amplified using primer pair LROR/LR5 (Rehner and Samuels 1994), and the cycling conditions indicated

in Martín and Winka (2000), except changing the elongation to 1.5 min at 72°C for the extension steps. Negative controls lacking fungal DNA were run for each experiment to check for contamination.

The PCR products were subsequently purified using the QIAquick Gel PCR Purification (Qiagen) according to the manufacturer's instructions or with 8 µl of 1:10 ExoSAP-IT® (USB Corporation, OH, USA). The purified PCR products were sequenced using the same amplification primers by Macrogen Inc. (Seoul, South Korea).

Sequencher 5.2.4 (Gene Codes Corp., USA) was used to obtain the consensus sequence from the two strands, both to the ITS and LSU markers of each isolate. BLAST searches with megablast option were used to compare the sequences obtained against the sequences in the National Center of Biotechnology Information (NCBI) nucleotide databases (Altschul et al. 1997). The new consensus sequences have been lodged in the EMLB-EBI database with the accession numbers indicated in Table 2.

Sequence alignment and phylogenetic analyses

The ITS and LSU nrDNA sequences obtained were aligned separately using Se-Al v2.0a11 Carbon Rambault (2002) for multiple sequences. The sequences were compared with homologous sequences of Lycopodaceae from GenBank (Table 2), mainly published in Larsson and Jeppson (2008) and Jeppson et al. (2012), two sequences of *Tulostoma* were included as outgroup. Where ambiguities in the alignment occurred, the alignment generating the fewest potentially informative characters was chosen. Alignment gaps were marked "-", unresolved nucleotides and unknown sequences were indicated with "N".

Maximum parsimony analyses (MP) were carried out separately for each alignment; as well as with the combined alignment of the two markers. Minimum length Fitch trees were constructed using heuristic searches with tree-bisection-reconnection (TBR) branch swapping, collapsing branches if maximum length was zero and using the MulTrees option in PAUP*4.0b10 (Swofford 2002); and a default setting to stop the analysis when reaching 100 trees. Gaps were treated as missing data. Nonparametric bootstrap support (bs) was tested for each clade (Felsenstein 1985), based on 10,000 replicates using the fast-step option. The consistency index, CI (Kluge and Farris 1969), retention index, RI (Farris 1989), and rescaled consistency index, RC (Farris 1989), were obtained.

Also, to each separate alignment, as well as with the combined ITS/LSU alignment, analyses were done by Bayesian approach (Larget et al. 1999; Huelsenbeck and Ronquist 2001) using MrBayes 3.1 (Ronquist and Huelsenbeck 2003). These analyses were performed assuming the general time reversible

model (Rodriguez et al. 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six categories (GTR+I+G) as selected by MrModelTest v. 2.3 (Nylander 2004). Two independent and simultaneous analyses starting from different random trees were run for 2,000,000 generations with four parallel chains, and trees and model scores saved every 100th generation. The default priors in MrBayes were used in the analysis. Every 1,000th generation tree from the two runs was sampled to measure the similarities between them and to determine the level of convergence of the two runs. The potential scale reduction factor (PSRF) was used as a convergence diagnostic and the first 25% of the trees were discarded as burn-in before stationary condition was reached. Both the 50% majority-rule consensus tree and the posterior probability (pp) of the nodes were calculated from the remaining trees with MrBayes. The phylogenetic trees were viewed with Figtree v. 1.3.1 ([http:// tree.bio.ed.ac.uk/software/figtree/](http://tree.bio.ed.ac.uk/software/figtree/)) and edited with adobe Illustrator CS3 v. 11.0.2 (adobe Systems).

Results

Molecular and morphological analyses

DNA was isolated from seventy-one specimens, first identified as belonging to the genus *Morganella* (Table 1), including the paratype of *M. mexicana* and holotypes of *M. albotipitata* Baseia & Alfredo, *M. arenicola*, *M. compacta* (G. Cunn.) Kreisel & Dring, *M. nuda* Alfredo & Baseia, *M. rimosa* Baseia & Alfredo and *M. velutina* (Berk. & M.A. Curtis) Kreisel & Dring. Unfortunately, the sequences obtained from the types of *M. compacta*, *M. mexicana*, *M. rimosa* and *M. velutina*, after blast search, were identified as belonging to Ascomycota. Finally, forty-six new ITS and LSU sequences related to Lycoperdaceae were generated and included in the alignments (Table 2).

From the ITS alignment, the final dataset has 787 unambiguously aligned nucleotide positions (455 constant, 97 parsimony-uninformative, and 235 parsimony-informative). The 100 most parsimonious trees gave a length of 1.006 steps, CI = 0.5089, HI = 0.4911, and RI = 0.8483. The trees obtained from the MP (strict consensus tree) and Bayesian analyses show similar topologies (data not shown).

From the LSU alignment, the final dataset has 926 unambiguously aligned nucleotide positions (772 constant, 69 parsimony-uninformative, and 85 parsimony-informative). The 100 most parsimonious trees gave a length of 320 steps, CI = 0.5719, HI = 0.4281, and RI = 0.8743. The trees obtained from the MP (strict consensus tree) and Bayesian analyses show similar topologies (data not shown).

Since the ITS and LSU trees showed essentially the same topology, a combined analysis ITS/LSU was done. Final ITS/LSU dataset has 1713 unambiguously aligned nucleotide positions (1227 constant, 166 parsimony-uninformative, and 320 parsimony-informative). The 100 parsimonious trees gave a length of 1268 steps, CI = 0.5080, HI = 0.4920, and RI = 0.8451. The topology of the parsimony strict consensus tree (Fig. 1 and Fig. S1) was essentially the same as the 50% majority rule combined consensus tree (not shown).

According to Fig. 1, all specimens under *Morganella* grouped in the *Lycoperdon* clade. The two sequences under *M. sulcatostoma* C.R. Alves & Cortez grouped close to the clades formed by specimens of the subgen. *Vascellum* and subgen. *Lycoperdon*; the revision of morphological features (table 1) confirmed the presence of capillitium in *M. sulcatostoma*. The new combination *L. sulcatostomum* is proposed.

The majority of the specimens under *Morganella* grouped together (bs = 89 %, pp = 1.0), distributed in two sister clades (A and B in Fig. 1). Clade A (top of the tree) includes 13 specimens under *M. arenicola* and one specimen under *M. afra* Kreisel & Dring; this relationship is not highly supported (bs = 84 %, pp = 0.61). In all specimens, the presence of cellular subgleba, exoperidium ornamentation that falls off, capillitium and paracapillitium (table 1), exoperidium composed of sphaerocysts and mycosclerids are present. Since in this clade specimens show features that distinguish these species from those of the subgenus *Morganella*, the new subgenus *Arenicola* is proposed.

Moreover, in clade A, the *M. arenicola* specimens appeared distributed in two subgroups; the relationship between these two subgroups has very high support (bs = 100%, pp = 1.0). One subgroup is formed by 11 specimens from Brazil, including the type of *M. arenicola* (bs = 100%, pp = 0.96), characterized by well-developed subgleba, verrucose exoperidium ornamentation, verrucae < 0.5 mm length, smooth endoperidium surface, punctate basidiospores, and the presence of capillitium and paracapillitium (table 1), mycosclerids found at the apical pore hyphae; the new combination *L. arenicola* is proposed and a synoptic updated description provided. The other subgroup (bs = 100%, pp = 1.0) is represented by two specimens from Brazil; although first identified as *M. arenicola*, UFRN-Fungos 655 and UFRN-Fungos 2554, they have unique morphological characters, such as the well-developed subgleba; the exoperidium ornamentation composed of deciduous spines 0.5–1 mm in length, leaving the endoperidium surface areolate from scars of the spines; punctate basidiospores; abundant capillitium and occasional paracapillitium (table 1); and mycosclerids found at the apical pore hyphae. We find reasons as

well-developed subgleba, capillitium present in contrast to *M. compacta* that has an absence capillitium and reduced subgleba, to describe UFRN-Fungos 655 and UFRN-Fungos 2554 as belonging to a new species: *Lycoperdon demoulinii*.

In clade B, *Lycoperdon* subgen. *Morganella*, three main groups were recognized, and a singleton (UFRN-Fungos 2570). From up to down, the clade B1 (bs = 99 %, pp = 1.0), included 22 specimens with granulose exoperidium, and large aculeate basidiospores (up to 7.5 µm diam). From them, 17 specimens appeared in a well-supported subgroup (bs = 100 %, pp = 0.87), characterized by a persistent exoperidium. Five specimens under *M. nuda* (ephemeral exoperidium), including the holotype UFRN-Fungos 1765, grouped together with the sequence under *M. fuliginea* (AF485065) from Paraguay (Krüger and Kreisel (2003). Even though we were not able to study the specimen from which sequence AF485065 was obtained, the molecular data suggest that the specimen that produced sequence AF485065 belongs to *M. nuda* instead of *M. fuliginea*. Based on morphological features and molecular analyses, two species are clearly differentiated in clade B1: *L. fuligineum*, and *L. nudum* comb. nov.; a synoptic updated description is provided for each species.

In clade B2 (bs = 100 %, pp = 1.0), four sequences obtained from *M. abostipitata* specimens, including the type (INPA239563), appeared together. These specimens show persistent exoperidium composed of chained subglobose to setoid sphaerocysts mixed with elongated hyphae, and medium aculeate spores (5–6 µm diam.) (Table 1). The new combination *L. albostipitatum* is proposed, and a synoptic updated description provided.

In clade B3, three sequences under *M. subincarnata*, including the sequence from GenBank AJ237626, grouped together (bs = 90 %, pp = 1.0), as the sister group of a clade formed by two sequences from GenBank, one under *M. purpurascens* (Berk. & M.A. Curtis) Kreisel & Dring (KP012918), and other under *M. sosinii* Yu. Rebriev & E. Bulakh (KG591769). Kreisel and Dring (1967) made the combination *M. purpurascens* (basionym *L. purpurascens* Berk. & M.A. Curtis), and *M. sosinii* as a new species recently described in Rebriev and Bulakh (2015). The collection UFRN-Fungos 2570 appeared basal in this group. Specimens under *M. subincarnata* show tuberclose to spinulose persistent exoperidium composed of isodiametric to pyriform sphaerocysts, and small aculeate spores (3.5–4.5(6.5) µm diam.). Larsson and Jeppson (2008) treat this taxon as *Lycoperdon subincarnatum* Peck. On the other hand, according to Kreisel and Dring (1967) and Ponce de León (1971), *M. purpurascens* is characterized by warty exoperidium ornamentation, surface-pitted endoperidium, with basidiospores smooth to minutely spiny; we adopted the

name *L. purpurascens* Berk. & M.A. Curtis. In Rebreiv and Bulakh (2015), *M. sosinii* have been described as a new species with an exoperidium ornamentation composed of granules and spines (0.1–0.2 mm length), endoperidium surface with “incomplete areolate pattern”, basidiospores echinulate, differing from *M. purpurascens* through the cellular subgleba; the new combination *L. sosinii* is proposed.

In clade B3, collection UFRN-Fungos 2570 grouped separately from the other species. The basidiomata show persistent exoperidium, composed of two kinds of elements: chained isodiametric to digitaliform sphaerocysts, and unchained elongated and thick-walled elements up to almost 200 µm; and large aculeate basidiospores (up to 7 µm diam.). Their unique morphological characters lead us to describe a new species: *L. exoelongatum*.

Taxonomy

Lycoperdon* subgen. *Arenicola Alfredo, M.P. Martín & Baseia, subgen. nov.

Mycobank: MB 818186

Etymology: Name refers to the basidiomata growth in sandy soil and the exoperidium encrusted with sand grains.

Holotype: Brazil, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal “Jornalista Luiz Maria Alves”, 05°48’38,7’’S, 035°11’39,9’’W in sand dunes, 22.06.2005, leg. B.D.B.Silva & J.S.Oliveira *s.n.* (UFRN-Fungos 1006!).

Type species: *Lycoperdon arenicola*

Diagnosis: This subgenus differs from subgenus *Morganella* by basidiomata growing in sandy soil; subgleba can be reduced (< 1/3 of basidiome) or well developed (> 1/3 of basidiome); exoperidium granulose to spiny (0.3–1.0 mm length); dehiscence hyphae composed of mycosclerids and interwoven hyphae with inflated terminations; capillitium and paracapillitium can be present and basidiospores punctate to slightly verrucose [A–B].

Description: Basidiomata immature, depressed globose, subglobose, pyriform to turbinate; subgleba well developed, cellular, occupying more than 1/3 of basidiome, cream (4A3); exoperidium surface velvety to spiny, incrustated with grains of sand, dark lilaceous (14F8) to black (14F4). Basidiomata mature, pyriform to turbinate, exoperidium incrustated with grains of sand, ornamentation of verrucae dark pink (14F6) or black (14F4) spines; the apical spines falling off with the age, leaving a smooth to scarred surface of the

endoperidium; endoperidium cream (4A3) to grayish yellow (4A3); gleba powdery, white when young, becoming light brown (5D5) to brown (6E8) with age.

Exoperidium base composed of chains of globose, subglobose sphaerocysts at the spine base and arranged in regular chains of cells in the spines (like in *L. perlatum*); apical exoperidium composed of mycosclerids with irregular form, apical dehiscence composed of interwoven hyphae with inflated terminations; basidiospores globose, punctate to slightly verrucose [A–B] in light microscope, and minute verrucae in SEM; capillitium aseptate and branched; paracapillitium septate and incrustated with amorphous hyaline glebal membrane.

Species included:

Lycoperdon afrum (Kreisel & Dring) Baseia, Alfredo, B.D.B Silva & M.P. Martín comb. nov.

Synonym: *Morganella afra* Kreisel & Dring, *Feddes Reppert.* 74: 117 (1967).

Lycoperdon arenicola (Alfredo & Baseia) Baseia, Alfredo & M.P. Martín comb. nov.

Synonym: *Morganella arenicola* Alfredo & Baseia, *Turkish Journal of Botany* 38: 595-596 (2014).

Lycoperdon demoulinii Baseia, Alfredo & M.P. Martín sp. nov.

1. *Lycoperdon* sp. ICN 177118

Previously identified as *Morganella afra* Kreisel & Dring, *Feddes Reppert.* 74: 117 (1967).

Synoptic description: Basidiomata mature depressed globose; subgleba reduced cellular. Exoperidium ornamentation minute verrucae, falling off with the age, leaving the endoperidium surface with tiny areoles. Gleba powdery.

Exoperidium composed of sphaerocysts that are globose or subglobose to ovoid. Endoperidium apex composed of mycosclerids of irregular forms; dehiscence hyphae aseptate and without inflated termination. Capillitium absent. Paracapillitium abundant. Basidiospores globose, 4–5 µm, slightly verrucose [B] in LM.

Specimens examined: BRAZIL, Rio Grande do Sul, Porto Alegre, Morro Santana, 30°01'58''W, 51°13'48''S, Atlantic rainforest on decaying woody, 26.03.2012, leg. L. Trierweiler-Pereira, LTP296 (ICN177118, ITS sequence GenBank KU958371, LSU sequence GenBank KU958372).

Notes: Previously identified as *Morganella afra* this specimen is characterized by its reduced and cellular subgleba, the tiny areolate surface of the endoperidium, and basidiospores slightly verrucose [B]. More analyzes including the type of *M. afra* are needed to clearly identify this specimen. This species differs from its sister species in the *Arenicola* clade, because *L. arenicola* and *L. demoulinii* show well developed subgleba and punctate basidiospores [A]; moreover, *L. arenicola* does not have an areolate

surface, and *L. demoulinii* has a surface well marked by scars in areolate format. The ICN177118 specimen differs from *L. subincarnatum* (= *M. subincarnata*), because the last one has reduced subgleba composed of compacted cells, areolate endoperidium surface and verrucose basidiospores [C] (Kreisel and Dring 1967; Ponce de Léon 1971). In our comparative analyses based on a specimen collected by C. G. Lloyd from USA (MA-Fungi 31310), the morphological features that distinguish *Lycoperdon* sp. ICN177118 from *L. subincarnatum* are mainly the tiny areoles in *L. afrum* vs the well-areolated endoperidium surface in *L. subincarnatum*; as well as the punctate basidiospores in *Lycoperdon* sp. ICN177118 vs the verrucose basidiospores in *L. subincarnatum*.

