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Phylogenetic analysis of the genus *Modicella* reveals an independent evolutionary origin of sporocarp-forming fungi in the Mortierellales

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ABSTRACT

Most studies of tissue differentiation and development have focused on animals and plants but many fungi form multi-cellular aggregations of spore-bearing tissue known as fruiting bodies or sporocarps. The ability to form sporocarps has arisen independently in several different evolutionary lineages of fungi. Evolutionary relationships of most sporocarp-forming fungi are well known, but the enigmatic zygomycete genus *Modicella* contains two species of sporocarp-forming fungi for which the phylogenetic affinities have not been explored based on molecular data. Species of *Modicella* have an uncertain trophic mode and have alternatively been considered members of the order Endogonales (which contains documented species of sporocarp-forming fungi) or the order Mortierellales (which contains no previously documented species of sporocarp-forming fungi). In this study we perform phylogenetic analyses based on ribosomal DNA of *Modicella malleola* from the Northern Hemisphere and *Modicella reniformis* from the Southern Hemisphere to determine the evolutionary affinities of the genus *Modicella*. Our analyses indicate that *Modicella* is a monophyletic genus of sporocarp-forming fungi nested within the Mortierellales, a group of microfungi with no previously documented sporocarp-forming species. Because *Modicella* is distantly related to all other known sporocarp-forming fungi, we infer that this lineage has independently evolved the ability form sporocarps. We conclude that the genus *Modicella* should be a high priority for comparative genomics studies to further elucidate the process of sporocarp formation in fungi.

1. Introduction

Most studies of tissue differentiation and development have focused on animals and plants but many species of fungi form complex, multi-cellular, spore-forming bodies called fruiting bodies or sporocarps. A sporocarp is an aggregation of fungal tissues where spores are produced prior to reproduction and/or dissemination. Sporocarps can take a wide variety in size and shapes ranging from coral fungi to cup fungi to epigeous mushrooms to hypogeous truffles to smooth resupinate crusts to indeterminate masses of spore-bearing tissues (Gerdemann and Trappe, 1974; Hansen and Pfister, 2006; Hibbett, 2007). Sporocarp formation in fungi is a complex developmental process that involves tissue aggregation and differentiation (e.g., Kamada et al., 2010; Lord and Read, 2011; Wang et al., 2012). Studies on the phylogenetic distribution of sporocarp-forming fungi across the major lineages indicate that most sporocarp-forming species are members of the Dikarya (a monophyletic clade comprised of the Ascomycota and Basidiomycota) and that the ability to form sporocarps has arisen multiple times within this group (James et al., 2006; Sugiyama et al., 2006; Taylor and Ellison, 2010). Sporocarp formation has also been documented in several other non-Dikarya fungi, including species of Endogonales in the Mortierellomycotina (e.g. *Endogone, Sclerogone,* and Youngiomyces) and Glomerales in the Glomeromycota (e.g. *Gigaglomus)* (Bidartondo et al., 2011; Gerdemann and Trappe, 1974; Redecker et al., 2013). *Geosiphon* (Glomeromycota), which is not closely related to other sporocarp-forming fungi in Glomeromycota, also produces multicellular aggregations of tissue during its symbiotic growth with cyanobacteria. However, these fungal tissues are usually not considered sporocarps (Gehrig et al., 1996). Most Dikarya species produce sexual spores inside their sporocarps but in many non-Dikarya fungi it is unclear as to whether or not their spores are the products of sexual recombination. Nonetheless, several non-Dikarya fungi form multi-cellular aggregations of spores that are encased within a protective layer of hyphae and are considered sporocarps. Although evolutionary relationships of most sporocarp-forming fungi are well documented, a few sporo-

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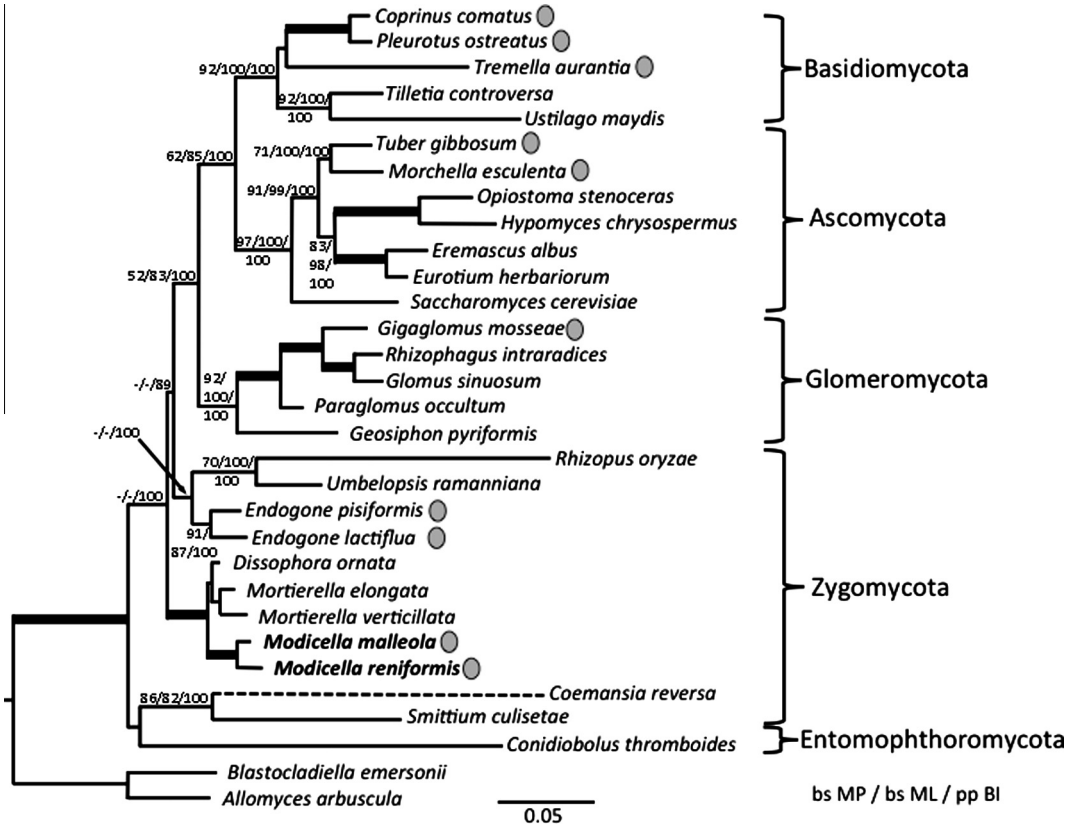

