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Perichaena megaspora, a new nivicolous species of myxomycete from the Andes

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Abstract: A new nivicolous species of *Perichaena* is described from the Andes in Argentina. The most conspicuous characteristics of *Perichaena megaspora* are the large spores and their ornamentation in the form of flattened warts. The 16–21 μm diam spores make the new species unique in the genus in which all other species have spores rarely reaching 15 μm diam. Twenty-two collections were found in the field during two consecutive years at 10 localities in Mendoza province Argentina, including one collection isolated from a moist chamber culture of ground litter. The new species was examined under stereomicroscope, light microscope and scanning electron microscope and micrographs of relevant details are included.

Key words: Amoebozoa, Argentina, Eumycetozoa, Myxogastria, SEM, species distribution, taxonomy

Introduction

The myxomycete genus *Perichaena* belongs to the order Trichiales and is the second largest genus in the family Arcyriaceae. It comprises almost 30 species (Lado 2012). Species belonging to the genus form sessile or stalked sporocarps or short to long plasmodiocarps with pale capillitium covered with various types of ornamentation in the form of warts, spines or rings, but never spirals, and with pale spores usually covered with warts or spines (Martin and Alexopoulos 1969, Farr 1976, Nannenga-Bremekamp 1991, Lado and Pando 1997). A few species of *Perichaena*, such as *P. chrysosperma* (Curr.) Lister, *P. corticalis* (Batsch) Rostaf., *P. depressa* Lib. and *P. vermicularis* (Schwein.) Rostaf., are common and distributed worldwide (e.g. Martin and Alexopoulos 1969), while other representatives of the genus are rare or known only from the type locality. Seven species of *Perichaena* have been reported from Argentina. Apart from the four cosmopolitan species mentioned above (see Lado and Wrigley de Basanta 2008), three other species have been reported from the country: *P. calongei* Lado, D. Wrigley & Estrada (Lado et al. 2009), *P. quadrata* T. Macbr. (Lado et al. 2011) and *P. pedata* (Lister & G. Lister) Lister ex E. Jahn (Wrigley de Basanta et al. 2010).

In the material collected during a survey of myxomycetes in South America (the Myxotropic project, www.myxotropic.org), we found numerous collections of an undescribed species of *Perichaena* that formed strikingly large spores. Morphological characters were found to be constant for all these specimens, collected over two consecutive years and at several localities and produced in one collection in moist chamber culture. Detailed examination of all samples and comparison with all the described species from the genus revealed that the South American material represents a distinct unknown taxon that we propose here as a new species.

Materials and methods

Specimens were collected from 10 localities in 2007 and 2008 in the Andes of Argentina (Figs. 1, 2). All the collection localities were georeferenced (Garmin, eTrex Vista HCx, Datum WGS84). For microscopic examination 21 specimens collected in the field were used and one collection obtained in moist chamber culture of substrate material collected in the field. Observations and measurements of the morphological characters were made under stereoscopic microscopes Nikon SMZ 1000 and SMZ 1500. The total height and length of the sporophores were measured (n = 56). Observations and measurements of microscopic characters were made on material mounted in Hoyer's medium, under a Nikon Eclipse E-600 light microscope, with a Nomarski interference contrast. Micrographs of microscopic characters were taken from material mounted in water or in Hoyer's medium with a Nikon DS-Fi1 camera head. The material is deposited in MA-Fungi, TLXM, KRAM, and in the personal collection of Diana Wrigley de Basanta (dwb). Spore measurements of all well developed specimens of the new species (50 per collection, 1050 in total) were made under an oil-immersion objective and include ornamentation. Values present in less than 1% of the measurements are given in parentheses in the descriptions provided below. The statistics were calculated with Statistica 6 software. Scanning electron microscopy (SEM) studies of the collections were made at the Royal Botanic Garden of Madrid. Critical-point drying was used for SEM preparations, and specimens were examined with a Hitachi S-3000N scanning electron microscope, at 15 kV.