2. *Lycoperdon allostipitatum* (Baseia & Alfredo) Baseia, Alfredo & M.P. Martín comb. nov. –*Mycobank*: MB 818188. **Fig. 2**

Holotype: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, 60°01'30''W, 03°06'07''S, Amazon rainforest on decaying wood, 14.11.2010, leg. J.M.F. Araújo, GF23 (INPA 239563!).

Basionym: *Morganella allostipitata* Baseia & Alfredo, *Mycosphere*, 3: 67 (2012) MycoBank: MB 564373.

Synoptic description: Basidiomata depressed globose to pyriform; subgleba reduced ($< \frac{1}{3}$ of basidiome) up to 2 mm in length, sustained by a white elongated subgleba (2–4 mm length). Exoperidium granulose, cracked, forming persistent tufts; endoperidium surface smooth. Gleba powdery.

Exoperidium composed of chains of sphaerocysts that are globose to subglobose, mixed with elongated hyphae weakly to strongly dextrinoid. Apical endoperidium without mycosclerids, and dehiscence hyphae aseptate and without inflated termination. Capillitium absent. Paracapillitium abundant. Basidiospores globose, 5–6 μ m, strongly verrucose [D] in LM, and aculeate in SEM. Complete description in Alfredo et al. (2012).

Specimens examined: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, 60°01'30''W, 03°06'07''S, Amazon rainforest on decaying wood, 22.01.2013, leg. T. Accioly, N.K. Ishikawa and R.V. Isla, TA0042b (UFRN-Fungos 2249); Ibidem, 18.01.2013, leg. T. Accioly and N.K. Ishikawa (UFRN-Fungos 2269); Ibidem, 19.02.2013, leg. T. Accioly, T.S. Cabral and N.K. Ishikawa (UFRN-Fungos 2572).

Notes: *Lycoperdon allostipitatum* was originally described from an Amazon rain forest on decaying wood (Alfredo et al. 2012). It is characterized by its large, white elongated subgleba (up to 4 mm length), the persistent exoperidium composed of sphaerocyst chains mixed with elongated hyphae (like in *L.*

velutinum), and globose basidiospores (5–6 µm), strongly verrucose [D]. As showed in the figures 2a of Kreisel and Dring (1967) and figures 14, 18 of Suárez and Wright (1996), *L. velutinum* have a mixed exoperidium cells, however, in our morphological analyzes the specimens under name *Morganella velutina* (NY00398807 – type, and NY00398809) have not mixed cells, only elongated cells, may be this fact is because the type specimens of *L. fuliginea* (= *M. mexicana*, Ellis no. 5013) have a mixed specimens of *M. velutina* and *M. fuliginea*. The others specimens under name *M. velutina* (NY00398806 and NY00398808) have exoperidium with cells non-elongated. The morphological and molecular analyses allowed us to confirm this species as belonging to the subgenus *Morganella* clade, jointly with the species: *L. exoelongatum* sp. nov., *L. fuligineum*, *L. nudum*, *L. purpurascens* and *L. subincarnatum*. Due to its elongated subgleba (2–4 mm high) and unique sphaerocysts, *L. allostipitatum* can be separated from these five species. The features shared among all these species are the strongly verrucose [D] basidiospores and abundant paracapillitium (table 1): moreover, all species grow on decaying wood.

3. *Lycoperdon arenicola* (Alfredo & Baseia) Baseia, Alfredo & M.P. Martín comb. nov. – MycoBank: MB 818189. **Fig. 3**

Holotype: BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, 05°50'35''S, 35°11'35''W, in dunes growing on sandy soil, 22.06.2005, leg. B.D.B. Silva and J.S. Oliveira. (UFRN-Fungos 1006!).

Basionym: *Morganella arenicola* Alfredo & Baseia, Turkish Journal of Botany, 38: 596, 2014. – Mycobank: MB 805081

Synoptic description: Basidiomata pyriform to turbinate. Subgleba well developed (> 1/3 of basidiome) cellular, cream-colored (4A3). Exoperidium ornamentation composed of minute spines (< 0.3 mm length) becoming wart-like or granulose with the age, brown (6E8), incrustated with grains of sand, ornamentation falling off from the apex to halfway down the stem of the basidioma at complete maturity. Endoperidium surface smooth, greyish brown (6D3). Gleba powdery brown (6E8).

Exoperidium composed of globose to pyriform sphaerocysts. Apical endoperidium composed of mycosclerids and hyphae with inflated terminations. Capillitium without pores and septa, paracapillitium abundant. Basidiospores globose, 3–5 µm, punctate [A–B] often [A] in LM, minute aculeus (< 0.5 µm) in SEM, acyanophilous and without reaction in Melzer's reagent.

Specimens examined: BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, 06°18'18''S, 35°21'40''W, in dunes growing on sandy soil, 01.05.2008, leg. B.D.B. Silva, A.G. Leite and J.J.S. Oliveira (UFRN-Fungos 941); ibidem, 12.07.2012, leg. J.O. Sousa, R.H.S.F. Cruz, J.C. Bezerra, T. Accioly and N.M. Assis (UFRN-Fungos 2581); ibidem, 17.07.2004, leg. M.M.B. Barbosa, I.G. Baseia and P.P.T. Lacerda (UFRN-Fungos 657); ibidem, 10.09.2005, leg. M.M.B. Barbosa and I.G. Baseia (UFRN-Fungos 649, duplicate MA-Fungi); ibidem, 26.04.2008, leg. B.D.B. Silva and E.P. Fazolino (UFRN-Fungos 729); ibidem, 08.07.2006, leg. B.D.B. Silva, A.G. Leite, I.G. Baseia and T.B.S. Ottoni (UFRN-Fungos 864); ibidem, 01.07.2006, leg. M.M.B. Barbosa, I.G. Baseia and P.P.T. Lacerda (UFRN-Fungos 656); ibidem, 26.05.2011, leg. I.G. Baseia and M.A. Sulzbacher (UFRN-Fungos 1510, duplicate MA-Fungi); ibidem, 06°23'07''S, 35°00'58''W, 16.06.2010, leg. B.D.B. Silva, D.S. Alfredo and I.G. Baseia (UFRN-Fungos 1367, duplicate MA-Fungi); ibidem, 14.05.2014, leg. D.S. Alfredo (UFRN-Fungos 2567).

Note: *Lycoperdon arenicola* was originally described in dune ecosystems in northern Brazil and, despite its occurrence in sandy soil and the presence of capillitium without pores and septa, both uncommon characteristics for this genus, it was described as a *Morganella* species by Alfredo et al. (2014a). However, revision of the morphological characters revealed the presence of a developed cellular subgleba and capillitium (Fig.3), typical characters of the genus *Lycoperdon*, which accords with the molecular data.

4. *Lycoperdon compactum* G. Cunn., Trans. New Zeal. Inst., 57: 195 (1926) – Mycobank: MB261548.

Fig. 4

Holotype: NEW ZEALAND, York Bay, growing on decaying wood, 01.02.1923, leg. E.H. Atkinson. (PDD 10140!).

Synonym: *Morganella compacta* (G. Cunn.) Kreisel & Dring, *Feddes Repertorium* 74: 116 (1967). Mycobank: MB334487.

Synoptic description: Basidiomata depressed globose to pyriform, subgleba reduced ($< \frac{1}{3}$ of basidiome), composed of compacted cells. Exoperidium ornamentation spiny (0.5–2 mm length), dark pyramidal spines (6F8) with the tips converging, falling off with age, sometimes remaining at the top of the basidiome. Endoperidium surface areolate, areoles 0.5–1 mm width; gleba powdery, brown (6E8).

Exoperidium composed of chains of sphaerocysts; at the base of spines the sphaerocysts are globose to subglobose, $11\text{--}17 \times 11\text{--}19 \mu\text{m}$, and at the apex of spines are oval with oblong truncate to obtuse apices,

6–12 × 12–42 µm. Endoperidium apex without mycosclerids, dehiscence without hyphae with inflated terminations. Capillitium absent. Paracapillitium abundant. Basidiospores globose, 3.5–4 µm, punctate [B] in LM, minute spines < 0.5 µm long in SEM.

Specimens examined: NEW ZEALAND, York Bay, growing on decaying wood, 03.12.1922, leg. G.H. Cunningham (PDD 1102); ibidem, 06.12.1930, leg. M. Hodgkins (PDD 16721); ibidem, 07.12.1930, leg. M. Hodgkins, (PDD 8525); ibidem, 08.12.1932, leg. E.E. Chamberlain. (PDD 4008); ibidem, 06.12.1945, leg. J.M. Dingley (PDD 4530); ibidem, 07.12.1946, leg. G.H. Cunningham. (PDD 5607); ibidem, 06.12.1948, leg. P.M. Ambler (PDD 11111); ibidem, 06.12.1951, leg. G.H. Cunningham (PDD 11565); ibidem, 03.12.1952, leg. J.M. Dingley (PDD 12039); 05.12.1955, leg. G.H. Cunningham (PDD16058); ibidem, 05.12.1997, leg. R.E. Beever (PDD 68943).

Notes: *Lycoperdon compactum* was described by Cunningham (1926) from New Zealand. The peculiar habit, growing on decaying wood, spines up to 2 mm length in the exoperidium, the areolate endoperidium surface, and the punctate basidiospores are the main features of *L. compactum*. Since this species has only paracapillitium, Kreisel and Dring (1967) transferred it to the genus *Morganella*.

Although mainly cited from New Zealand, *L. compactum* has also been reported from Costa Rica (Calonge et al. 2005), and from Brazil (Barbosa et al. 2011); however, Brazilian specimens were misidentified. Alfredo et al. (2014a), reviewed the collection of *L. compactum* (including the holotype PDD 10140) and compared it with the Brazilian specimens (UFRN-Fungos 1006, UFRN-Fungos 649, UFRN-Fungos 650, UFRN-Fungos 651, UFRN-Fungos 657, UFRN-Fungos 864, UFRN-Fungos 941, UFRN-Fungos 656, UFRN-Fungos 729, UFRN-Fungos 1367, UFRN-Fungos 1510); no Brazil specimens belong to *L. compactum*. During our molecular analyses, although exhaustive attempts were made to amplify the type or paratype of *L. compactum*, we were unsuccessful. For this reason, leave *L. compactum* as *incertae sedis*, although due to the absence of paracapillitium, probably it belongs to subgenus *Morganella*; moreover, this species has morphological features like *L. purpurascens*, *L. sosinii* and *L. subincarnatum*.

5. *Lycoperdon demoulinii* Baseia, Alfredo, & M.P. Martín sp. nov. – MycoBank: MB 816279. Fig. 5

Etymology: In honor of to Dr. Vincent Demoulin, for his august contribution to the study of the genus *Lycoperdon*.

Diagnosis: This species is characterized by its exoperidium with black spines (14F4) 0.6–1.0 mm in length, leaving the endoperidium surface areolate, basidiospores globose 4.0–4.5 µm. *Lycoperdon*

demoulinii can be differentiated from *L. arenicola* by its areolate endoperidium surface, and it is different from *L. compactum* (= *Morganella compacta*) due to the presence of capillitium and mycosclerids in the apical endoperidium.

Holotype: BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, 35°21'40''S, 06°18'18''W, in dunes growing on sandy soil, 24.06.2006, leg. M.M.B. Barbosa and I.G. Baseia (UFRN-Fungos 655!).

Description: Basidiomata immature, epigeous, pyriform to turbinate 9 width × 12 mm length; subgleba well-developed, cellular, cream-colored (4A3); exoperidium with spine ornamentations 0.6–1.0 long., incrustated with grains of sand, black (14F4). Basidiomata mature, epigeous 16–20 width × 11–21 mm in length, pyriform to turbinate. Exoperidium incrustated with grains of sand, ornamentation of black spines (14F4) 0.6–1.0 mm length at the apical portion and black verrucae (14F3) at the basal portion; the apical spines falling off with the age. Endoperidium yellowish white (3A2) to yellowish gray (3A3), surface marked by areoles format. Gleba powdery brown.

Exoperidium basal composed of sphaerocysts globose to subglobose, 20–39 × 19–27 µm, disposed in non-regular chain at the base of the spines, which remains on endoperidium surface after the spines fallen off, walls 1–2.2 µm thick, while the spines are formed by regular chains of sphaerocysts (like in *L. perlatum*), globose to pyriform, 21–28 × 14–18 µm < 1.5 µm thick. Apical endoperidium composed of hyphae interwoven with inflated terminations, 8–17 µm diam., walls 1.3–2.2 µm thick, mixed with mycosclerids, irregular shape, 40–75 × 10–34 µm weakly dextrinoid, wall 2–3.7 µm thick. Capillitium 3–5 µm diam., without pores and septa, walls 1–1.8 µm thick. Paracapillitium absent. Basidiospores globose, 4–4.5 µm, punctate [A-B] often A in LM.

Specimens examined: BRAZIL, Rio Grande do Norte, Natal, Parque Estadual Dunas de Natal, 35°21'40''S, 06°18'18''W, 11.05.2011, leg. B.D.B. Silva, A.G. Leite and J.J.S. Oliveira (UFRN-Fungos 2554).

Habitat: Atlantic rainforest growing on sand dune.

Notes: After molecular analyses, and the revision of the morphological features, *Lycoperdon demoulinii* can be clearly distinguished from *L. arenicola*: in *L. demoulinii* the exoperidium ornamentation is formed by big spines (0.6–1.0 mm long) that fall off leaving an endoperidium surface marked by areoles; while in *L. arenicola* these features are not present. *Lycoperdon americanum* Demoulin and *L. echinatum* Pers. are species morphologically closest to *L. demoulinii* by they have exoperidium ornamentation of spines and

endoperidium surface areolate, being *L. demoulinii* has the smaller ones spines than *L. americanum* (3 mm long) (Demoulin 1972a) and *L. echinatum* (3–6 mm long) (Demoulin 1982; Calonge 1996); also, the basidiospores are distinctive between them, the Brazilian species are mostly punctate [A] (although B can be found) versus the basidiospores verrucose [C] in these two species (Demoulin 1972a; 1982).

6. *Lycoperdon exoelongatum* Accioly, Baseia & M.P. Martín, sp. nov. – MycoBank: MB 816241. **Fig. 6**

Etymology: Name refers to the elongated unchained elements of the exoperidium.

Holotype: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, at Manaus Botanical Garden area, Amazon rainforest, growing on decaying wood, 19.01.2013, leg. T. Accioly, N.K. Ishikawa and R. Vargas-Isla 020 (UFRN-Fungos 2570!).

Diagnosis: Epigeous basidiome with a white and cottony subgleba. Exoperidium with tiny tufts when young, becoming velutinous in aged specimens, composed of chained spherocysts, and also by unchained thick walled elongated elements up to almost 200 μm ; basidiospores up to 7 μm , with aculeate ornamentation.

Description: Basidiomata epigeous, depressed globose to subglobose, slightly umbonate, 15.5 mm (wide) $\times$ 10 mm (high). Subgleba white, cottony, up to 3 mm length. Rhizomorphs white, slender. Exoperidium not persistent, formed by a tomentum organized in tufts, light yellow to light orange (4A5 to 5A4) mainly apparent at the base of basidiomata, wearing out and giving a rugose aspect with a felted texture, greyish brown (6D3) towards the apex, finally, giving a velutinous aspect and orange (6A2) color in old specimens; endoperidium smooth, cream (4A3) and dehiscence by a lacerated apical pore. Subgleba elongated, 2.5 mm high, composed of compacted cells, cream to pale orange (4A3 to 5A3). Gleba cottony, furfuraceous, greyish orange to brownish orange (5B3 to 5C3).