Fig. 1. Maximum likelihood phylogeny (−lnL = 20431.972378) based on ribosomal DNA (18S, 5.8S, and 28S) depicts the position of *Modicella* species among major clades of terrestrial fungi, including representatives of Ascomycota, Basidiomycota, Glomeromycota, and Zygomycota *sensu lato*. *Allomyces* and *Blastocladiella* are aquatic, flagellated fungi that served as outgroups. Fungi that form sporocarps are indicated by filled circles. Statistical support is indicated above nodes as follows: maximum parsimony boostrap/maximum likelihood bootstrap/Bayesian posterior probability. Thickened branches indicate nodes that received maximum statistical support with all three phylogenetic methods.

carp-forming fungi that belong to early diverging fungal lineages have not been recently studied and their phylogenetic affiliations are unclear. Among the non-Dikarya fungi, the evolutionary relationships of two prominent genera remain unknown: *Modicella* and *Densospora* (McGee, 1996; Wagner et al., 2013).

The genus *Modicella* Kanouse is comprised of two recognized species that form truffle-like sporocarps usually collected on soil or within decomposing plant material (Gerdemann and Trappe, 1974; Kanouse, 1936). *Modicella* sporocarps are composed of hyaline, smooth- and thin-walled sporangia that contain hyaline, thin-walled sporangiospores encased in extensive aggregations of fungal hyphae. The genus *Modicella* has a long taxonomic history. Kanouse (1936) proposed the genus *Modicella* but she did not transfer any species to the genus. Gerdemann and Trappe (1974) later transferred two species to *Modicella* from their original placement in the genus *Endogone* (*Modicella malleola* (Harkn.) Gerd. & Trappe and *Modicella reniformis* (Bres.) Gerd. & Trappe) (Bresadola, 1896; Harkness, 1899). *Modicella* was included in the Endogonales by Gerdemann and Trappe (1974) following Thaxter (1922) but Walker (1923), Trappe and Shenck (1982), Benny et al. (1987), and Wagner et al. (2013) have accepted *Modicella* as a member of the Mortierellales with an unresolved taxonomic placement. The hypothesis that *Modicella* belongs to the Mortierellales is based on the formation of an acolumellate sporangium and a garlic-like odor similar to that produced by some species of *Mortierella* (Benny et al. 1987; Gams et al., 1972). However, there are no documented cases of sporocarp formation within the order *Mortierellales* and the phylogenetic affinities of the genus *Modicella* have not yet been studied based on molecular data.

The two *Modicella* species are morphologically variable but can be separated from one another based on biogeography, average size of the sporangiospores, and the number of spores per sporangium. The type species, *M. malleola*, was described by Harkness (1899) but was first illustrated by Thaxter as *Endogone malleola* (Thaxter, 1922 – figures 72–78). This species typically has a large but variable number of sporangiospores per sporangium and the sporangiospores are smaller on average than those found in *M. reniformis* (Gerdemann and Trappe, 1974; Thaxter, 1922). *M. malleola* has been collected primarily in Europe, North America, and Taiwan (Wu and Chen, 1986) but one anomalous collection from New Zealand may represent either a disjunct distribution or an accidental introduction of *M. malleola* into the Southern Hemisphere.

The second species, *M. reniformis*, was originally described by Bresadola (1896) based on Brazilian specimens collected by Möller (Thaxter, 1922). This species was first illustrated by Thaxter (1922) as *Endogone reniformis* (Thaxter, 1922 – figures 60–71). *M. reniformis* has since been collected in a wide range of habitats from tropical rainforest to cool, temperate forests in Brazil, Chile, and Argentina. *M. reniformis* has an average of four sporangiospores per sporangium and rarely has more than twelve sporangiospores per sporangium. We also observed that spores of *M. reniformis* are surrounded by a clear zone of apparently gelatinous material that was not observed around the spores of *M. malleola* (Fig. 3).