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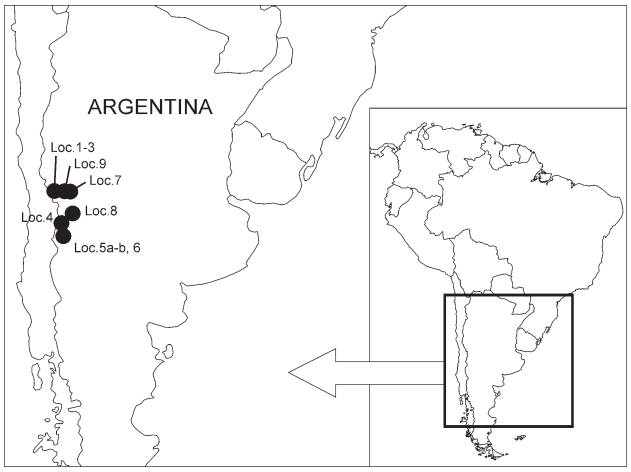


FIG. 1. Distribution of *Perichaena megaspora*. For explanation of the localities, see specimens examined.

Color notations in parentheses are from the ISCC-NBS color name charts illustrated with centroid colors (Anonymous 1976).

Moist chamber cultures of ground litter (leaves and twigs) of an unidentified plant from the Asteraceae were prepared according to Wrigley de Basanta et al. (2009). The cultures were kept at room temperature (21–23 C), the pH was recorded at 24 h and the cultures were observed 3 mo. All sporocarps in one moist chamber culture were considered to represent one collection, irrespective of the number of sporocarps produced or the time of incubation.

TAXONOMY

Perichaena megaspora A. Ronikier, Lado et D. Wrigley, sp. nov. FIGS. 3–17
MycoBank MB801832

Sporophores in groups, rarely scattered, short plasmodiocarps to sporocarps, sessile, attached to the substrate (FIG. 3). Plasmodiocarps or sporocarps subglobose to allantoid, 0.62–3.00(–6.25) mm long, 0.65–1.12 mm wide, 0.48–1.00 mm high. Sporotheca dark yellowish brown (75. deep y Br–78. d. y Br). Hypothallus membranous, yellowish brown (75. deep y Br–78. d. y Br), common for a group of sporophores. Stalk absent. Peridium double (FIGS. 4, 9), two layers closely adhered, so that they appear as one thick unit, orange-yellow (70. l. OY–71. m. OY) or olive brown (94. l. Ol Br) to colorless by transmitted light, covered with slightly darker yellow granular matter, inner surface of the inner layer distinctly warted, sometimes embossed with outlines of the spores by light microscope and SEM (FIGS. 4, 10–11), dehiscence irregular. Capillitium abundant, dense, delicate, built up of long tubules with many branches, forming a loose net-like structure, with few free ends, and some swellings, capillitial tubules strongly flattened, 3–6(–8) μm wide, variously ornamented from almost smooth to covered with warts, yellowish brown (74. s. y Br–75. deep y Br) in mass, yellow (86. l. Y–87. m. Y) by transmitted light, ends blunt (FIGS. 5–6, 10, 12–14). Spores yellowish brown (74. s. y Br) to orange-yellow (72. d. OY) in mass, yellow (87. m. Y–88. d. Y) to olive brown (94. l. Ol Br) by transmitted light, (15–)15.5–22(–25) μm in diam, globose or slightly ovoid, thick-walled and darker on one side, thin-walled (germ pore) and paler on the other side, loosely and irregularly covered with big warts by transmitted light, pilate by SEM, the pila up to 1 μm wide and less than 1 μm high, sometimes fused (FIGS. 7, 8, 15–17). Plasmodium unknown.

HOLOTYPE: ARGENTINA. MENDOZA: Malargüe, Los Molles, Las Leñas, Portezuelo Ancho, 12 km from Las Leñas on the track to Valle Hermoso, 35°05′49″S 70°08′20″W, 2805 m, 5 Mar 2007, on twigs of an Asteraceae, *C. Lado, A. Estrada & D. Wrigley de Basanta,* Lado 18626 (MA-Fungi 82108). *Isotypes* at KRAM (M-1556) and TLXM (FIG. 1; Loc. 5a, 2).