Exoperidium composed of branched chains of sphaerocysts 18.8–27.3 $\mu\text{m} \times$ 4.5–8.8 μm , irregularly cylindrical, short or elongated, to digitiform at the terminal portions, mixed with setoid to claviform unchained hyphal elements 49.3–198.9 $\mu\text{m} \times$ 7.4–12.4 μm , with thickened walls up to 4 μm . Endoperidium composed of hyphae (3.3)3.6–5(5.6) μm wide interwoven, branched, septate, with pores, hyaline in 5% KOH. Paracapillitium 3.2–6.3 μm (diam.), branched, densely covered with glebal membranes. Basidiospores globose (4.7)5.4–6.6(7) μm diam., aculeate, with spiny projections up to 1 μm in length and width, some spines with rounded or recurved apex.

Specimens examined: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, 03°05'42"S, 59°59'13"W, growing on decaying wood, 19.01.2013, T. Accioly, N.K. Ishikawa & R. Vargas-Isla 020 (UFRN-Fungos 2570).

Notes: Several *Lycoperdon* species first described under *Morganella* present basidiospores with similar dimensions and aculeate ornamentations, similar to those of *L. exoelongatum*, such as *L. allostipitatum*, *L. fuligineum*, and *L. velutinum* (Ponce de León 1971; Suárez and Wright, 1996; Trierveiler-Pereira et al. 2010; Alfredo et al. 2012). However, some authors, such as Kreisel and Dring (1967), and Ponce de León (1971), did not include the spore ornamentation in basidiospore measurements. In our morphological revision (Table. 2), the basidiospores sizes of *L. fuligineum* (= *M. fuliginea*) and *L. velutinum* (= *M. velutina*), are up to 6.5 µm diameter, including ornamentation. Apart from the velvety exoperidium, *L. velutinum* can be easily confused with *L. exoelongatum*, since both species have non-chained elongated elements in the exoperidium; however, holotype and published descriptions of *Lycoperdon velutinum* (= *Morganella velutina*, = *mexicana*) differs macroscopically from *L. exoelongatum* in the absence of a white proeminent elongated subgleba and in the velvety exoperidium, which is more rough, felted, in *L. exoelongatum*. Microscopically, at the exoperidium, *L. exoelongatum* differs from *L. velutinum* by presenting its elongated unchained elements always setose, while *L. velutinum* exhibits most commonly paraphisoid or clavate elements, rarely setose. At the spores, *L. velutinum* have smaller ones (3.8 – 5.5 µm). Although are both aculeate, under electron microscope is observed that the swallows at the basis of each aculeous is striated in *L. velutinum*, being theses swallows smooth in *L. exoelongatum*. Furthermore, basidiospores of *L. velutinum* presents confluent cristae between some aculei, which are always independent in *L. exoelongatum* (Kreisel and Dring 1967; Ponce de Leon, 1971; Morales-Zurcher et al. 1974; Suárez and Wright 1996).

On the other hand, older specimens of *L. fuligineum* and *L. allostipitatum* can also present a velvety exoperidium, due to the wear and tear of the conic-pyramidal ornamentations exhibited in the younger basidiomata. Despite this, neither *L. allostipitatum* or *L. pyriforme* presents unchained elongated elements in the exoperidium. Beyond this, *L. allostipitatum* presents a more conspicuous elongated subgleba in relation to that of *L. exoelongatum* and, also, presents striate basal swallow on basidiospores aculeo which some are confluent (Alfredo et al. 2012). *Lycoperdon subincarnatum* has also been reported as presenting a velvety exoperidium, but this is formed by sparse spines up to 1 mm in length, and the velvety character is probably due to the wear and tear of the exoperidium ornamentations; *L. subincarnatum* also presents

smaller basidiospores (3.5–5 µm) and echinulate ornamentation (Coker and Couch 1928; Cunningham 1944; Ponce de León 1971; Cortez 2009), instead of aculeate ones as in *L. exoelongatum*.

7. *Lycoperdon fuligineum* Berk. & M. Curtis, in Berkeley, *J. Linn. Soc. Bot.* 10(46): 345 (1868) – MycoBank: MB216221. **Fig. 7**

Synonym: Morganella fuliginea (Berk. & Curt.) Kreisel & Dring, *Feddes Repert.* 74:113 (1967) – MycoBank: MB 334488.

Synonym: Morganella mexicana Zeller, *Mycologia* 40 (6): 650 (1948) – MycoBank: MB 288452.

Holotype: Mexico, Sonora, near Guaymas, leg. Thomas H. McBride (NY00839021).

Synoptic description: Basidiomata depressed globose, subglobose to pyriform, growing on decaying wood. Subgleba reduced (< 1/3 of basidiome) composed of compacted cells. Exoperidium minute warts or spines (< 0.5 mm in long.), persistent, brown (6E6) to dark brown (6F8) with age. Endoperidium surface smooth, cream (4A3). Gleba powdery, brown (6E8).

Exoperidium base like the apex, composed of chains of sphaerocysts (like in *L. perlatum*) that are globose, subglobose, ellipsoid, with truncate apices, except at the top of the chain where they are pyriform. Endoperidium composed of interwoven hyphae that are hyaline and with wall <0.5 µm. Mycosclerids and inflated terminations absent. Capillitium absent. Paracapillitium abundant. Basidiospores globose, 4.5–7 µm, strongly verrucose [D] in LM, aculeate in SEM; spines 0.5–1.5 µm long; the density of ornamentation is of 7–15.9 (mean = 10.84) warts by a reduced area of 10 µm.

Specimens examined: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, 59°58'07"W, 2°55'41"S, Amazon rainforest, growing on decaying wood, 25.12.2010, leg. R. Braga-Neto, GF38 (INPA 239561); ibidem, 17.02.2013, leg. T. Accioly, T.S. Cabral, N.K. Ishikawa and I.G. Baseia. (UFRN-Fungos 2571); ibidem (UFRN-Fungos 2578); ibidem, 16.01.2013, leg. T. Accioly (UFRN-Fungos 2579); Reserva Florestal do Cuieiras, ZF-2, 60°11'W, 02°38' S, 20.02.2013, leg. T. Accioly, T.S. Cabral and N.K. Ishikawa (UFRN-Fungos 2575); Ceará, Tianguá, APA Serra de Ibiapaba, 40°59'30"W, 3°43'56"S, remnant of Atlantic rainforest, growing on decaying wood, 19.04.2012, leg. D.S. Alfredo, DSA73 (UFRN-Fungos 1768); Pará, Belém, Floresta Nacional de Caxuanã, 51°27'20"W, 1°42'15"S, Amazon rainforest, growing on decaying wood, 10.08.2007, leg. T.B. Gibertoni (UFRN-Fungos 606); Paraíba, Areia, Reserva Ecológica Estadual Mata do Pau-Ferro, remnant of Atlantic rainforest, growing on rainforest, 35°42'15"W, 6°58'12"S, 22.07.2014, leg. D.S. Alfredo, DSA240 (UFRN-Fungos 2560); Ibidem, DSA241 (UFRN-Fungos 2561); ibidem, DSA242 (UFRN-Fungos 2562); ibidem, DSA243 (UFRN-Fungos 2563); ibidem, DSA247

(UFRN-Fungos 2566); Mamanguape, Reserva Biológica Guaribas, 39°39.811'W, 07°05.993'S, Atlantic rainforest, growing on decaying wood, 28.07.2012, leg. B.D.B. Silva, J.O. Sousa and M.A. Sulzbacher (UFRN-Fungos 1971); ibidem (UFRN-Fungos 1972); Rio Grande do Sul, São Leopoldo, 51°08'50"W, 29°45'37"S, Atlantic rainforest, growing on decaying wood, 01.01.1929, leg. Rick (PACA 13802); ibidem, 51°08'50"W, 29°45'37"S, 01.01.1940, leg. Rick (PACA13765); Rio Grande do Norte, Baía Formosa, R.P.P.N. Mata Estrela, 35°02'15"W, 6°37'47"S, Atlantic rainforest, growing on decaying wood, 18.03.2006, leg. I.G. Baseia, P.P.T. Lacerda and M.M.B. Barbosa (UFRN-Fungos 371); ibidem (UFRN-Fungos 2582); ibidem (UFRN-Fungos 2586). VENEZUELA, Cerro de la Neblina, Rio Yatua in Amazon territory, growing on decaying wood, (NY00398806, as *M. velutina*); Aragua, 1.5 km up the trail on mountain behind the hotel at Rancho Grande, growing on wood, 13.06.1968, leg. K.P. Dumont (NY00398808, as *M. velutina*); Yaracuy, Nirgua, in mountain, rotted wood, 07.07.1971, leg. K.P. Dumont, J.H. Haines, G.J. Samuels and G.S. Buting (NY00398727); Bolivar, El Portachuelo, growing on decaying wood, 22.06.1971, leg. K.P. Dumont, J.H. Haines and E. Moreno (NY00398728).

Other specimens examined: USA, New Jersey, Newfield, growing on decaying wood, 08.1887, leg. J.B. Ellis n. 5013 (NY00839022! *Morganella mexicana* paratype designated in Zeller 1948; *pro parte!*); idem (NY00839023 *pro parte!*).

Notes: The type of *Morganella mexicana* Zeller was designated by Zeller (1948) from a Mexican collection located in the Morgan Herbarium at the University of Iowa (Mexico, Sonora, near Guaymas, leg. Thomas H. McBride); moreover, Zeller (1948) included other collections under *M. mexicana*, as paratypes. One of these collections is B. Ellis 5013 from New Jersey (USA); different authors (as indicated in the revision labels) have studied this collection and noted that specimens belong at least to two species: *M. mexicana* and *M. velutina*. Specimens of B. Ellis 5013 are distributed in NY00839022 and NY00839023. In agreement with the revision done in 1991 by Suarez, specimens in NY00839022 belong not only to *M. velutina*, but also to *M. mexicana*; also NY00839023 is a mixed collection of *M. mexicana* and *M. velutina*. The specimens of *M. mexicana* have exoperidium ornamentation composed by a chain of sphaerocysts, such as the exoperidium *perlutum*-type mentioned in Kreisel (1973: 96) of *M. fuliginea*; Kreisel and Dring (1967) consider *M. mexicana* synonymous to *M. fuliginea*.

In the *L. fuligineum* protologue, Berkeley and Curtis (1868) did not indicate a voucher, nor a specific locality; they just wrote that all collections in the paper Berkely and Curtis (1868) are from Cuba. Kreisel and Dring (1967) indicate that the type of *L. fuligineum* is deposited in K; however, it has not been located.

The authors labelled the “type” in exsiccate NY00839021 under name *Morganella mexicana*. This one was asked for us under loans, but it is not possible, and I agreed for that, because the specimens are a little part of a basidioma. In determination by Dring 1966 (as indicate in the exsiccate) wrote “this seems to be all that is left of the type collection, the material at Iowa has been lost” and his assigned D.M. Dring. Photos these exsiccate is available in C. V. Starr Virtual Herbarium website (http://sweetgum.nybg.org/science/vh/specimen_details.php?irn=946535).

On the other hand, the GenBank sequence AJ485065 under *L. fuligineum* grouped in our analyses inside *L. nudum*; however, the persistent exoperidium clearly distinguishes *L. fuligineum* from *L. nudum* (exoperidium ephemeral), and probably the voucher from which AJ485065 sequence was obtained is misidentified.

8. *Lycoperdon nudum* (Alfredo & Baseia) Baseia, Alfredo & M.P. Martín comb. nov. – MycoBank: MB 818190. **Fig. 8**

Holotype: BRAZIL, Paraíba, Areia, Reserva Ecológica Estadual Mata do Pau-Ferro, 35°42'15"W, 6°58'12"S, remnant of Atlantic rainforest, growing on decaying wood, 17.06.2012, leg. D.S. Alfredo DSA105 (UFRN-Fungos 1765!).

Basionym: *Morganella nuda* Alfredo & Baseia, *Nova Hedwigia* 98: 460 (2014). MycoBank: MB 801797.

Synoptic description: Basidiomata depressed globose to pyriform; reduced subgleba (< 1/3 of basidiome), composed of compacted cells, cream (4A3). Exoperidium ornamentation granulose, composed of hyphae aggregated in small tufts, falling off partially or completely with the age. Endoperidium orange when young (5A2) to yellowish brown (5D8), surface smooth, occasionally some exoperidium remnants remain at the base. Gleba powdery, brown (6E8).

Exoperidium base similar to the apex, composed by a chain of sphaerocysts globose, subglobose, lageniform, from the base to the top of the chain; and oval to ovoid some with acute apexes. Endoperidium composed of interwoven hyphae, hyaline, with wall < 0.5 µm. Mycosclerids and inflated terminations absent. Capillitium absent. Paracapillitium abundant. Basidiospores globose, 5.5–7.5 µm, strongly verrucose [D] in LM, aculeate in SEM, spines 0.5–1 µm long; the density of ornamentation is of 11.9–15.7 (mean = 13.61) warts by a reduced area of 10 µm.

Specimens examined: BRAZIL, Paraíba, Areia, Reserva Ecológica Estadual Mata do Pau-Ferro, 35°42'15"W, 6°58'12"S, remnant of Atlantic rainforest, growing on decaying wood, 18.06.2012, leg. D.S.

Alfredo (UFRN-Fungos 1766); ibidem, 35°44'24''W, 6°58'23''S, 22.07.2014, leg. D.S. Alfredo DSA245 (UFRN-Fungos 2565); ibidem, 21.07.2014, 35°44'13''W, 06°58'26''S, leg. D.S. Alfredo DSA238b (UFRN-Fungos 2568); Rio Grande do Sul, Santa Maria, Três Barras, 53°48'25''W, 29°41'03''S, Atlantic rainforest, growing on decaying wood, 14.03.2008, leg. V.G. Cortez n. 056/08 (ICN 154541 as *M. fuliginea*).

Notes: *Lycoperdon nudum* was firstly reported from areas of “Brejos”, in accordance with Veloso et al. (1991) and Tabarelli and Santos (2004), these are areas of high altitude (up to 1.100 m) with an average annual rainfall of 1200 mm of Atlantic Forest surrounded by typical xerophilic vegetation (Caatinga). Yet, the “Brejos” are considered ecological disjunctions of Atlantic Forest with high biodiversity, considered as a refuge ecological due its different and unique phytophysiology being an important center of endemism (Santos et al. 2007). The ephemeral exoperidium distinguishes *L. nudum* from *L. fuligineum*. All the specimens under *L. fuligineum* analyzed in this work showed persistent exoperidium, and all them grouped together in the same clade (Fig. 1). In general, specimens of *L. nudum* have bigger spores, 5.5–7.5 µm including ornamentation, with the spines 0.6–1 µm in long (Table 2), compared to those of *L. fuligineum*. Suárez and Wright (1996), in the revision of *Morganella* from South America, mention basidiospores of 3.2–4.7(5.4) µm, but they did not include the ornamentation (spines 0.4–2.2 µm long). Moreover, the density of number of wart by a reduced circumference of 10 µm is of 11.9–15.7 (mean = 13.61) versus the found in *L. fuligineum* (7–15.9 (mean = 10.84)). The fact of *L. nudum* to be found in same areas that some specimens of *L. fuligineum* (UFRN-Fungos 1768, UFRN-Fungos 2560, Fungos 2561, UFRN-Fungos 2562, UFRN-Fungos 2563, UFRN-Fungos 2566) bring to us a question: why the specimens of *L. nudum* have an ephemeral exoperidium while the specimens of *L. fuligineum* is a persistent one under the same climatic conditions? This question yet is difficult to answer, but the molecular data show us that we have at least a little difference between these two species and maybe they are a complex species. More DNA markers are need to clarify it, so by now we prefer to treat them as two distinct species.

9. *Lycoperdon purpurascens* Berk & M.A. Curtis, *Proceedings of the American Academy of Arts and Sciences* 4: 124 (1858). (Figure 4 in Ponce de León, 1971) – MycoBank: MB 166087.

Holotype: Bonin Island, U.S.N. Pacific Exped. 1853 – 1856, leg. C. Wright (Type).