The trophic mode of the two *Modicella* species has remained controversial. Species of *Endogone* and *Gigaglomus* form sporocarps that are superficially similar to those produced by species of *Modicella*. The genera *Endogone* and *Gigaglomus* contain fungi with a wide variety of trophic modes, including ectomycorrhizal (Walker,

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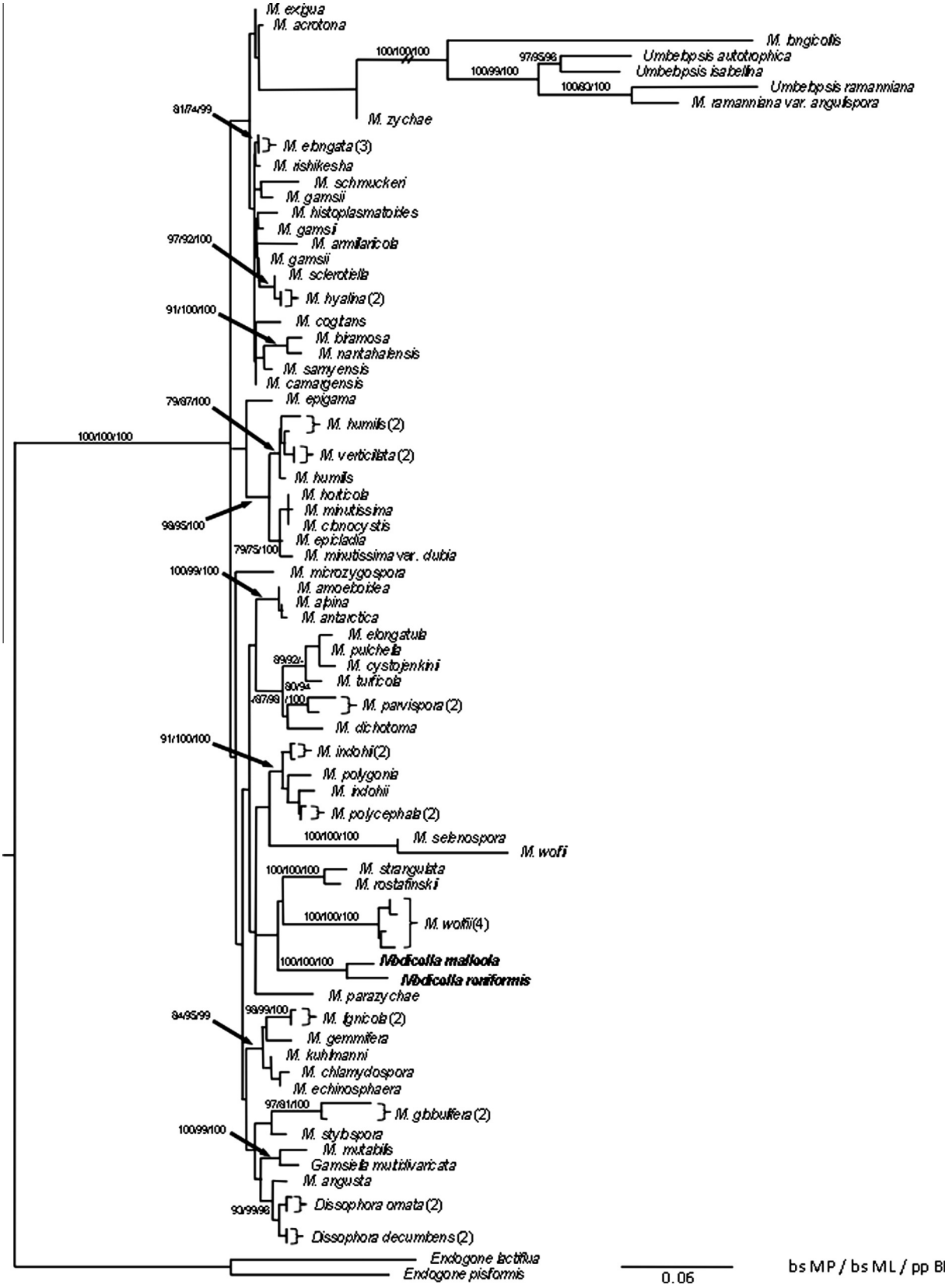

Fig. 2. Maximum likelihood phylogram (−lnL = 20431.972378) based on ribosomal DNA (18S, 5.8S, and 28S) depicting the phylogenetic position of *Modicella* species within the Mortierellales. *Umbelopsis* spp. and *Endogone* spp. served as outgroup taxa. Statistical support is indicated above nodes as follows: maximum parsimony boostrap/ maximum likelihood bootstrap/Bayesian posterior probability.