Other specimens examined: ARGENTINA. MENDOZA: Las Heras, Uspallata, Parque Provincial Aconcagua, Los Horcones, route RN-7, km 1222, 32°49′22″S 69°56′18″W, 2906 m, 23 Feb 2007, (FIG. 1; Loc. 1), on a woody shrub, Lado 18501 (MA-Fungi 82109). Lujan de Cuyo, Uspallata, Parque Provincial Volcán Tupungato, Las Cuevas, old road RN-7 to Cristo Redentor, 32°48′41″S 70°04′33″W, 3479 m, 23 Feb 2007, (FIG. 1; Loc. 2), on living twigs of a woody shrub, Lado 18508 (MA-Fungi 82110). Las Heras, Uspallata, Parque Provincial Aconcagua, Las Cuevas, Quebrada del Navarro, route RN-7, km 1229, 32°49′03″S 70°00′45″W, 2991 m, 23 Feb 2007, (FIG. 1; Loc. 3), on living and dead twigs of a woody shrub, Lado 18513 (MA-Fungi 82111), Lado 18514 (MA-Fungi 82112), Lado 18516 (MA-Fungi 82113). San Rafael, El Sosneado, Minas de Azufre, route RP-220, 74 km NW from El Sosneado, by Atuel river, 34°40′50″S 70°05′35″W, 2257 m, 2 Mar 2007, (FIG. 1; Loc. 4), on leaves of an Asteraceae, Lado 18574 (MA-Fungi 82114), Lado 18575 (MA-Fungi 82115). Malargüe, Los Molles, Las Leñas, Portezuelo Ancho, 12 km W from Las Leñas on the track to Valle Hermoso, 35°05′49″S 70°08′20″W, 2805 m, 5 Mar 2007, (FIG. 1; Loc. 5a), on leaves and twigs of an Asteraceae, Lado 18631 (MA-Fungi 82116), Lado 18647 (MA-Fungi 82117, KRAM M-1557), Lado 18648 (MA-Fungi 82118), Lado 18651 (MA-Fungi 82119), Lado 18654 (MA-Fungi 82120, KRAM M-1558), Lado 18656b (MA-Fungi 82121), Lado 18661a (MA-Fungi 82122), Lado 18664 (MA-Fungi 82123). Malargüe, Los Molles, Las Leñas, Portezuelo Ancho, 12 km W from Las Leñas on the track to Valle Hermoso, 35°05′48″S 70°08′17″W, 2789 m, 25 Feb 2008, (FIG. 1; Loc. 5b), on litter of an Asteraceae in moist chamber culture, dwb 3183. Malargüe, Los Molles, Las Leñas, Vírgen de los Arrieros, 10 km W from Las Leñas on the track to Valle Hermoso, 35°05′29″S 70°07′32″W, 2715 m, 5 Mar 2007, (FIG. 1; Loc. 6), on leaves of an Asteraceae, Lado 18667 (MA-Fungi 82124). Las Heras, Uspallata, Los Penitentes, route RN-7, km 1211, 32°50′29″S 69°49′50″W, 2600 m, 11 Mar 2007, (FIG. 1; Loc.

FIGS. 2–8. Collecting site of the new *Perichaena* species: locality 5a. 3–8. *Perichaena megaspora* (Lado 18626, HOLOTYPE). 3. Sporophores. 4. Two-layered peridium with the inner surface of the inner layer distinctly warted. 5. Capillitium details. 6. Fragments of the capillitium with characteristic swellings. 7–8. Spore ornamentation observed by light microscope. Bars: 3 = 1 mm, 4–8 = 20 µm.

7), on woody remains and living twigs of a Papilionaceae, Lado 18773 (MA-Fungi 82125), Lado 18788 (MA-Fungi 82126). San Carlos, Reserva Natural Laguna del Diamante, 6 km W of Refugio General Alvarado, 34°13′29″S 69°26′20″W, 2875 m, 24 Feb 2008, (FIG. 1; Loc. 8), on twigs of *Berberis* sp., Lado 19247 (MA-Fungi 82127). Las Heras, Uspallata, Los Penitentes, route RN-7, km 1211, 32°50′30″S 69°49′54″W, 2594 m, 1 Mar 2008, (FIG. 1; Loc. 9), on woody remains of *Adesmia* sp., Lado 19570 (MA-Fungi 82128).