Synonym: *Morganella purpurascens* (Berk. & M.A. Curtis) Kreisel & Dring, *Feddes Repertorium* 74:115 (1967) – MycoBank: MB 334490.

Complete description: Ponce de León (1971).

Habitat: tropical deciduous forest, tropical zones, growing on decaying wood, on rotting wood.

Notes: This Indo-Pacific species is characterized by a low basidiospores ornamentation, weakly developed exoperidium, often pitted endoperidium, and compact subgleba. The endoperidium surface pitted like a thimble, and basidiospores slightly verrucose are the most characteristic of *L. purpurascens*. Others species have an endoperidium surface marked with areoles left by exoperidium ornamentation: *L. afrum*, *L. subincarnatum* and *L. demoulinii*. However, the scars in those species are larger than those found in *L. purpurascens* (Kreisel and Dring, 1967; Ponce de León, 1971). Moreover, *L. subincarnatum* has reduced subgleba and verrucose basidiospores [C] (Ponce de León, 1971), while *L. demoulinii* has well-developed subgleba and punctate basidiospores [A]. The species *M. costaricensis* M.I. Morales, described by Morales et al. (1974), is morphologically similar to *L. purpurascens*; however, *M. costaricensis* has cellular subgleba, and smooth basidiospores [A], slightly ovate. In our molecular analyses.

10. *Lycoperdon sulcatostomum* (C.R. Alves & Cortez) Baseia, Alfredo & M.P. Martín comb. nov. –
Mycobank: MB 818191

Holotype: BRAZIL, Paraná, Palotina, Parque Estadual de São Camilo, growing on rotting fallen leaves of Brazilian Queen Palm, *Syagrus romanzoffiana*, 17.01.2011, leg. A.J. Ferreira and V.G. Cortez 17–18 (UPCB 72893).

Basionym: *Morganella sulcatostoma* C.R. Alves & Cortez, *Nova Hedwigia*, 96: 410 (2013) –
MycoBank: MB 564889

Specimens examined: BRAZIL, Paraná, Foz do Iguaçu, Parque Nacional do Iguaçu, 54°35'11"W, 25°32'49"S, on rotting fallen leaves, 13.12.2010, leg. L.Trierveiler-Pereira LTP140 (ICN 177032); ibidem (ICN 177033).

Notes: In our analyses, this species did not group in any of the subgenera described by Larsson and Jeppson (2008), nor in the new subgenus described in this paper. *Lycoperdon sulcatostomum* is characterized by its sulcate apical pore, exoperidium ornamentation falling off, surface endoperidium cover by mycosclerids, velutinous, basidiospores slightly verrucose [B], and presence of capillitium with rare septa, without pores and paracapillitium (Table 1).

10. *Lycoperdon velutinum* Berk. ex Massee, *Monograph of the Myxogastres*, 18(1892) – Mycobank: MB 197111. **Fig. 9**

Holotype: Venezuela, Colonia Tover, Amazon rainforest, growing on decaying wood, leg. Fendler (NY00398807!).

Synonym: *Morganella velutina* (Berk. ex Massee) Kreisel & Dring, *Feddes Repertorium*, 74: 114 (1967). Mycobank: 334492

Synoptic description: Basidiomata depressed globose. Subgleba none or when present, reduced (<1/3 of basidiome) and composed of compacted cells. Exoperidium ornamentation persistent, velutinous (carpet-like) up to 0.15 mm in long. Endoperidium smooth, without scars on surface. Gleba powdery, brown (6E8).

Exoperidium composed of elongated hyphae, some clavate at the top, dextrinoid, up to 140 µm long. Endoperidium apex without mycosclerids, dehiscence without hyphae with inflated terminations. Capillitium absent. Paracapillitium abundant. Basidiospores globose, strongly verrucose [D] in LM, aculeate in SEM.

Specimens examined: USA, New Jersey, Newfield, growing on decaying wood, 08.1887, leg. J.B. Ellis, n. 5013 (NY00839022 as *M. mexicana*, *pro parte*!); ibidem, (NY00839023 as *M. mexicana*, *pro parte*!); VENEZUELA, Colonia Tover, Amazon rainforest, growing on decaying wood, leg. Fendler (NY00398809).

Notes: *Lycoperdon velutinum* is mostly characterized by its velutinous, carpet-like exoperidium ornamentation, composed of dextrinoid elongated hyphae, and strongly verrucose [D]. basidiospores. Kreisel and Dring (1967), transferred *L. velutinum* to *Morganella* genus, due to the absence of capillitium and abundant paracapillitium. As indicated above, *L. fuligineum* and *L. velutinum* are close enough to have been often confused as with the mixed collection J. B. Ellis 5013. However, these species are easily separated by the exoperidium that is totally different (Figs. 6 and 10).

Excluded taxon

Morganella rimose Baseia & Alfredo

According to Agerer (2002), the rhizomorphs of lycoperdaceous fungi are composed of two kinds of hyphae: the thinner outer part has hyphae that are aseptate, hyaline in 5% KOH, dextrinoid in Melzer's reagent; and the other thicker inner (vessel-like) hyphae are septate or with dissolving septa, hyaline in 5%

KOH and weakly dextrinoid with characteristic dissolving septa. All specimens included in this study of subgenera *Arenicola* and *Morganella*, have the same rhizomorph features described in Agerer (2002) for lycoperdaceous fungi; and, similar to those described for *Calvatia nodulata* Alfredo & Baseia in Alfredo et al. (2014b). However, in *M. rimosa* the rhizomorphs are formed by one kind of hypha, septate, with clamp connections, hyaline, and with the occurrence of oleoacanthocystidia; these features are similar to those in the members of the gomphoid-phalloid clade described in Agerer (2006). We have compared the type of *M. rimosa* (UFRN-Fungos 1680) and specimens of *Lycogalopsis solmsii* (NY00792715); there are no morphological differences in the rhizomorphs, or in the rest of the characters:

Lycogalopsis solmsii E. Fisch., Berichte der Deutschen Botanischen Gesellschaft 4: 197 (1886) – MycoBank: MB 209949.

Synonym: Morganella rimosa Baseia & Alfredo, *Mycosphere* 3: 68 (2012) – MycoBank: MB 564371.

Discussion

Kreisel and Dring (1967) segregated *Morganella* from *Lycoperdon* due to the absence of the capillitium and association of the paracapillitium with corrugated glebal membranes. However, some species within *Lycoperdon* can develop in wood, such as *L. pyriforme* (Larsson and Jeppson 2008); and, *Lycoperdon* species may present paracapillitium, such as in *L. foetidum* and *L. perlatum* (Demoulin 1972b). Demoulin (1972b), based only morphological features, already have been treated the genus *Lycoperdon* as paraphyletic one, which showed *Morganella* is related to the *L. cokeri*, and *L. follicolum* (a Brazilian species) could occupy an intermediary one position. Unfortunately, we have not received the specimens of *L. follicola* to our study. Yet, the author draws attention to the occurrence of abundant paracapillitium being a character more evolved character than capillitium (with or none pores). Based on molecular analyses of ITS and LSU markers, Larsson and Jeppson (2008) treated *Morganella* as a subgenus of *Lycoperdon*. With a broader sampling than the previous study, we state the position of seven species in *Lycoperdon* subgen. *Morganella*; the absence of capillitium and the reduced (<1/3 of basidioma) compact cellular subgleba are autapomorphies in this subgenus.

Three species are included in the new subgenus *Arenicola*, delimited by subgleba reduced to well developed, cellular gleba with capillitium and paracapillitium, peridium composed of mycosclerids and/or hyphae with inflated terminations.

Following the concept of synapomorphy and autapomorphy adopted by Assis and Rieppel (2011) and Hörandl and Stuessy (2010), the subgleba cellular, the glebal membrane which can disappear with the age in *Lycoperdon*, and the occurrence of paracapillitium can be considered synapomorphies of *Lycoperdon* and the subgenera *Arenicola* and *Morganella*.

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References

- Agerer R (2002) Rhizomorph structures confirm the relationship between Lycoperdales and Agaricaceae (Hymenomycetes, Basidiomycota). *Nova Hedwigia* 75: 367–385
- Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. *Mycol Progress* 5: 67–107. doi: 10.1007/s11557-006-0505-xv
- Alfredo DS, Leite AG, Braga-Neto R, Baseia IG (2012) Two new *Morganella* species from the Brazilian Amazon rainforest. *Mycosphere* 3(1): 66–71
- Alfredo DS, Baseia IG (2014) *Morganella nuda*, a new puffball (Agaricaceae, Basidiomycota) in the upland forests of the Brazilian semi-arid region. *Nova Hedwigia* 98(3-4): 459–466
- Alfredo DS, Accioly T, Baseia IG (2014a) *Morganella arenicola*, a new species records from North and Northeast Brazil. *Turk J Bot* 38: 1–5
- Alfredo DS, Rodrigues ACM, Baseia IG (2014b) *Calvatia nodulata*, a new gasteroid fungi from Brazilian Semiarid region. *Journal of Mycology* 2014(2): 1–7. doi: 10.1155/2014/697602
- Altschul SF, Madden TL, Schäffer AA et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acid Res* 25: 3389–3402
- Alves CR, Cortez VG (2013a) *Morganella sulcatostoma* sp. nov. (Agaricales, Basidiomycota) from Paraná State, Brazil. *Nova Hedwigia* 96(3-4): 409–417
- Alves CR, Cortez VG (2013b) *Calvatia guzmanii* sp. nov. (Agaricaceae, Basidiomycota) from Paraná State, Brazil. *Phytotaxa* 85: 35–40

738 Assis LCS, Rieppel O (2011) Are monophyly and synapomorphy the same or different? Revisiting the role
 739 of morphology in phylogenetics. *Cladistics* 27: 94–102
 740 Barbosa MMB, Silva MA, Cruz RHSF, Calonge FD, Baseia IG (2011) First report of *Morganella compacta*
 741 (Agaricales, Lycoperdaceae) from South America. *Mycotaxon* 116: 381–386. doi: 10.5248/116.381
 742 Berkeley MJ, Broome MRCE (1847) Enumeration of the fungi of Ceylon. Part II. *Botanical Journal of the*
 743 *Linnean Society* 14: 29–141
 744 Berkeley MJ, Curtis MA (1868) Fungi Cubenses (Hymenomycetes). (Continued.). *Bot J Linn Soc London,*
 745 *Botany* 10(46): 321–341
 746 Calonge FD (1998) Flora Mycológica Ibérica vol. 3. Gasteromycetes, I. Lycoperdales, Nidulariales,
 747 Phallales, Sclerodermatales, Tulostomatales. J. Cramer, Stuttgart
 748 Calonge FD, Mata M, Carranza J (2005) Contribución al catálogo de los Gasteromycetes (Basidiomycota,
 749 Fungi) de Costa Rica. *An Jard Bot Madrid* 62(1): 23–45
 750 Coker WC, Couch JN (1928) The Gasteromycetes of the Eastern United States and adjacent Canada. New
 751 York, Dover.
 752 Cortez VG, Baseia IG, Silveira RMB (2011) *Lycoperdon ovoidisporum* sp. nov. from Brazil. *Sydowia* 63:
 753 1–7
 754 Cortez VG (2009) Estudos sobre Fungos Gasteroides (Basidiomycota) no Rio Grande do Sul, Brasil.
 755 Doctoral Thesis, Universidade Federal do Rio Grande do Sul
 756 Cunningham GH (1926) Lycoperdaceae of New Zealand. Transactions and proceedings of the New Zealand
 757 Institute 57:187–217
 758 Cunningham GH (1944) The Gasteromycetes of Australia and New Zealand. Dunedin, N.Z. John McIndoe
 759 Demoulin V (1972a) Espèces nouvelles ou méconnues du genre *Lycoperdon* (Gastéromycètes). *Lejeunia*
 760 62: 1–28
 761 Demoulin V (1972b) Le Genre *Lycoperdon* en Europe et en Amérique du Nord. Étude Taxonomique et
 762 Phytogéographique. Doctoral Thesis, Université de Liège
 763 Demoulin V (1976) Species of *Lycoperdon* with a setose exoperidium. *Mycotaxon* 3: 275–296
 764 Demoulin V (1983) Clé de détermination des espèces du genre *Lycoperdon* presentes dans le sud de
 765 l'Europe. *Revista de Biologia* 12Ç 65–70
 766 Farris JS (1989) The retention index and the rescaled consistency index. *Cladistics* 5: 417–419
 767 Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–
 768 791
 769 Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes –application to the
 770 identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118
 771 Homrich MH, Wright JE (1988) South American Gasteromycetes. II. The genus *Vascellum*. *Can J Botany*
 772 66: 1285–1307
 773 Hörandl E, Stuessy TF (2010) Paraphyletic groups as natural units of biological classification. *Taxon* 59(6):
 774 1641–1653
 775 Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:
 776 754–755

777 Jeppson M, Larsson E, Martín MP (2012) *Lycoperdon rupicola* and *L. subumbrinum*: two new puffballs
 778 from Europe. Mycol Progress 11(4): 887–897. doi: 10.1007/s11557-011-0804-8
 779 Kirk PM, Cannon PF, David JC, Stalpers JA (2008) Ainsworth & Bisby's Dictionary of the Fungi. 10th ed.
 780 CAB Publishing
 781 Kluge AG, Farris JS (1969) Quantitative phyletics and the evolution of anurans. Syst Zoology. 18: 1–32
 782 Kornerup A, Wanscher JH (1978) Methuen handbook of colors. Third edition. Eyre Methuen. London
 783 Kreisel H, Dring DM (1967) An emendation of the genus *Morganella* Zeller (Lycoperdaceae). Feddes
 784 Repertorium 74(1–2): 109–122
 785 Kreisel, H (1973) Die Lycoperdaceae der DDR, Nachträgen 1962-1971. Bibliotheca Mycol 36:1-13
 786 Kreisel H, Hausknecht A (2002) The gasteral Basidiomycetes of Mascarenes and Seychelles. Österr. Z.
 787 Pilzk 11: 191–214
 788 Krüger D, Kreisel H (2003) Proposing *Morganella* subgen *Apioperdon* subgen nov for the puffball
 789 *Lycoperdon pyriforme*. Mycotaxon 86: 169–177
 790 Larget B, Simon DL (1999) Markov chain Monte Carlo algorithms for the Bayesian analysis of
 791 phylogenetic trees. Mol Biol Evol 16: 750–759
 792 Larsson E, Jeppson M (2008) Phylogenetic relationships among species and genera of Lycoperdaceae based
 793 on ITS and LSU sequence data from north European taxa. Mycol Res 112: 4–22
 794 Lewinsohn TM, Prado PI (2005) How many species are there in Brazil? Conserv Biol 19: 619–624
 795 Martín MP, Winka K (2000) Alternative methods of extracting and amplifying DNA from lichens.
 796 Lichenologist 32: 189–196
 797 Mittermeier RA, Fonseca GD, Rylands AB, Brandon K (2005) uma breve história da conservação da
 798 biodiversidade no Brasil. Megadiversidade 1(1): 14–21
 799 Morales MI, Nassar M, Saenz JA (1974) Lycoperdaceae of Costa Rica. I. The genus *Morganella*. Revista
 800 de Biología Tropical 21: 221–227.
 801 Nylander JAA (2004) MrModeltest v. 2. Program distributed by the author. Uppsala: Evolutionary Biology
 802 Center, Uppsala University
 803 Peres CA (2005) Porque precisamos de megareservas na Amazônia. Megadiversidade 1(1): 175–180
 804 Ponce de León P (1971) Revision of the genus *Morganella* (Lycoperaceae). Fieldiana Botany 349(3): 27–
 805 44
 806 Rambaut A (2002) Se-Al. Sequence alignment editor. Version 2.0a11. Oxford: Department of Zoology,
 807 University of Oxford
 808 Rebriev YA, Bulakh EM (2015) *Morganella sosinii* sp. nov. (Agaricaceae) from the Russian Far East.
 809 Mycologiya i Phytopathologiya 49(5): 293–296
 810 Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of Gliocladium analyzed from nuclear large
 811 subunit ribosomal DNA sequences. Mycol Res 98: 625–634
 812 Rodriguez F, Oliver JL, Marin A, Medina JR (1990) The general stochastic model of nucleotide
 813 substitution. Journal of Theoretical Biology 142: 485–501
 814 Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models.
 815 Bioinformatics 19: 1572–1574

Silva BDB, Sousa JO, Baseia IG (2011) Discovery of *Geastrum xerophilum* from the Neotropics. Mycotaxon 118: 355–359

Stearn WT (2010) Botanical Latin. Timber Press, Portland

Suárez VL, Wright JE (1996) South American Gasteromycetes V: The genus *Morganella*. Mycologia 88(4): 655–661

Swofford DL. PAUP* (2002) Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0 b. Sinauer Associates, Sunderland, Massachusetts

Tabarelli M, Santos AMM (2004) Uma breve descrição sobre a história natural dos Brejos Nordestinos. In: Pôrto KC, Cabral JJP, Tabarelli M (ed.) Brejos de Altitude em Pernambuco e Paraíba: História natural, ecologia e conservação, v. 9. Ministério do Meio Ambiente (MMA), Brasília, pp 17–24

Trierveiler-Pereira L, Kreisel H, Baseia IG (2010) New data on puffballs (Agaricomycetes, Basidiomycota) from the Northeast Region of Brazil. Mycotaxon 111: 411–421

Veloso HP, Filho ALRR, Lima JCA (1991) Classificação da vegetação brasileira, adaptada a um sistema universal. IBGE, Rio de Janeiro

Winka K, Ahlberg C, Eriksson OE (1998) Are there lichenized Ostropales? Lichenologist 30: 455–462

White TJ, Bruns T, Lee SJWT, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), PCR protocols: a guide to methods and applications, Academic Press, USA, pp 315–322

Zeller SM (1948) Notes on certain Gasteromycetes, including two new orders. Mycologia 40: 639–668

Competing interests: The authors have declared that no competing interests exist.