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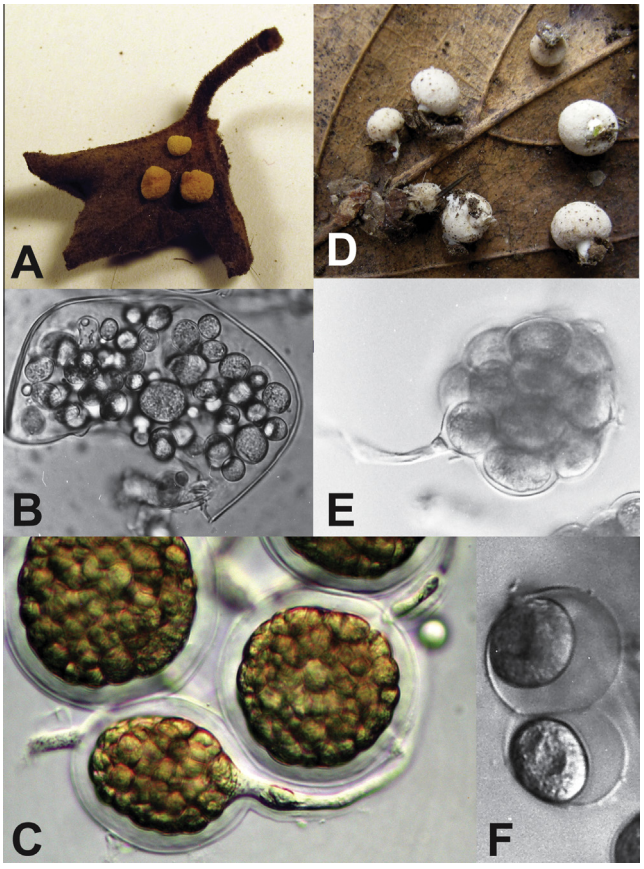

Fig. 3. Morphology of *Modicella* species. (A) Dried yellowish sporocarps of *Modicella malleola* specimen FLAS-F-56295 on leaf debris. (B) Ruptured sporangium from *M. malleola* specimen FLAS-F-56295 reveals a large number of sporangiospores that are variable in size and maturity. (C) Intact sporangia from *M. malleola* specimen FLAS-F-56295; each sporangium is attached to a single subtending hypha. (D) Fresh white sporocarps of *Modicella reniformis* collection EN253 from Argentina. (E) Intact sporangium of *M. reniformis* collection FH5185 from Brazil; note that *M. reniformis* has fewer large spores per sporangium as compared with *M. malleola*. (F) Sporangiospores from *M. reniformis* collection FH5185 show accumulation of an apparently gelatinous material within the sporangiospores of *M. reniformis*.

1985), arbuscular mycorrhizal (Redecker et al., 2000) and endophytic with non-vascular plants (Bidartondo et al., 2011). Some authors have considered *Modicella* species as putative mycorrhizal symbionts (e.g. Manoharachary et al., 2002; Nemec et al., 1981; Stuessy, 1992; Wu and Chen, 1986) but that trophic mode seems unlikely since fresh specimens were readily cultured under axenic conditions (Walker 1923). Walker (1923) collected fresh specimens of *M. malleola* in Nebraska and was able to germinate spores and obtain pure cultures on a variety of artificial media types. Similarly, collection notes by J.M. Trappe (personal communication) record the substrate of *M. malleola* to range from soil, on twigs and leaves, on stones among mosses, and most tellingly, on charcoal brickettes and ashes in a steel barbeque stand raised 1.5 m above the ground on a steel post. The observations of Walker (1923) and Trappe (personal communication) indicate that *M. malleola* has saprotrophic capability, although this does not preclude some kind of symbiotic association with plants. Recent studies have identified some species of Mortierellales as endophytes in healthy plants (e.g. Narisawa et al., 1998; Summerbell, 2005), indicating that some species of Mortierellales may play dual trophic roles as both plant symbionts and saprobes. DNA sequence data from *Modicella* species followed by comparisons with environmental sequences from GenBank may help to clarify the trophic role(s) of these fungi.

Due to the rarity of *Modicella* species, the phylogenetic affiliations of these fungi have not been documented. The purpose of this study was to use herbarium specimens and freshly collected *Modicella* sporocarps to explore the evolutionary relationships and putative ecology of this poorly studied fungal genus. Specifically, we asked the following questions: (1) What are the phylogenetic affinities of the two known *Modicella* species? (2) Do the two *Modicella* species form a monophyletic group? (3) Does the genus *Modicella* represent an independent evolutionary origin of sporocarp formation in the Mortierellales? and (4) Are species of *Modicella* saprobes or plant mutualists?

2. Materials and methods

2.1. Morphological analyses

Modicella species were obtained as dried herbarium specimens consisting of sporocarps or parts of sporocarps, except for collection Trappe 12428 which was obtained fresh in the field and sent directly by J. Trappe to G. Benny. Institutional designations are those of Index Herbariorum (http://sweetgum.nybg.org/). Sporangia and sporangiospores were photographed and measured after wetting fungal material in 95% ethanol followed by mounting in distilled water or 2% KOH. Microscope mounts were made using the procedures of Benny and Blackwell (2004) and Benny (2008).

2.2. DNA extraction and sequencing

Molecular analyses of *Modicella* species relied on one specimen of *M. malleola* collected in Spain during 2005 (BM-350) and one specimen of *M. reinformis* collected in Argentina during 2011 (EN253). DNA was extracted using the CTAB extraction technique (Gardes and Bruns, 1993). The resulting DNA pellet was resuspended in 25 µl sterilized TE buffer (10 mM Tris–HCl, pH 8.0; 1 mM EDTA) at 40 °C, and DNA solutions were diluted 10-fold with sterilized distilled water for use in PCR reactions. The following primer sets were used for 18S rDNA: NS24 (Gargas and Taylor, 1992) and NSSU1088R (Kauff and Lutzoni, 2002), NS1 and NS4 (Vilgalys and Hester, 1990); 28S rDNA: LROR (Rehner and Samuels, 1994) and LR5 (Vilgalys and Hester, 1990); and ITS rDNA; ITS1f (Gardes et al., 1991) and ITS4 (White et al., 1990). All PCR reactions were performed using Apex Taq DNA Polymerase (Genesee Scientific, San Diego, CA, USA) according to the manufacturer's recommendations. Successful amplicons were electrophoresed on 1.5% agarose gels stained with SYBR Green I (Molecular Probes, Eugene, OR, USA) and then purified with Exonuclease I (EXO) and Shrimp Alkaline Phosphatase (SAP) enzymes (NEB, Ipswich, MA, USA). Purified PCR products were then sequenced using amplification primers in both directions and BigDye version 3.1 (Applied Biosystems Inc., Foster City, CA, USA) and the sequences were determined with an ABI3700 DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Raw sequence data were analyzed and edited using Sequencher v. 4.1.4 software (Gene Codes Corporation, Ann Arbor, MI, USA).

2.3. Phylogenetic analyses of molecular data

DNA sequences of the ribosomal DNA operon (including 18S, ITS1-5.8S-ITS2, and 28S) from *M. reniformis* (specimen EN253 from Argentina) and *M. malleola* (specimen BM-350 from Spain) were first subjected to BLAST searches to determine the likely phylogenetic placement. BLAST analysis suggested a phylogenetic affinity within Mortierellales so two different phylogenetic analyses were performed: (1) a broad-scale analysis to place *Modicella* species within the larger fungal phylogeny (including an array of fungi that

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produce fruiting bodies), and (2) a phylogenetically constrained phylogeny that focused on placement of *Modicella* species within the Mortierellales. Although both analyses utilized the ribosomal DNA operon, the phylogeny focused on Mortierellales was able to include more nucleotides because the alignment had fewer ambiguous positions. The ITS1 and ITS2 regions were excluded from both analyses because they were too divergent to be aligned.

The broader-scale phylogenetic analysis used 18S, 5.8s, and 28S rDNA sequences from 31 taxa representing all major fungal lineages, including 29 terrestrial taxa and two flagellated fungi that served as the outgroups (*Allomyces* and *Blastocladiella*). Sequences were aligned individually for each locus using the MUSCLE software package (Edgar, 2004) and the alignments were inspected and adjusted manually with ambiguous regions excluded using Mesquite 2.73 (Maddison and Maddison, 2010). Preliminary phylogenetic analyses suggested no strong incongruence among the different rDNA regions so all three genes were concatenated to produce an alignment of 2805 nucleotides (1638 bp of 18S, 157 characters of 5.8s, 1010 characters of 28S). Phylogenetic trees were estimated using three methods; maximum parsimony (MP) maximum likelihood (ML) and Bayesian inference (BI) using PAUP* 4.0 (Swofford, 2002) for MP, Garli-1.0 (Zwickl, 2006) for ML and MrBayes 3.1.2 for BI (Huelsenbeck and Ronquist, 2001) on the CIPRES Science Gateway V. 3.1 (www.phylo.org). Analyses were conducted applying the GTR+Γ+I substitution model and rate heterogeneity with unlinked parameters for each partition. One thousand ML bootstrap replicates were computed for MP and ML. For Bayesian phylogenetic estimations, parallel runs of four chains were computed to 20 million generations, with sampling every 1000 generations. Trees were sampled when an equivalent posterior probability plateau was achieved between runs. Statistical support was recognized as significant with bootstrap values $\geqslant$70% for ML and MP, and $\geqslant$95 posterior probability for BI.

The phylogenetically constrained analysis of *Modicella* within Mortierellales incorporated data for 83 isolates representing 63 taxa, with two species of *Endogone* serving as the outgroup taxa. The overall analysis was similar to that described above. The concatenated dataset consisted of 2698 nucleotides (996 bp of 18S, 339 characters of 5.8s, 1361 characters of 28S). The majority of the sequences in both analyses were obtained directly from GenBank except for sequences of *Modicella* species. All sequences are deposited in GenBank (Supplemental Table S1) with the nucleotide alignments deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S14261).

3. Taxonomy

Modicella Kanouse, Mycologia 28: 60. 1936.

Sporocarps hemispherical above and flattened or flattened with a cavity below, white when fresh, some shade of yellow to orange when dry, filled with sporangia. Sporangia subtended by a single hypha, multispored, smooth, and thin-walled, acollumellate, some shade of yellow. Sporangiospores smooth, thin-walled, globose to ovoid to irregular, and light yellow. Subtending hyphae with a septum immediately below the sporangium. Fresh sporocarps emitting an onion- or garlic-like odor. Zygospores not observed.