Table 1 Specimens studied in this work with voucher numbers, country, and the main morphological features considered. Collections which sequences were obtained ITS and LSU sequences are indicated with (+)

Table 2 All the specimens used in the molecular phylogenetic analyses with voucher number and GenBank Accession Numbers. When ITS and LSU have different numbers, the first number corresponds to ITS and the second to LSU. In black are indicated the new sequences generated in this study.

Table Supplementary 3 Specimens studied in this work with voucher numbers, basidiospores which were classified in C and D, measures without ornamentation and with the density of warts by a reduced area of 10 µm

Table supplementary 4 Comparative between three semi-cryptic species of *Lycoperdon*.

Fig. 1 Phylogenetic tree obtained from parsimony analysis of ITS and LSU combined alignment. Clades and subclades as described in the text. Numbers above branches are parsimony bootstrap (bs) and posterior probability (pp) values. *Bovista*, *Calvatia*, *Disciseda*, *Mycenastrum* and *Tulostoma* as outgroups. Vouchers numbers are indicated as in Table 1 and 2.

Fig. 2 *Lycoperdon albstipitatum* (Holotype INPA 239563). a. dry specimen. b. detail of apical pore. c. detail of exoperidium ornamentation. d. elongated dextrinoid hyphae from exoperidium ornamentation. e. basidiospores strongly verrucose [D] in LM. f. scanning electron microscopy of basidiospores. Scale bars (a) 10 mm; (b) 1 mm; (c) 0.2 mm; (d) 50 μ m; (e) 5 μ m; (f) 2 μ m

Fig. 3 *Lycoperdon arenicola* (Holotype UFRN-Fungos 1006). a. basidiomata *in situ*. b. dry specimen in cross section. c. sphaerocysts from exoperidium ornamentation. d. detail of capillitium and paracapillitium mixed. e. basidiospores punctate [A] in LM. f. scanning electron microscopy of basidiospores. Scale bars (a–b) 10 mm; (c) 50 μ m; (d) 20 μ m; (e) 5 μ m; (f) 2 μ m

Fig. 4 *Lycoperdon compactum* (PDD 10140). a. dried specimens; b. detail of exoperidium ornamentation; c. detail of endoperidium surface areolate; d. sphaerocysts forming the spines from exoperidium ornamentation; e. basidiospores punctate [A] in LM; f. scanning electron microscope of basidiospores. Scale bars: (a) 5 mm; (b–c) 1 mm; (d) 50 μ m; (e) 5 μ m; (f) 2 μ m

Fig. 5 *Lycoperdon demoulinii* (Holotype UFRN-Fungos 655). a. dry specimen; b. detail of exoperidium ornamentation; c. detail of endoperidium surface areolate; d. details of spines from exoperidium; e. sphaerocysts forming the spines; f. mycosclerids from apical endoperidium; g. capillitium and basidiospores punctate in LM. Scale bars (a) 10 mm; (b) 2 mm; (c) 2 mm; (d) 50 μ m; (e–f) 20 μ m; (g) 5 μ m

Fig. 6 *Lycoperdon exoelongatum*. a. basidiome *in situ*; b. dry specimen; c. detail of tufts in exoperidium of young basidiome; d. detail of exoperidial surface in aged basidiome; e. paracapillitium covered with glebal membrane; f. basidiospores strongly verrucose [D] in LM; g. scanning electron microscopy of basidiospores; (H) exoperidial elements. Scale bars: (a, b, d) bar = 5 mm.; (c) bar = 0.5 mm; (e) bar = 20 μ m; (f) bar = 10 μ m; (g) bar = 1 μ m; (h) bar = 50 μ m

Fig. 7 *Lycoperdon fuligineum*. a. Paratype of *Morganella mexicana* (NY00839022, J.B.Ellis 5013), dried specimens; b. dry specimens of *Morganella mexicana* mixed with *M. velutina* (NY00839023, J.B. Ellis 5013); c. detail of exoperidium ornamentation; d. sphaerocysts from exoperidium ornamentation; e. basidiospores strongly verrucose [D] in LM; f. scanning electron microscope of basidiospores. Scale bars: (a–b) 10 mm; (c) 0.2 mm; (d) 50 μ m; (e) 5 μ m; (f) 2 μ m

Fig. 8 *Lycoperdon nudum* (Holotype UFRN-Fungos 1765). a. dried specimen; b. detail of exoperidium falls off; c. detail chain of exoperidium ornamentation; d. basidiospores strongly verrucose [D] and

880 paracapillitium in LM; e. scanning electron microscope of basidiospores. Scale bars: (a) 2 mm; (b) 2 mm;
881 (c) 50 µm; (d) 5 µm; (f) 2 µm
882 **Fig. 9** *Lycoperdon velutinum*. a. dried specimens of type (NY00398807); b. dried specimens of the mixed
883 basidiomes with *L. fuliginum* (NY00839022); c. exoperidium ornamentation; d. elongated hyphae from
884 exoperidium; e. basidiospores strongly verrucose [D] in LM (type); f. scanning electron microscope of
885 basidiospores (type). Scale bars: (a) 10 mm; (b) 5 mm; (c) 0.2 mm; (d) 50 µm; (e) 5 µm; (f) 2 µm

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Revision of taxa of *Lycoperdon* (Agaricomycetidae, Basidiomycota) from Brazil previously described under *Morganella*: a new subgenus, two new species and five combinations

Mycological Progress

Dear Editor,

We have revised the manuscript MYPR-D-16-00254 and we have prepared this rebuttal letter. Thank you very much for your comments and Reviewer 1; our manuscript has improved a lot.

Reviewers' comments:

Reviewer #1: While the proposal to treat *Morganella* as a subgenus of *Lycoperdon* was made by Larsson and Jepsson in 2008, Alfredo and Baseia in 2012 and twice in 2014 have described new species under *Morganella*. This wise attitude not to follow a taxonomy based on dogmatic cladistic concepts is abandoned in this manuscript, leading to the recombination of several of the new taxa. No justification is given for this, while the literature supposedly cited include a paper that could have been used to defend a paraphyletic *Lycoperdon* (Hörandl and Stuessy, 2010). The petition published in *Taxon* 56: 5, 2005 would have added weight to such an attitude and it should be remembered that *Lycoperdon* is obviously paraphyletic on morphological grounds, appearing as such in the phylogeny proposed in Demoulin's thesis of 1971, 36 years before Larsson and Jeppson sequencing. One is however free to adopt a taxonomic philosophy, but an explanation of why authors abandon a genus that has been universally accepted, including by themselves, for more than a half-century would be welcomed.

Authors: Last year we have submitted a manuscript to PlosOne Journal with the title “When monophyly lies on paraphyly: Tropical data confirms *Morganella* as a congener of *Lycoperdon* (Agaricomycetidae, Basidiomycota). In this paper we consider that *Morganella* genus as monophyletic within a paraphyletic *Lycoperdon*, and we described two new species in the *Morganella* group. PLOS ONE reviewers considered that “the paraphyletic concept is

a rather obsolete” (in reference mostly to the Hörandl e Stuessy (2010)), and for this interpretation the manuscript was rejected. We were strongly advised to keep on *Morganella* as proposed by Larsson and Jeppson (2008); and we decided to rewrite the paper following these authors.

About of *Morganella* type species: we request loan of material for the main Brazilian collections, but not all loans were taken for various reasons.

Reviewer #1: Anyway, a better title would be "Revision of species previously reported from Brazil under *Morganella*". Enumeration of novelties is not usual in titles and anyway would need revision if advices formulated below are followed.

Authors: Title: Changed.

Reviewer #1: One will note that the included species have not all been recorded from Brazil, while *M. costaricensis*, which could be expected there (reported in Bolivia) is only fleetingly mentioned.

Authors: we request loan of material, but not all loans were taken for various reasons.

Reviewer #1: Three notes on descriptive vocabulary will not be repeated below:
-pseudostipe is not a recommendable term for the subgleba of Lycoperdaceae
-mycoslereid is a seldomly used word that would be more correctly spelled mycosclerids.
- eucapillitium is an irritant, perfectly superfluous word for capillitium. It is not because there are paramedical professions that doctors should be called "eudoctors"!

Authors: We made changes in the text.

Reviewer #1: Glebal membranes are not mentioned in the descriptions while, even if not unique to that genus, an essential character of *Morganellas*.

Authors: Glebal membranes are mentioned in the text and figures.

Reviewer #1: L. 26-28. Move the first sentence to the introduction. This has nothing to do in an abstract. Give the number of Brazillian specimens revised and then speak of the extralimital specimens studied, supposedly for comparison. The qualification of some types is inadequate, see below.

Authors: Changed.

Reviewer #1: L.32. "following the literature" is meaningless. The literature is not homogeneous and L.85-86 it appears it is a very limited literature that has been followed.

Authors: Changed.

Reviewer #1: L.33. Add " 5' end of".

Authors: changed.

Reviewer #1: L. 37-38. Keywords must include *Morganella* and preferably also *Lycoperdon*. If necessary drop phylogenetic analyses and puffballs.

Authors: *Morganella* is not include, because it already on the title. Other suggestions were changed.

Reviewer #1: L. 48-51. The summary of Kreisel and Dring position is incorrect. It could be "Those authors segregated *Morganella* from *Lycoperdon* on the basis of the absence of capillitium, only paracapillitium accompanied by corrugated glebal membranes being present, and a diaphragm being absent". Definition of paracapillitium and glebal membranes could follow if deemed necessary.

Authors: Changed.

Reviewer #1: L. 52 After *M. mexicana*, add "a synonym of *Lycoperdon fuligineum* Berk. et Curt."

Authors: 52: changed.

Reviewer #1: L. 54. It is unusual to give a plural, especially Latin to capillitium and eucapillitium should be forgotten

Authors: Done.

Reviewer #1: L. 61. Replace ", "by "of".

Authors: Done.

Reviewer #1: L. 67. Index fungorum is the bibliographic data base. It is the associated Species fungorum that can be considered a taxonomic data base.

Authors: Channged.

Reviewer #1: L.85-86. The reference considered "specific literature for lycoperdaceous fungi are totally inadequate. Those cited are three previous papers by the authors of the manuscript and one of a poor usually forgotten work. Fundamental work on Lycoperdaceae are practically never cited. A minimum list would include the Czexh flora edited by Pilat in 1958, Die Lycoperdaceae der DDR of Kreisel (1962), Gastéromycètes de Belgique of Demoulin (1968), Gastéromycètes de Corse of Moyersoen and Demoulin (1996), Gasteromiceti epigei of Sarasini (Italy, 2005), papers by Dring on Africa (important for Brazil!) and Mornand for France and Jeppson for Scandinavia. For those who only read English, there is the Flora mycologica iberica of Calonge (1998) and the British puffballs of Pegler, Laessoe and Spooner(1995). For *Morganella* the essential paper is that of Kreisel and Dring, and of course there is a revision for South America by Suarez and Wright. Those two papers should be much more used as a starting point for revising the genus in Brazil. The authors cannot be asked to do their literature at this stage, but what should be done is drop the Ponce de Leon reference and write " the specimens were studied as in our previous papers (the three Alfredo ref.)".

Authors: In Alfredo et al. (2012), Alfredo and Baseia (2014) and Alfredo et al. (2014 a, b) (cited in text) we included in “Materials and Methods”, the references Zeller (1948), Kreisel and Dring (1967), Ponce de Leon (1971) and Suarez and Wright (1996). These references were followed in concordance with expert authors and who treated specifically of *Morganella* genus.

It is a little surprising for us that the reviewer suggesting the literatures that treat specifically of species from Europe. Of course, Dring (1964) should be inserted in our manuscript and we have already added.

Moreover, in lines 85-86, Kreisel and Dring (1967), Morales et al. (1974) and Suarez and Wright (1996) were added. These references were included in the Notes section to discuss taxonomic matter.

Reviewer #1: L. 87-88. KOH is not a good mounting medium for Lycopedaceae, even if some authors stick to it by tradition. It is not understandable that the superior lactophenol

cotton blue is used for capillitium and paracapillitium and not spores. This explains some lack of accuracy in spore descriptions and poor quality of some photographs.

Authors: All spore's images with light microscope were made in cotton blue: Fig. 3 – *L. arenicola*, Fig. 5 *L. exoelongatum*, Fig. 6 – *L. fuligineum*, *L. nudum*, Fig. 10 *M. velutina*, we have repeated spore pictures of Figs. 2, and 4.

Reviewer #1: The very important point as to whether spores are measured with or without ornamentation is not mentioned here. From l. 461-464 one can conclude that measurements include the ornamentation. This is a methodological heresy, mixing two independent characters, the volume of the spore body and the height of the ornamentation. It is only justified when observation conditions are bad (low quality microscope, poor mounting medium). I feel that papers making that mistake should be rejected but one cannot ask to redo all the measurements. Too bad.

Authors: The spore measures were given including the ornamentation, although, we have also, made measures without ornamentation, but not include in the preliminary text. Now, as indicated in “current lines 95-96” the basidiospores measures were done including and excluding ornamentation (Table Supl.3). All photograph in light microscope were made in accordance with Alfredo and Baseia (2014) Alfredo et al. (2014 a, b), and the optical equipment: microscope Nikon Eclipse NI with Nikon camera and software of support to measures.

Reviewer #1: L. 91-92. Put the ref. Silva et al. at the end "the preparation being as described by Silva et al.(2011)".

Authors: changed.

Reviewer #1: L. 92-93. I wonder what the use of Stearn could have been.

Authors: In the book “Botanical Latin” by Willian T. Stearn in 2010, the author in the page 340 give in Fig. 32 shapes of spores, illustration some terms as used in Mycology. The term used by us “criteria” was wrong. We changed the sentence.

Reviewer #1: L. 96. It should be stated from what the DNA is extracted. In a Lycoperdaceae it could be from a culture, a fresh immature fruitbody, a dried one or the spores which are the only living cells in mature fruitbodies. Spores are the best to retrieve DNA from old

herbarium specimens and ripe specimens are the best for morphological identification. They however may be hard to break and it is surprising no method for crushing spores is mentioned. If none has been used, this may explain the negative result with old herbarium specimens.