Type species: *Endogone malleola* Harkness

Modicella malleola (Harkness) Gerdemann & Trappe, Mycologia Mem. 5: 67. 1974. = *Endogone malleola* Harkness, Proc. Calif. Acad. Sci, Ser. 3, 1: 280. 1899.

Sporocarps 1–5 mm in diameter, yellow or buff to orange in color when dry *in herbario*, reported as white when fresh, hemispherical above, base flattened or flat with a cavity; sporangia visible on outer surface, hyphae lighter in color. Sporangia 30–127 μm long × 25–95 μm in diameter, globose to ovoid, ellipsoid, or irregular in shape, hyaline when fresh but becoming yellow to light-yellow after storage, thin-walled (ca. 1.5 to 2.5 μm thick at maturity; filled with sporangiospores; acolumellate. Hypha at base of sporangium absent or up to 600 μm long × 4–11.5 μm in diameter, thin- and smooth-walled. Sporangiospores 6.5–23 × 4–22 μm, round, ellipsoid, to ovoid or irregular, light yellow, contents granular to vacuolate. Zygospores not observed. Subtending hyphae with a septum immediately below the sporangium.

Holotype. U.S.A. CALIFORNIA. Marin County, Mt. Tamalpais, on the surface of the ground in dense shade under *Sequoia sempervirens* (D. Don) Endlicher, collected by H.W. Harkness No. 103, December, sometime before 1899 (BPI).

Specimens examined. CANADA. MANITOBA. Riding Mountain National Park, Jackfish Creek at Lake Andy, on silty organic debris of *Carex* in a marshy area dominated by *Carex-Salix*, collected by S.A. Redhead No. 2990, 22 August 1979, det. by R. Fogel (MICH). ITALY(?) Collecting location unknown, on ground among leaf litter in winter; in the Naples herbarium, collected by Cesati. Rabenhorst's Fungi Europaei 2516 (as *Endogone microcarpa* Tul.; BPI, K, NY). NEW ZEALAND. Collecting location and habitat unknown, James Mitchell No. 11 (Thaxter 5215), 20 October 1921 (FH). SPAIN. Cordoba, El Cañuelo, sporocarps along a riverbank within partially decomposed leaf litter of *Ulmus minor*, collected by B. Moreno-Arroyo, BM-350, 17 December 2005 (OSC, FLAS) (GenBank: KF053133, KF053135, KF053131). U.S.A. CALIFORNIA. Los Angeles County, Claremont, Rancho Santa Ana Botanic Garden, sporocarps produced on leafy debris under *Fremontodendron californicum* Cor., ca. 50 m NE of the main building, collected by R.K. Benjamin, 19 Dec. 1965 (FLAS); same collection data except 16 Nov. 1976 (FLAS-F-56295); Marin County, Mill Valley, collected by N.L. Gardner No. 169, 23 Nov. 1904, (UC 1273111). NEBRASKA. Lancaster County, Lincoln, abundant in the woods near Lincoln on soil and leaf litter, collected by Leva B. Walker, 18 Sept. 1921 (BPI); same collection data except 6 October 1921, Missouri Botanical Garden Herbarium No. 58675 (BPI); same collection data except Sept. 1921, Thaxter No. 5214 (FH). OREGON. Josephine County, Wolf Creek Park, on charcoal or on the apothecia of a phoenicoid *Peziza* fruiting on charcoal located in a steel barbeque stand raised 1.5 meters off of the ground, collected by J.M. Trappe, 2 Apr. 1992, Trappe 12428 (OSC); Benton County, NE foot of Forest Peak, under Douglas fir and moss, collected by J.M. Trappe No. 2649 (OSC 34790); at Soap Creek, in humus or on soil under Douglas fir, collected by J.M. Trappe, 19 Apr. 1971 (OSC 34820); Paul Dunn State Forest, on road west from Tampico Road following the north boundary, in moss with *Corylus cornuta*, *Rhus diversiloba*, and *Pseudotsuga menziesii*, collected by D. Louma (OSC 44714); Coos County, Coquille Falls Natural Area, on twig in humus, collected by J.M. Trappe, 26 May 1971 (OSC 30943 and OSC 34459); Curry County, Winchuck Forest Camp, on soil among roots of Douglas fir and *Umbellularia californica*, collected by J.M. Trappe, 25 May 1971 (OSC 31016); Linn County, 2 miles west of Roaring River Fish Hatchery, on soil and oak leaves, collected by J.M. Trappe, 7 Apr. 1971 (OSC 34781); Polk County, 3 miles southeast of Airlie, under Douglas fir, moss, and sparse herbaceous cover, collected by J.M. Trappe (OSC 34783). WASHINGTON. King County, Seattle, Denny Park, epigeous on moss, collected by D. Hosford No. 271, 4 May 1968, (OSC 29299).

Modicella reniformis (Bresadola) Gerdemann & Trappe, Mycologia Mem. 5: 68. 1974.

=*Endogone reniformis* Bres., Hedwigia 35: 297. 1896

=*Endogone argentina* Speg., An. Mus. Nac. Buenos Aires 6: 300. 1899.