Authors: In general, we have very good DNA isolation from gleba. Our unsuccessful with herbarium specimens because the sequences obtained belong to other fungus, such as *Penicillium*, Ascomycota); in some samples (generally old and bad preserved in herbaria) we repeated many times the DNA isolation, but always the sequence belonged to other fungus.

Reviewer #1: L. 125-126. A strange sentence concerning ambiguities. Rephrase.

Authors: This sentence is well written; it is part of general methodology when performing manual alignment. We have not changed it.

Reviewer #1: L. 132 If gaps, which are abundant in ITS, are treated as missing data, a lot of information is lost. There are ways to treat indels as single events if one wishes. Like for spore measurements one cannot however ask to redo everything.

Authors: Reviewer is right, however, not include in this paper, one of the authors (M.P.M.) have done different analyses using many alignments, not only from Lycoperdaceae, but also from many other genera of fungi, and when an alignment is well done, include or exclude gaps as a five characters does not change too much the position of the final clades. Also, in the preliminary combined alignment of *Lycoperdon* performed to this paper (with less sequences), we include the gaps as a five characters, and the results were similar.

Reviewer #1: L. 182-184. The presence of mycosclerids was not mentioned in the original description, thus "confirmed" should be replaced by a formulation with "are present". In *L. arenicola* it seems the mycosclerids are localized at the dehiscence of the endoperidium while in *sulcostomatum* they cover the endoperidium (as in *L. marginatum*). Just mentioning the presence of mycosclerids is thus ambiguous. I would prefer to speak of "thick walled irregular shaped spherocysts as pore cells" in the first case and "covering the endoperidium from which it derives" (to be demonstrated) in the second.

Authors: The concept of mycosclereids follow Homrich and Wright (1988) in the page 1287, fig. 2 and page 1290, fig. 15 - "mycosclerids from apical pore region"; and in the page 1293,

fig. 25 - “from apical pore region” likely in the page 1294 - 1295, fig. 33 and 37) describe the same concept adopted by the reviewer “thick walled irregular shaped”. Here we choice follow the term mycosclerids likewise Homrich (1988).

Reviewer #1: L. 184. What are the features that distinguish clade A and B? It is clear that with their capillitium, well developed subgleba and terricolous habitat, *Morganella arenicola* and the related new species are true Lycoperdon. *Morganella afra* is a true *Morganella* and its position in the ITS-LSU tree is anomalous and should be verified with more collections, preferably from Africa.

Authors: The clade A have characters distinctive from clade B such as: subgleba well - developed and cellular, capillitium present and paracapillitium absent, and the mycosclerids from apical portion; in clade B those characters are not found. In posterior studies we will include more specimens from Africa and others regions not well known.

Reviewer #1: L. 199. "We find reasons". Those reasons should be given. A comparison with *M. compacta* would be welcomed since this was done when *M. arenicola* was described.

Authors: Changed. Also, changed in the discussion of *L. demoulinii*.

Reviewer #1: L. 212. It is doubtful that *M. allostipitata* is a good species. This may be the classical *M. velutina* (see below). There is not much of an updated description as claimed.

Authors: No changed. We have analyzed the type of *M. velutina* NY00398807, and the e sphaerocysts in the exoperidium are distinct from those found in *L. allostipitatum* (type and others specimens revised in our study). The fig. 2 and fig. 10 shows that they are different species and *L. allostipitatum* is an and distinct and authentic Brazilian species.

Reviewer #1: L. 224. What is a "digitaliform spherocyst"?

Authors: Changed by digitiform as cited in Suarez and Wright (1996) to describe a form in finger-like.

Reviewer #1: L. 225-226. It is not because Larsson and Jeppson place *Morganella subincarnata* in Lycoperdon that it is a "current name". The correct sentence should be 'Larsson and Jeppson treat this taxon as *Lycoperdon subincarnatum* Peck'. There is nothing

updated in the provided description of this well-known species, which is outside the scope of the paper as defined in the title.

Authors: Yes, reviewer is right. Sentence changed.

Reviewer #1: L. 228. How different is the endoperidium between *M. subincarnata* and *purpurascens*?

Authors: Changed - removed phrase “not like in *L. subincarnatum*” because in fact, this sentence is not good here.

Reviewer #1: L. 229. Stop again of speaking of current name. "We adopt the name *Lycoperdon purpurascens* would be correct.

Authors: Changed.

Reviewer #1: L. 230. "has been described" instead of "is described".

Authors: Changed.

Reviewer #1: L. 231. Replace "due to" by "with an "

Authors: Changed.

Reviewer #1: L. 232. Drop "verrucose" which is not in the original description. Add "differing from *M. purpurascens* through the cellular subgleba".

Authors: Changed.

Reviewer #1: L. 242-273. The description of a new subgenus *Arenicola* of *Lycoperdon* is definitely premature. The description only applies to *L. arenicola* and the new species. *Morganella afra* grows on wood and the presence of mycosclerids as pore cells would be surprising. The authors have only studied one collection of this species, previously unreported outside Africa. *L. arenicola* and the related new species are definitely *Lycoperdon* s. str., for which a subgeneric classification is premature, given the number of species not taken into account in this study nor in that of Larsson and Jeppson. Those authors even used a paraphyletic subg. *Utraria* in contradiction with their taxonomic principles, thus acknowledging the preliminary nature of their classification.

Authors: The new subgenus is based on morphological features, as well as differences in ITS and LSU. We kept the description of the new subgenus; thus we maintain our taxonomic principles.

Reviewer #1: Taxon sampling should be enlarged before describing new subgenera of Lycoperdon but also the DNA sequence sampling with independent loci from the ribosomal one, where ITS are specially poorly informative in Lycoperdaceae.

Authors: We continue collecting in different areas around the World, and checking herbarium collections to enlarge the sampling to a future work.

Reviewer #1: L. 275-303. Delete the key., which is useless. A key should be taxonomically or geographically limited but not to the studied species. Further the key use inadequate terminology and would hardly work.

Authors: Removed.

Reviewer #1: L. 305-302. The treatment of *Morganella afra* is inadequate for the only collection seen is a Brazilian one of which the identity should be checked by comparison with authentic African material. Comparison to the well-known boreocontinental *M. subincarnata* has no point. The holotype of *M. afra* is in Kew, together with 4 paratypes and if there is a problem with the K material, there are two paratypes in BR, one in P and one in Kreisel's herbarium. More recent collections may exist in those herbaria and in those holding African fungi.

Note that in the description of the Brazilian material the areolate endoperidium is emphasized while in the original description it is qualified of "smooth to very minutely areolate". Further in the original description the spore ornamentation is qualified of "minutely asperulate to short-spined", which is more than "slightly verrucose".

At this moment the Brazilian collection should be referred to by its number and not the name *Morganella afra*. If a description of this collection is included it could be mentioned that this collection could be related (which I doubt) to *M. afra* at the most.

Authors: Changed.

Reviewer #1: L. 334-367. I find no reason to believe *Morganella allostipitata* is not a synonym of *M. velutina*. The only collection on which the name was based shows a whitish subgleba which is an occasional anomaly, perhaps more frequent in *Morganella*, among *Lycoperdaceae*. It would have been important to state if such a white subgleba was a constant feature of the three additional collections mentioned here. It was incredible that the original description made a comparison with *M. fuliginea* and not *M. velutina*, while its fig. 13 fits so well fig. 2A of the exoperidium of *M. velutina* in Kreisel and Dring, as well as fig. 18 of Suarez and Wright. Until convincingly demonstrated otherwise, the four collections reported here should be named *Morganella* (or *Lycoperdon*) *velutina*.

Authors: About the fig 2A in Kreisel and Dring (1967) and fig. 18 in Suárez and Wright (1996): The dry specimen analyzed by those authors Ellis no. 5013 is a mixed collection of *M. mexicana* and *M. velutina*. This fact, probably taken to misinterpret the description of the exoperidium in *M. velutina*. As discussed by us, the specimens of *M. velutina* do not show two types of cells as indicated by Kreisel and Dring (1967) and Suarez and Wright (1996) figures. The other collections of *M. velutina* also show the same, only one type of cells from exoperidium (cells elongated). *Lycoperdon allostipitatum* (= *M. allostipitata*) showed often two types of dextrinoid cells from exoperidium and all specimens elongated subgleba (2–4 mm).

Reviewer #1: L. 394-395. *Lycoperdon arenicola* may be a good species and a true *Lycoperdon*. The combination is thus justified, even for those who maintain the genus *Morganella*. It is strange that this terricolous species with capillitium was originally described in *Morganella*. One should note that the correct orthography in *Lycoperdon* is *L. arenicola*, as in the text, and not *L. areniculum* as in the legend of fig. 3. Names in -cola are substantive in apposition, not adjectives.

Authors: ok! Ps.: We are humbly acknowledging that we have made a mistake, and we have corrected here. Last year we had an opportunity to work and observe European specimens of *Lycoperdon* and *Morganella* in MA-Fungi herbarium, LG herbarium (Belgium) and Kew Botanical Gardens, which helped us understand little more the Brazilian specimens/species. The figures in PDF file is with low resolution (this one was generated by submission system

of Mycological Progress. However, our JPEG or TIF files is a good one resolution. Although the spores in *L. arenicola* can be [B] sometimes, but is often [A].

Reviewer #1: L. 337 (or 397?). It should be specified whether the capillitium presents septa and/or pores. Spores are probably B as demonstrated by the SEM picture. The qualification of A results from poor observation in KOH.

Authors: changed.

Reviewer #1: L.409. It should be mentioned if the gleba is cellular. Note than in the externally similar *L. echinatum* group, the cells are somewhat ill defined with a cottony aspect of the whole structure.

Authors: Changed.

Reviewer #1: L. 413 Scar is not usual for areole. "... marked by areoles" is enough but the colour should be given.

Authors: Changed.

Reviewer #1: L. 415-417. The description of the anatomy of the exoperidium is not clear. Among what are "spherocysts scattered at the base of the spines"? What is a "complete spine as in *L. perlatum*"? Fig. 4 does not help very much. It should be clearly stated how are the spherocysts at the base and top of the spines (different or not) and if the areoles are made up of spherocysts similar to those of the base of the spines. Those spherocysts are important characters in the externally similar *L. echinatum* group, used to separate the American vicariant *L. americanum* from the European *L. echinatum* (Demoulin 1972a).

Authors: Changed.

Reviewer #1: L. 419. Specify the presence or absence of pores and septa in the capillitium. It is impossible to get an idea of spore ornamentation, given the low magnification and use of KOH in fig.4 and absence of a SEM picture.

L. demoulinii may be a good species but it should be described more accurately and compared to species of the *L. echinatum* group.

Authors: Changed.

Reviewer #1: L. 430-432. *L. exoelongatum*. The epithet is not well chosen as it is meaningless.

Authors: Name refers to the elongated unchained elements of the exoperidium.

Reviewer #1 L.435. Subgleba, not pseudostipe

Authors: Changed. We have changed pseudostipe by elongated subgleba along the text and figures.

Reviewer #1: L. 435-438. The diagnosis is not discriminant toward *Morganella velutina* (including *M. allostipitata*).

Authors: Neither *Lycoperdon velutinum* or *L. allostipitata* presents exclusively setose unchained thick-walled elongated elements in the exoperidium.

Reviewer #1: L. 440. Subgleba not pseudostipe. Is it that the subgleba is white and cottony in section that is meant?

Authors: Changed.

Reviewer #1: L. 441. Micrometres not millimetres I guess. Drop "peridium two-layered", a general feature of the genus. On l. 441 the exoperidium is said to be persistent and on l. 442 to wear out. The description of the exoperidium is anyway not clear.

Authors: Some changes made. The exoperidium do not detach, but it change its overall aspect with the age.

Reviewer #1: L. 451. It is hard to believe there would be pores in endoperidial hyphae.

Authors: Since it is not a main taxonomical trait it was cut from the text.

Reviewer #1: L. 458-464. The purpose of this discussion of spore size is not clear. It is however useful to make clear the authors measure with the ornamentation included, but this should have been stated in Material and methods.

Authors: Done.

Reviewer #1: L. 466-467. What is the difference between a cystidioid and a setoid shape? There is no illustration and in mycology, setae are a category of cystidia.

Authors: There is an illustration of elongated thick walled setoid elements of exoperidium in the plate of *L. exoelongatum* (Fig. X, G). It can be compared with illustrations in Suarez and Wright (1996).

Rhomboid-cystidioid shape are more prone to a clavate contour, with the basis prone to be slender than the apex. In the other hand, setoid-cystidioid shape is clearly setoid (Fig. X, G), slendering towards de apex.

Reviewer #1: L. 467-471. The authors have a typological species concept of another age. It is not because one finds a difference with a type specimen that one has a different species. Types should be used to fix nomenclature when taxonomy is clarified. Good taxonomy proceeds through establishing the variability of taxa. Only when this variability is defined can it be known if a type collection falls in its range. This is the way Kreisel and Dring and Wright and Suarez have worked and they have well described the variability of exoperidial elements in *Morganella velutina* and I do not see any reason not to consider this supposedly new taxon will be one more synonym of *M. velutina*. *M. fuliginea* has a different exoperidium structure and is not worth discussing here, anymore than the completely different *M. subincarnata*. On another hand there is no convincing argument to claim *M. albostipitata* is distinct. Development of the subgleba is quite variable in Lycoperdaceae, depending on growth conditions, and fig. 5b is extremely similar to 2a, including the whitish subgleba. Remains the difference in ITS, which is however based on a single collection. In addition to usual sequencing errors and DNA contaminations, Lycoperdaceae are subject to easy contamination of fruitbodies by the tiny wind-blown spores of others. More sequences, to confirm the present one and to establish the ITS variability in *Morganella velutina* are thus needed before drawing conclusions and describing a new species.

Authors: Holotype and published descriptions of *Lycoperdon velutinum* (= *Morganella velutina*, = *mexicana*) differs macroscopically from *L. exoelongatum* in the absence of a white proeminent pseudostipe/subgleba and in the velvety exoperidium, which is more rough, felted, in *L. exoelongatum* (Kreisel & Dring 1967; Ponce de Leon, 1971; Morales et al. 1974; Suárez & Wright 1996).

Microscopically, at the exoperidium, *L. exoelongatum* differs from *L. velutinum* by presenting its elongated unchained elements always setose, while *L. velutinum* exhibits most

commonly paraphisoid or clavate elements, rarely setose. At the spores, *L. velutinum* have smaller ones (3.8 – 5.5 µm). Although are both aculeate, under electron microscope is observed that the swallows at the basis of each aculeous is striated in *L. velutinum*, being theses swallows smooth in *L. exoelongatum*. Furthermore, basidiospores of *L. velutinum* presents confluent cristae between some aculei, which are always independent in *L. exoelongatum* (Kreisel & Dring 1967; Ponce de Leon, 1971; Morales et al. 1974; Suárez & Wright 1996).

Concerning to *Lycoperdon albotipitatum*, this species clearly differs from *L. exoelongatum* by the absence of elongated unchained elements. Besides that, the swallows of aculei are striate and some aculeo are confluent in *L. albotipitatum* (Alfredo et al. 2014). Not to mention the high supported phylogenetical delimitation in the tree.

We also know that only ITS sequence could be not sufficient to identify new species, however, in this study also morphological features support it.

Reviewer #1: L. 486-487. A neotype cannot be designated for *L. fuligineum*. Even if the holotype studied by Kreisel and Dring and Suarez and Wright (who gave good SEM pictures of the spores) had been lost, this collection is part of the Fungi Cubenses Wrightiani and there is at least one isotype in the Curtis herbarium at FH. It is anyway hard to believe Kew would have lost this precious holotype. One should be aware it could either be stored under *Lycoperdon* or *Morganella*.

Authors: Reviewer is right. We are really sorry for our mistake. A neotype is not designed to *L. fuligineum*.

Reviewer #1: L. 542. It is stated B. et C. did not indicate a "voucher". This is incorrect. Wright's number 705 is mentioned. One should remember looking at the exsiccata that it bears this number and also 506, which is the number of the species in the publication.

At most one could designate an epitype, but this should follow a serious attempt to revise the original material and demonstrate some diagnostic feature cannot be observed, for example a DNA sequence that would be impossible to obtain. Given the absence of a taxonomic problem with this species at the moment I do not see the need of an epitype.

Note that according to Stevenson (1973) book on exciccati, sets of the Fungi Cubenses are probably complete at K and FH and partial at BPI, BUF, UPS, NY and NYS.

Authors: improved

Reviewer #1: L. 490. The common neotropipcal *Morganella fuliginea* has a long list of synonyms established by Kreisel and Dring, to which Suarez and Wright have added *Morganella puiggarii* (Speg.)Kreisel & Dring. For that species described from Brazil, Kreisel and Dring had based themselves on publications only, while Suarez and Wright studied many collections of *M. fuliginea* and "*puiggarii*", several from Brazil. It is strange that here a single name(*M. mexicana*) is cited. It may not be necessary to list again all the synonyms but one could write "For synonymy, see Suarez and Wright (1996.)". A discussion of the Brazilian *M. puiggarii* would have been expected.

Authors: see above.

Reviewer #1: L. 497. Replace "that" by "where they".

Authors: Changed.

Reviewer #1: "long" not "length".

Authors: Changed.

Reviewer #1: L.530-541. The discussion of Ellis 5013 is confuse. It seems to state that after Suarez and Wright reported this was a mixed collection, it had been split, but incorrectly, the two parts remaining mixtures. This could be made clearer and shorter.

Authors: Changed.

Reviewer #1: L. 542-545. This erroneous paragraph has been discussed under L. 486-487.

Authors: Changed.

Reviewer #1: L. 575-580. *Morganella nuda* was first described, based on three collections from the same locality. Those were rain-washed as apparent from the picture and line drawings of microscopical details were of poor quality. On those drawings the paracapillitium looked like a capillitium with pseudosepta and the exoperidial spherocysts seem to originate in the endoperidium. Given the fact that the exoperidium of *M. fuliginea*

has been reported to be occasionally caducous, the impression was that this was one more synonym of *M. fuliginea*.

It is here reported that three more collections would have a caduceus exoperidium and would form a clade in the ITS-LSU tree with the three original ones. It is also mentioned that the spores are in general bigger (5.5-7.5 against 4.5-7 including ornamentation, the spines being 0.5-1 against 0.5-1.5). This is hardly significant and in the photographs in fig. 6 and 7, if the scale bar is correct, *M. fuliginea* would have spores about 4 μ m without the ornamentation (5 with) and *nuda* 5 without and 7 with the ornamentation, in light microscopy. With the SEM, *M. fuliginea* would have 4 without and 5.4 with the ornamentation, while for *M. nuda* it would be 3.75 and 5.6. The scale thus do not agree between fig. 7d and 7e. If one wants to use the spore size, one should measure without the ornamentation (eventually also with, so as to by subtracting the two values and dividing by two get the height of the spines) with good observation condition. Further a sufficient number of spores by specimen and specimens by species to obtain a significant result would be necessary. It thus remains to be convincingly established if *M. nuda* is a good species or should be included in the morphological and ITS variation of *M. fuliginea*.

Authors: Firstly, the specimens of *L. nudum* are reported from areas of “Brejos”, which are areas of rainforest surrounded by typical xerophily vegetation (Caatinga). A traditional work about that (Veloso et al. 1991), considered it an ecological disjunction of the Atlantic Forest, with a high biodiversity. Also, are treated as an ecological refuge by displayed different and unique phytophysiology characteristic making part of a relic vegetation inserted in the middle of adverse ecological situations.

When comparing the specimens of *L. fuligineum* (= *M. fuliginea*) vs *L. nudum* (*M. nuda*) we noted different features as we have discussed in the text. Moreover, if we call all the B1 clade of *M. fuliginea*, this will have repeat in the entire tree and then, we will be underestimated our results. In fact, these situations bring at the light a discussion about “what does it do feel better the taxonomist”? Does ITS and LSU are good to work with phylogeny to the reviewer? Here seem that the #reviewer 1 is skeptical to the ITS region, but if he/she liked this marker maybe we had not problems with this results and would be discussed other things. We know about the problems with ITS (one of the authors is coauthors in those general papers related

to ITS and other markers, such as Schoch et al. 2012; Stielow et al. 2015), but in our study, we have done a careful morphological analyses of different features. We appreciate very much all the exhaustive revision made by this anonymous reviewer, and our paper has been improved very much that's to theirs comments. We would have like to merge all their comments with our data analyses and, however, in some points we cannot agree totally. This is Science.

Reviewer #1: L. 582-599. Apparently no material of this Indo-Pacific species has been studied and one may wonder why it is included (because of the existence of a sequence under that name in GenBank?).

Authors: We include the sequences from GenBank, to confirm that they did not fit with any of the Brazilian species.

Reviewer #1: L. 590. Scar is not a good word for areole.

Authors: changed.

Reviewer #1: L. 588-599. The description is not very clear and too long. For a taxon that is not really relevant to the paper, if one wants to include it (but then why not *L. costaricensis*?) it could suffice to write: "This Indo-Pacific species is characterized by a low spore ornamentation, weakly developed exoperidium, often pitted endoperidium, and compact subgleba". The discussion of differences with *L. sosinii* could be left to that taxon, if included.

Authors: Changed.

Reviewer #1: L. 601-612. One more taxon of little relevance to the subject of the paper. Included because of the existence of a sequence in Gen Bank? It is distinguished from *M. pururascens* by by the cellular subgleba and stronger spore ornamentation.

Authors: Changed (removed).

Reviewer #1: L. 614-633. *Morganella subincarnata*, a well known boreocontinental species of the Northern Hemisphere, is one more species hardly relevant to the studied area. Even if one collection has been studied, one may question the interest of including a species that has little chance to occur in Brazil and is described at length in several North-American

publications like Coker and Couch, and has recently been illustrated in colour by Sarasini (*Gasteromiceti epigei*, 2005).

Authors: Changed (removed).

Reviewer #1: L. 621 and 627. Usually, the endoperidium is pitted and not or only weakly areolate.

Authors: Changed (removed).

Reviewer #1: L. 628. It is not true that the areolation is similar in *Morganella subincarnata* and *compacta*. The former is more pitted than areolate, while the latter has a true brown areolation like in *L. echinatum*. This is such a distinct species that it is not worthwhile discussing its difference from *M. subincarnata*. The discussion of similarities with *M. purpurascens* and *sosinii* should also be left out. It is not necessary and is incorrect.

Authors: Removed.

Reviewer #1: L. 635-714. The category "Incertae sedis" is made up of two classical *Morganella* and one strange recently described puffball. It is hair-raising to see that two of the best known *Morganella* (*compacta* and *velutina*) are considered "Incertae sedis" because the authors failed to obtain ITS sequences.

The situation of *Morganella sulcostomata* is different. Here they have a sequence but they consider the species is incertae sedis because they claim it does not fit in Larsson's and Jeppson or their subgenera of *Lycoperdon*. In the tree fig.1 it is in true *Lycoperdon* and with the (undue) importance given by those authors to ITS-LSU is certainly not of uncertain position. Naming subgenera in *Lycoperdon* s. str. Is premature with so few extraeuropean species present in the available trees . One will note Larsson and Jeppson kept subg. *Utraria* for two different clades.

This section Incertae sedis should disappear and the three species included in the general treatment.

Authors: Changed.

Reviewer #1: L. 650. Spores of *M. compacta* are not punctate, but present minute spines. Even in the poor picture 9e, probably taken with KOH, it is evident those spores are verrucose.

Authors: Changed.

Reviewer #1: L. 665. The Brazilian specimens reported as *M. compacta* should be cited.

Authors: Changed.

Reviewer #1: L. 666. It is stated that DNA extraction was unsuccessful with the type and paratype. One concludes that more recent material was not tried. Table 1 is ambiguous and should make the difference between failed attempt and no attempt.

Authors: L. 666: the PDD herbarium gave us the permission to DNA extraction for two exsiccate PDD 10140 and PDD 11111. The others (cited in the text) we had not permission.

Reviewer #1: L. 684. The peridium is not composed of mycosclerids, otherwise it would disintegrate *Calvatia* like. The original description shows the mycosclerids cover the endoperidium, causing the fall of the exoperidium as in *L. marginatum*.

Authors: Changed.

Reviewer #1: L. 685. The endoperidium is not smooth but velutinous, as correctly stated in Table 1. In that table, the spores should be B, if not C. The capillitium was said to be absent in the original description and if it now has been observed, this should be emphasized and illustrated. This would confirm it is not a *Morganella* s. str.

The presence of spherocysts on the endoperidium is a rare feature that indicates a possible relationship with *L. marginatum*. It is anomalous that this taxon that in the tree of Larsson and Jeppson comes close to *L. perlatum*, that is not so far from *L. sulcostomatum* in the present tree, has not been included, even in the rather superfluous supplementary material tree.

Authors: L. 685: Changed. About the sequences: We have added numerous sequences from species not included in Larsson and Jeppson alignment. It is well known that the position of some sequences can be modified in different analyses when incorporating new sequences, and more when they belong to different species, not included in the previous studies.

Reviewer #1: L. 690-691. A NY collection is qualified of holotype of *M. velutina*. This is impossible. When publishing the name, Massee indicated this was a collection from the Berkeley herbarium, which is in Kew. What is in NY could be an isotype not seen by Berkeley nor Massee. As mentioned by Kreisel and Dring, the type in K is made up of two Fendler's collections (235 and 236). Of those, Suarez and Wright selected as lectotype

Authors: We have used the same specimens mentioned in Ponce de Leon (1971), the specimen have labelled type. The reviewer can easily see the negative number photo in the Ponce de Leon and here. The exsiccate is labelled as “Neotropical types and authentic specimens”.

Reviewer #1: L. 709-710, Is it true that the Ellis 5013 collection has induced people in believing *Morganella velutina* and *fuliginea* were a single species? I would rather say "L. fuligineum and velutinum are close enough to have been often confused as with the mixed collection J. B. Ellis 5013."

That those two species have been confused emphasizes the absurdity to consider *M. velutina* is incertae sedis. It certainly belongs to the same generic or infrageneric taxon than *M. fuliginea*. The sentence speaking of amplification from type or paratype is not only absurd, but incorrect for there are no paratypes in this case.

Authors: L. 709 – 710: Changed.

Reviewer #1: L. 716. There is a single excluded taxon, hence taxon, not taxa.

Authors: Changed.

Reviewer #1: L. 717-731. Start with the name (*Moganella rimosa*). It is strange to have to use strand anatomy to discover that *Morganella rimosa* is in fact *Lycogalopsis solmsii*. This well-known pantropical fungus is only superficially similar to Lycoperdaceae. It would have been enough to say that re-examination of the original material has shown the presence of clamps which rules out the affiliation with Lycoperdaceae and redirected attention toward *Lycogalopsis solmsii*. On another hand it is interesting to note that cord anatomy fits the affiliation to the gomphoid-phalloid clade, as established by Demoulin et al. on molecular ground (Acta Mycol. 98: 15-20, 2013).

Authors: Changed.

Reviewer #1: L. 734. Kreisel and Dring did not separate *Morganella* from *Lycoperdon* through the presence of paracapillitium, which occurs in some *Lycoperdon* s. str. and of course *Vascellum*. What they emphasized was the total absence of capillitium and association of the paracapillitium with corrugated glebal membranes. They discussed the similarities with *Vascellum* and *Lycoperdon pyriforme*, known as a wood-inhabiting fungus since the 18th century (no need to refer to Larsson and Jeppson for that!).

Authors: Changed.

Reviewer #1: L. 736-738. I do not think one can find in Demoulin 1972b that presence of paracapillitium in *Lycoperdon* s. str. depends of the stage of development. That work is primarily based on the study of mature fruitbodies and if paracapillitium is mentioned it is when it is present in such fruitbodies. One may note that in *L. perlatum* there is a great variability in the abundance of paracapillitium.

On another hand, in this reference (Demoulin's thesis) one will find a phylogenetic tree (fig. 25) and comment p.265 that suggest on morphological grounds that *Morganella* derives from *Lycoperdon foliicola* (a Brazilian species not mentioned in this manuscript). Including *Morganella* in *Lycoperdon* is thus no consequence of molecular data but of the adoption of a cladistic philosophy that bans a paraphyletic *Lycoperdon*.

Authors: Changed.

Reviewer #1: L. 747. It has been previously stated it is premature to describe a subgenus *Arenicola*.

Authors: Based on our molecular analyzes and following the concepts adopted by Larsson and Jepson (2008) we keeping on our decision about subgenus *Arenicola*.

Reviewer #1: L. 749-752. This choice of most important characters is not in line with the experience of most authors, at least for macroscopic peridial characters (including the inadequate word scar). Neither of the two cited references (Demoulin 1976 and Homrich and Wright 1988) support this. Those references seem to have been picked at random

Authors: changed. About the characters: in accordance with our molecular data, plus our observation of morphological features are itself in the tree. For example, in the Clade A, the two species are separating by their endoperidium surface, which is areolate in *L. demoulinii* and smooth in *L. arenicola*. Clade B1, *L. nudum* has exoperidium falling off, while *L. fuligineum* has persistent exoperidium; other subject important is: in Clade B1, B2, B3 the basidiospores are very similar, although, the exoperidium features, as to their ephemeral or persistent nature, the types of sphaerocysts that make up the exoperidium; the surface endoperidium can be areolate or smooth, are morphological information that support each clade.

In the Demoulin (1976), the author call attention to the use of exoperidial characters. because they seem strongly influenced by ecology. However, the author use those elements to delimit species. Of course, we must be caution all the time with these situation, but in *L. allostipitatum* vs. *L. velutinum* the macro morphological exoperidium are quite different, *L. allostipitatum* has verrucose exoperidium which is velutinous in *L. velutinum*; the elongated subgleba was not found it in all specimens of *L. velutinum* studied here. About our experience, I have not comments.

Reviewer #1: Table 1. Distinguish (for example with 0 and -) cases where sequences could not be obtained and those where no attempt was made.

Authors: Changed.

Reviewer #1: Subgleba compact versus cellular would be more informative.

Authors: Done.

Reviewer #3: This is a poorly prepared manuscript, and, thus, a major revision must be done.

Authors: Done.

Electronic Supplementary Material (animations, movies, audio, FASTA etc.) - will be published

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Electronic Supplementary Material (animations, movies, audio, FASTA etc.) - will be published

Fig. S1_Morganella_ITS_LSU.pdf

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audio, FASTA etc.) - will be published**

Table S3 and S4_Measures Spores.docx

Lycoperdon subgen. *Arenicola* subg. nov.

Lycoperdon subgen. *Morganella*

Lycoperdon subgen. *Vascellum*

Lycoperdon subgen. *Lycoperdon*

Lycoperdon subgen. *Ultraria*

Lycoperdon subgen. *Apiopordon*

Bovista

Calvatia

L. arenicola comb. nov.

L. demoulinii sp. nov.

L. nudum comb. nov.

L. fuligineum Berk. & M.A. Curtis

L. albstipitatum comb. nov.

L. subincarnatum Peck.

L. purpurascens Berk. & M.A. Curtis

L. sosinii comb. nov.

L. exolongatum sp. nov.

L. sulcatostomum comb. nov.

L. caudatum, RGC920818

Scale bar: 0.5 μm

Table 1 Specimens studied in this work with voucher numbers, country, and the main morphological features considered. Collections which ITS and LSU sequences were obtained are indicated with (+), sequences were not obtained (-), sequences were not attempt (0).