Sporocarps (1.5–)3–6(–9) mm in diameter, hemispherical, white when fresh, yellow to orange upon drying, containing many sporangia. Sporangia globose, ovoid, reniform, triangular, to irregular, 32–70 μm long × 24–64 μm in diameter, hyaline when fresh

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but becoming yellow after storage, thin-walled, containing 3–8 (usually 4) sporangiospores; subtended by a single hypha. Subtending hypha may be absent or relatively short, 2.5 μm long × 4 μm in diameter, Sporangiospores 17–37 μm long × 15–25 μm in diameter, globose or ellipsoid to irregular in shape, thin-walled, light yellow, with granular contents.

Holotype. BRAZIL. SANTA CATARINA. Blumenau, in leaves, collected by A. Moiler No. 45d, 1891 or 1892 (Thaxter 518), exact collection date unknown but part of type sent by Lindau to Thaxter in December 1921 (FH).

Specimens examined. ARGENTINA. BUENOS AIRES. Santa Catalina, near Buenos Aires, in decaying leaves in the forest, collected by C. Spegazzini, 3 Nov. 1890 (Holotype of *E. argentina* Speg.,Thaxter 5184) (FH, K 27906). CATAMARCA. Cuesta del Clavillo, buried within the soil and leaf litter in *Alnus acuminata* forest, collected by E. Nouhra, 28 May 2011, 1876 meters elevation (27° 20.786′ S; 65° 58.300′ W) (CORD, EN253) (GenBank: KF053134, KF053136, KF053132). BRAZIL. Bono Principia, Municipio, Montenegro, 1928, sent by J. Rick to Thaxter at FH, habitat data and exact collection data unknown (FH); San Salvador, in humus, collected by J. Rick, 6 June 1945 (PACA 22736); in leaves, collected by J. Rick, 3 April 1943 (PACA 23153). RIO GRANDE DO SUL. Arroio do Meio, collected by J. Rick, 1920, in the Herbarium of J.B. Weir No. 16708 (BPI); Bono Principia, collected by J. Rick, Oct. 1928; Nova Petropolis, collected by J. Rick No. 46, 1923, no other collection data (BPI, FH); Santa Cruz, det. J. Rick, 1927, no other collection data (FH); Sao Leopolda, in leaves, collected by J. Rick, no date or collection data, identified as *E. reniformis* by G. Bresadola, G. Bresadola No. 75 (BPI), det. J. Rick, 1929 (FH), in leaves, collected by Braun, 1929, det J., Rick (PACA 12956, 12957); same collection data, No. 215 (BPI); J. Rick, 1929, no other collection data (FH). SANTA CATARINA. Sao Canisio do Porto Novo, along the Uruguay River, collected by J. Rick (Thaxter 5032, 5185 [Rick 758], 5186) 1928, no other collection data (FH). CHILE. MAGELLANES. Punta Arenas, under leaf cover beside "aquaduct" brook in woods, collected by R. Thaxter 7316 (Fungus Hypogeous 15), identified by Thaxter as *E. argentina* Speg. 4 Mar. 1906, (FH).

4. Results

The broad-scale phylogenetic analyses of *M. malleola* and *M. reniformis* within the context of terrestrial fungi from GenBank placed the genus *Modicella* in a well-supported lineage among Mortierellales (*Mortierellla* spp. and *Dissophora ornata*) (Fig. 1). Phylograms generated by MP (best tree score = 3168 steps), ML ($-\ln L$ = 20431.972378) and BI (estimated arithmetic mean of marginal likelihood value for two runs = −20457.44) methods were congruent and all but the most early-diverging fungal lineages were strongly supported by high bootstrap and posterior probability values. Consistent with previous studies, the Dikarya and the Dikarya + Glomeromycota clade were supported as monophyletic lineages whereas Zygomycota sensu lato was not supported as a monophyletic group. The sporocarp-forming *Modicella* species were only distantly related to other sporocarp-forming fungi in several other major lineages, including species of *Coprinus, Pleurotus, Tremella* (Basidiomycota), *Morchella* (Ascomycota), *Gigaglomus* (Glomeromycota) and *Endogone* (Zygomycota). *Modicella* species are estimated to be among the most early-diverging fungi for which a sporocarp has been documented.

The phylogenetic analyses of the genus *Modicella* within the Mortierellales using MP (best tree score = 2912 steps), ML ($-\ln L$ = 20431.972378) and BI (estimated arithmetic mean of marginal likelihood for two runs = −18988.80) methods recovered phylograms with similar topologies. As with previous studies that have used ribosomal genes to resolve the phylogeny of the Mortierellales, we obtained weak statistical support for all of the basal nodes. However, we documented many of the same major lineages as previous studies (e.g. Wagner et al., 2013). The two *Modicella* species form a well-supported monophyletic group nested within the Mortierellales. *Modicella* species group with *Mortierella wolfii*, *Mortierella parazychae*, *Mortierella strangulata* and *Mortierella rostafinskii* in *Mortierella* clade 5 sensu Wagner et al. (2013) (Fig. 2). The ITS rDNA sequences of *M. malleola* and *M. reniformis* group closely with two environmental sequences obtained from a Texas prairie soil (Supplemental Fig. 1). Sequence EU490028 was generated from soil sampled beneath a C4 grass whereas EU490165 came from soil beneath mesquite (*Prosopis glandulosa*) (Hollister et al., 2010).