Species	Specimens	Country	ITS/LSU sequences	Subgleba	Exoperidium ornamentation	Endoperidium surface	Capillitium	Paracapillitium	Spores ornamentation	Spores size (μ)
<i>Lycoperdon afrium</i>	ICNI177118	Brazil	+	Reduced and compact	Minute verrucae, not persistent	Minute areole	Absent	Present	B	4–5×4–5
<i>Lycoperdon albositpitatum</i>	INPA 239563, holotype	Brazil	+	Reduced and compact	Spiny, persistent	Smooth	Absent	Present	D	5.2– 6×5–6
	UFRN-Fungos 2249	Brazil	+	Reduced and compact	Spiny, persistent	Smooth	Absent	Present	D	5.1–5.8×5.1–5.9
	UFRN-Fungos 2569	Brazil	+	Reduced and compact	Spiny, persistent	Smooth	Absent	Present	D	5.2– 5.8×5–5.7
	UFRN-Fungos 2572	Brazil	+	Reduced and compact	Spiny, persistent	Smooth	Absent	Present	D	5.1–5.8×5.1–5.9
<i>Lycoperdon arenicola</i>	UFRN-Fungos 1006, holotype	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	3–4×3–4
	UFRN-Fungos 649	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	3.8–4.5×3.8–4.5
	UFRN-Fungos 656	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4–4.5×4–4.5
	UFRN-Fungos 657	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4–4.5×4 – 4.5
	UFRN-Fungos 729	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4–4.5×4–4.5
	UFRN-Fungos 864	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4–4.5×4–4.5
	UFRN-Fungos 941	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	3–4×3–4
	UFRN-Fungos 1367	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	3.5–4.5×3.5–4.5
<i>Lycoperdon compactum</i>	UFRN-Fungos 1510	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4 – 4.5×4–4.5
	UFRN-Fungos 2567	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	4–4.5×4–4.5
	UFRN-Fungos 2581	Brazil	+	Well developed and cellular	Verrucose, not persistent	Smooth	Present	Present	A	3–4×3–4
	PDD 10140, holotype	New Zealand	-	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 1102	New Zealand	-	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 16721	New Zealand	0	Reduced compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 4530	New Zealand	0	Reduced compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0

	PDD 8525	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 4008	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–3.8
	PDD 5607	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–3.8
	PDD 11111	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 11565	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD 12039	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
	PDD16058	New Zealand	0	Reduced and compact	Spiny not persistent	Areolate	Absent	Present	D	3.5–4.0
<i>Lycoperdon demoulinii</i>	UFRN-Fungos 655, Holotype	Brazil	+	Well developed and cellular	Spiny, not persistent	Areolate	Present	Present	A	4–5×4–5
	UFRN-Fungos 2554	Brazil	+	Well developed and cellular	Spiny, not persistent	Areolate	Present	Present	A	4–4.5×4–4.5
	UFRN-Fungos 2570, Holotype	Brazil	+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	4–4.5×4–4.5
	INPA 239561	Brazi	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5–6.8×5–6.8
<i>Lycoperdon exoelongatum</i> <i>Lycoperdon fuliginum</i>	UFRN-Fungos 606	Brazi	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.5–7.0×5.1–6.8
	UFRN-Fungos 371	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.8–6.4×5.5–6.3
	UFRN-Fungos 1768	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	6–6×4.6–6.3
	UFRN-Fungos 1971	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.3–6.1×5–5.9
	UFRN-Fungos 1972	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	4.8–5.6×4.8–5.5
	UFRN-Fungos 2560	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.8–6.4×5.5–6.3
	UFRN-Fungos 2561	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5–6.5×5–7
	UFRN-Fungos 2562	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5–6×5–5.9
	UFRN-Fungos 2563	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	6–7×6–7
	UFRN-Fungos 2566	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.3–5.8×5.1–5.9
	UFRN-Fungos 2571	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.5–7×5.1–6.8
	UFRN-Fungos 2575	Brazil	+	Reduced and compact	Warty, persistent	Smooth	Absent	Present	D	5.3–6.1×5–5.9
	UFRN-Fungos	Brazil	+	Reduced	Warty, persistent	Smooth	Absent	Present	D	5.3–5.8×5.1–5.9

	2578			+	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.3–6.1×5–5.9
Lycoperdon nudum	UFRN-Fungos 2579	Brazil		+	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5–6×5–5.9
	UFRN-Fungos 2582	Brazil		+	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.5– 7×5.1–6.8
	UFRN-Fungos2586	Brazil		+	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	4.4–5.6×4.2–5.4
	NY00839022 (<i>pro parte, M. mexicana</i> , paratype)	USA		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	4.5–5.5×4.5–5.5
	NY00839023 (<i>pro parte, M. mexicana</i>)	USA		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.4–6.7×5.2–6.5
	NY00398806	Venezuela		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	52–6.5×5.1–6.5
	NY00398808	Venezuela		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.1–6.1 ×5.1–6.1
	NY00398727	Venezuela		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	4.9–5.9×4.9–5.8
	NY00398728	Venezuela		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.1–5.9×5.1–5.7
	PAC A13765	Brazil		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	5.1–5.7×4.8–5.6
Lycoperdon sulcatotomum	PAC A13802	Brazil		-	Reduced and compact	persistent Warty,	Smooth	Absent	Present	D	4.8–5.6×4.8–5.5
	ICN 154541	Brazil		+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	5.6–7.5×5.4–7.5
	UFRN-Fungos 1765, Type	Brazil		+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	5.6–6.6×5.4–6.6
	UFRN-Fungos 1766	Brazil		+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	5.6–6.6×5.4–6.6
	UFRN-Fungos 2565	Brazil		+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	5.6– 7.5×5.7–7.5
	UFRN-Fungos 2568	Brazil		+	Reduced and compact	Spiny, not persistent	Smooth	Absent	Present	D	5–6×5–6
	ICN177032	Brazil		+	Reduced and compact	Spiny, not persistent	Surface velutinous	Present	Present	B	5–6×5–6
	ICN177033	Brazil		+	Reduced and compact	Spiny, not persistent	Surface velutinous	Present	Present	B	5.1–5.6×4.9–6.1
	NY00398807, holotype	Venezuela		-	Reduced and compact	Velutinous, persistent	Smooth	Absent	Present	D	4.5–6.3×4.5–6.1
	NY00398809	Venezuela		-	Reduced and compact	Velutinous, persistent	Smooth	Absent	Present	D	4.9–6.2×5–6.1
Lycoperdon velutinum	NY00839022 (<i>pro parte</i>)	USA		-	Absent	Velutinous, persistent	Smooth	Absent	Present	D	5.7–6.5×5.6–6.5
	NY00839023 (<i>pro parte</i>)	USA		-	Absent	Velutinous, persistent	Smooth	Absent	Present	D	

Table 2 All the specimens used in the molecular phylogenetic analyses with voucher number and GenBank Accession Numbers. When ITS and LSU have different numbers, the first number corresponds to ITS and the second to LSU. In black are indicated the new sequences generated in this study.

Species	Herbarium voucher	GenBank Accession Number
		ITS/LSU
<i>Lycoperdon afrom</i>	ICN177118	KU958371, KU958372
<i>Lycoperdon albobostipitatum</i>	INPA 239563-TYPE!	KU958363, KU958364
<i>Lycoperdon albobostipitatum</i>	UFRN-Fungos 2249	KU958361, KU958362
<i>Lycoperdon albobostipitatum</i>	UFRN-Fungos 2569	KU958357, KU958358
<i>Lycoperdon albobostipitatum</i>	UFRN-Fungos 2572	KU958359, KU958360
<i>Lycoperdon altimontanum</i>	MJ4270	DQ112588
<i>Lycoperdon arenicola</i>	UFRN-Fungos 1006-TYPE!	KU958303, KU958304
<i>Lycoperdon arenicola</i>	UFRN-Fungos 649	KU958297, KU958298
<i>Lycoperdon arenicola</i>	UFRN-Fungos 656	KU958291, KU958292
<i>Lycoperdon arenicola</i>	UFRN-Fungos 657	KU958299, KU958300
<i>Lycoperdon arenicola</i>	UFRN-Fungos 729	KU958295, KU958296
<i>Lycoperdon arenicola</i>	UFRN-Fungos 864	KU958293, KU958294
<i>Lycoperdon arenicola</i>	UFRN-Fungos 941	KU958305, KU958306
<i>Lycoperdon arenicola</i>	UFRN-Fungos 1367	KU958287, KU958288
<i>Lycoperdon arenicola</i>	UFRN-Fungos 1510	KU958289, KU958290
<i>Lycoperdon arenicola</i>	UFRN-Fungos 2567	KU958285, KU958286
<i>Lycoperdon arenicola</i>	UFRN-Fungos 2581	KU958301, KU958302
<i>Lycoperdon atropurpureum</i>	MJ3269	DQ112586
<i>Lycoperdon atropurpureum</i>	MJ6150	DQ112587
<i>Lycoperdon caudatum</i>	RGC920818	DQ112633
<i>Lycoperdon cretaceum</i>	MJ4105	DQ112597
<i>Lycoperdon cretaceum</i>	MJ4302	DQ112598
<i>Lycoperdon decipiens</i>	MJ4330	DQ112582
<i>Lycoperdon decipiens</i>	MJ7715	DQ112583
<i>Lycoperdon demoulinii</i>	UFRN-Fungos 655-TYPE!	KU958307, KU958308
<i>Lycoperdon demoulinii</i>	UFRN-Fungos 2554	KU958309, KU958310
<i>Lycoperdon dermoxanthum</i>	MJ4856	DQ112579
<i>Lycoperdon echinatum</i>	MJ6498	DQ112578
<i>Lycoperdon ericaeum</i>	MJ5395	DQ112605
<i>Lycoperdon ericaeum</i>	MJ4866	DQ112606
<i>Lycoperdon excipuliforme</i>	MJ6467	DQ112590
<i>Lycoperdon exoelongatum</i>	UFRN-Fungos 2570-TYPE!	KU958355, KU958356
<i>Lycoperdon frigidum</i>	Eckblad151507	DQ112560
<i>Lycoperdon frigidum</i>	MJ7716	DQ112561
<i>Lycoperdon frigidum</i>	MJ4273	DQ112562
<i>Lycoperdon frigidum</i>	MJ4088	DQ112564
<i>Lycoperdon frigidum</i>	Lange901009	DQ112563
<i>Lycoperdon frigidum</i>	Lange191	DQ112559
<i>Lycoperdon fuligineum</i>	TENN59070	AF485065
<i>Lycoperdon fuligineum</i>	INPA 239561	KU958351, KU958352
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 606	KU958347, KU958348
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 371	KU958353, KU958354
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 1768	KU958327, KU958328
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 1971	KU958321, KU958322
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 1972	KU958323, KU958324
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2560	KU958333, KU958334
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2561	KU958335, KU958336
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2562	KU958337, KU958338
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2563	KU958345, KU958346
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2566	KU958341, KU958342
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2571	KU958329, KU958330
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2575	KU958325, KU958326
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2578	KU958331, KU958332
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2579	KU958349, KU958350
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2582	KU958339, KU958340
<i>Lycoperdon fuligineum</i>	UFRN-Fungos 2586	KU958343, KU958344
<i>Lycoperdon lambinonii</i>	Demoulin4622	DQ112575
<i>Lycoperdon lambinonii</i>	MJ5245	DQ112576
<i>Lycoperdon lividum</i>	Dobremez19740514	DQ112599
<i>Lycoperdon lividum</i>	MJ4005	DQ112600
<i>Lycoperdon mammiforme</i>	MJ4841	DQ112567
<i>Lycoperdon molle</i>	MJ4557	DQ112565
<i>Lycoperdon molle</i>	MJ4260	DQ122566
<i>Lycoperdon muscorum</i>	MJ7717	DQ112604
<i>Lycoperdon nigrescens</i>	MJ5376	DQ122577
<i>Lycoperdon niveum</i>	MJ484	DQ112568

Species	Herbarium voucher	GenBank Accession Number
		ITS/LSU
<i>Lycoperdon niveum</i>	MJ5267	DQ112569
<i>Lycoperdon niveum</i>	MJ4068	DQ112571
<i>Lycoperdon niveum</i>	MJ4109	DQ112570
<i>Lycoperdon niveum</i>	MJ5594	DQ112572
<i>Lycoperdon norvegicum</i>	MJ5453	DQ112631
<i>Lycoperdon nudum</i>	ICN 154541	KU958317, KU958318
<i>Lycoperdon nudum</i>	UFRN-Fungos 1765-TYPE	KU958319, KU958320
<i>Lycoperdon nudum</i>	UFRN-Fungos 1766	KU958315, KU958316
<i>Lycoperdon nudum</i>	UFRN-Fungos 2565	KU958311, KU958312
<i>Lycoperdon nudum</i>	UFRN-Fungos 2568	KU958313, KU958314
<i>Lycoperdon perlatum</i>	MJ4684	DQ112630
<i>Lycoperdon pratense</i>	MJ4864	DQ112554
<i>Lycoperdon pratense</i>	MJ5880	DQ112556
<i>Lycoperdon pratense</i>	SAH20000915	DQ112555
<i>Lycoperdon purpurascens</i>	MEL:2382736	KP012918
<i>Lycoperdon pyriforme</i>	MJ4849	DQ112558
<i>Lycoperdon pyriforme</i>	ICN177101	KU958367, KU958368
<i>Lycoperdon pyriforme</i>	UFRN-Fungos 155	KU958365, KU958366
<i>Lycoperdon radiculatum</i>	Parker970911	DQ112608
<i>Lycoperdon rupicola</i>	MJ4304	DQ112580
<i>Lycoperdon rupicola</i>	Vetter407	DQ112581
<i>Lycoperdon sosinii</i>	VLA 15520	KC591769
<i>Lycoperdon subincarnatum</i>	TNS Kasuya B286	KF551244
<i>Morganella subincarnata</i>	REG106/81	AJ237626
<i>Morganella subincarnata</i>	—	KM373265
<i>Lycoperdon</i> sp. 4	MJ4285	DQ112573
<i>Lycoperdon</i> sp. 4	MJ4277	DQ112574
<i>Lycoperdon subumbrinum</i>	MJ6394	DQ112601
<i>Lycoperdon subumbrinum</i>	MJ6377	DQ112602
<i>Lycoperdon turneri</i>	MJ5251	DQ112594
<i>Lycoperdon turneri</i>	Lange0895	DQ112596
<i>Lycoperdon umbrinum</i>	MJ4556	DQ112591
<i>Lycoperdon umbrinum</i>	MJ4556a	DQ112593
<i>Lycoperdon umbrinum</i>	MJ4559	DQ112592
<i>Lycoperdon utriforme</i>	MJ5388	DQ112607
<i>Lycoperdon</i> sp. 02	UFRN-Fungos 814	KU958375, KU958376
Outgroup		
<i>Bovista aestivalis</i>	MJ1122	DQ112620
<i>Bovista cretacea</i>	MJ4301	DQ112610
<i>Bovista cretacea</i>	ANMH11622	DQ112611
<i>Bovista furfuraceae</i>	MJ5435	DQ112622
<i>Bovista graveolens</i>	Widegren030816	DQ112618
<i>Bovista limosa</i>	AMJ5226	DQ112615
<i>Bovista limosa</i>	BMJ3971	DQ112614
<i>Bovista nigrescens</i>	S&J980905	DQ112612
<i>Bovista paludosa</i>	MJ5207	DQ112609
<i>Bovista plumbea</i>	MJ4856	DQ112613
<i>Bovista promotorii</i>	MJ7770	DQ112621
<i>Bovista tomentosa</i>	MJ5433	DQ112616
<i>Bovista tomentosa</i>	Steike951015	DQ112617
<i>Calvatia candida</i>	MJ3514	DQ112624
<i>Calvatia craniformis</i>	Steinke001017	DQ112625
<i>Calvatia gigantea</i>	MJ3566	DQ112623
<i>Disciseda bovista</i>	MJ5078	DQ112627
<i>Disciseda candida</i>	MJ3588	DQ112626
<i>Mycenastrum corium</i>	MJ5467	DQ112628
<i>Tulostoma kotlabae</i>	Mrazek1300	DQ112629
<i>Tulostoma squamosum</i>	MJ5467	DQ415732