5. Discussion

This is the first phylogenetic analysis to include *M. malleola* and *M. reniformis,* the only two described species in the genus *Modicella*. Although previous authors have debated whether species of *Modicella* belong to Endogonales or Mortierellales (e.g. Gerdemann and Trappe, 1974; Trappe and Shenck, 1982) this study convincingly shows that *Modicella* species are unique, sporocarp-forming members of the Mortierellales and are not closely related to other species of sporocarp-forming fungi. Although multi-cellular sporocarps have independently evolved in Ascomycota, Basidiomycota, Glomeromycota and other Zygomycota (e.g. *Endogone, Sclerogone,* and *Youngiomyces* in Endogonales), the genus *Modicella* appears to represent an additional independent evolutionary event leading to sporocarp formation (Fig. 1). The discovery that *Modicella* is nested within the Mortierellales indicates that *Modicella* species may be the earliest diverging fungal group to produce sporocarps.

Trappe and Shenck (1982) and Benny et al. (1987) considered the genus *Modicella* to belong in Mortierellales because *Modicella* species have an acolumellate sporangium, hyphal septae that are similar to those of *Mortierella* spp., and a garlic-like odor like that found in some species of *Mortierella* (Gams et al., 1972). However, garlic-like odors are found in many terrestrial truffle-like fungi (Trappe et al., 2009) and the phylogenetic signal of sporangial morphology can be misleading (Benny and Blackwell, 2004; O'Donnell et al., 2001), so the taxonomic position of *Modicella* has remained unsubstantiated. However, the phylogenetic analyses of *Modicella* within the context of diverse terrestrial fungi (Fig. 1) and within the Mortierellales (Fig. 2) indicate that the genus *Modicella* is monophyletic and nested within the Mortierellales. Our analyses indicate that *Modicella* species are allied with the *M. wolfii* and the *M. strangulata* groups (Fig. 2) and may belong to *Mortierella* clade 5 of Wagner et al. (2013). However, an earlier analysis by (Petkovits et al. (2011) did not resolve the *M. wolfii* and the *M. strangulata* groups as close relatives, so more analyses based on additional genes will be necessary for better phylogenetic resolution. In addition to the phylogenetic data presented here, analysis of the septal ultrastructure in *M. malleola* sporocarps further corroborates an evolutionary link with the Mortierellales (G. Benny, unpublished data).

The *Modicella* specimens we examined in this study generally fit the descriptions for the species as given by Thaxter (1922) and Gerdemann and Trappe (1974) (Fig. 3). However, we noted wide ranges in sporangia and sporangiospore sizes, even within individual sporocarps. Two ITS rDNA environmental sequences from a Texas prairie soil (EU490028, EU490165) were closely related but not identical to the sequences derived from *Modicella* sporocarps (Supplemental Fig. 1). This sequence variation, along with the wide distributions of the two described species and the high morphological diversity within and among specimens, suggests that there are

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probably more cryptic *Modicella* species that will be revealed through future DNA sequencing of fresh specimens and soil samples. We also suspect that some of the morphological variation that we observed may be due to specimen age. Examination of R.K. Benjamin's collections from California revealed that sporangia of *M. malleola* tend to become thinner as they mature. Young specimens have relatively thick sporangial and hyphal walls, but as sporangia mature the walls of spores and subtending hyphae appear to grow thinner. We suspect that the size of the sporangiospores may also change with age, but we have been unable to fully document this phenomenon.

Species of *Modicella* are only intermittently collected but they have wide distributions and have been collected in a variety of habitats during many months of the year. The wide distribution and habitat variation along with a lack of plant-associated DNA sequences in GenBank and the field observations by Walker (1923) and Trappe (personal communication) suggests that, like other members of the Mortierellales, *Modicella* species are probably soil-associated saprotrophs and not mycorrhizal. Given the inconspicuous nature of their small sporocarps that fruit directly on an array of substrates in contact with soil, we expect that members of this genus are overlooked by most biologists and could be more common than the collection records indicate. Alternatively, since *Modicella* species produce sporocarps that emit a garlic-like odor, it is possible that specimens are difficult to find in nature because they are consumed by animals. Gerdemann and Trappe (1974) noted that spores of *M. malleola* stick together in a mass, even after they have been removed from the sporangium. This morphological feature and the gelatinous coating of spores seen in *M. reniformis* might very well be evolutionary adaptations to animal mycophagy, but more work is needed to test these hypotheses.

Although the sporocarps of *Modicella* species are appear difficult to find, we expect that fresh material can probably be readily cultured on a wide variety of artificial growth media (Walker, 1923). In the future, we suggest that both sporocarp-producing *Modicella* species will be excellent candidates for genome sequencing and will provide rich material for genomic comparisons with closely related species of Mortierellales that do not produce sporocarps (Wang et al., 2011).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2013.10.001.

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