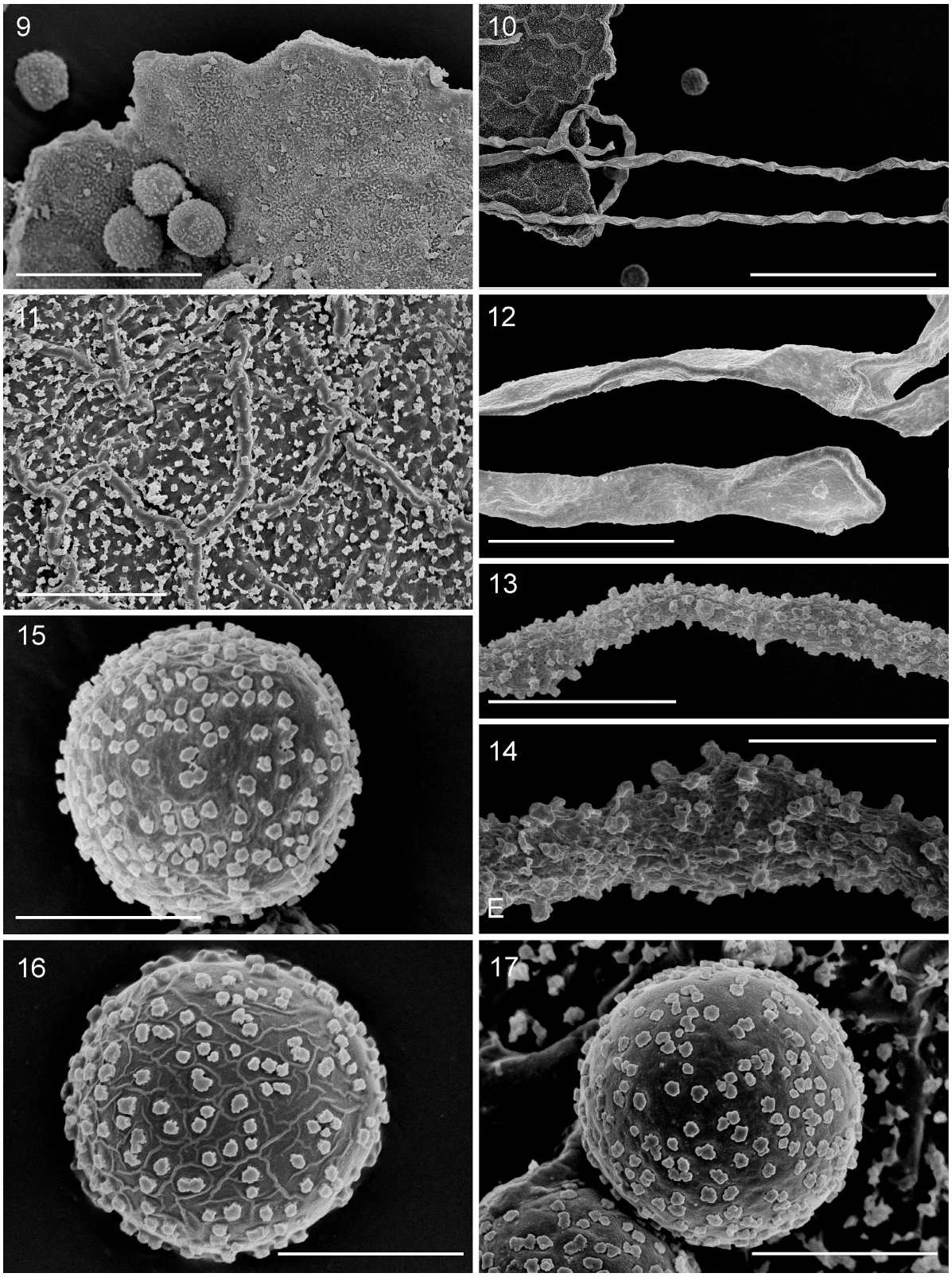
Etymology: From Latin: *mega* = large, *spora* = spore. The epithet refers to the unusually large spore.

Habitat: Dead or living branches of Andean bushes (Asteraceae, Papilionaceae, Berberidaceae).

Distribution: Known from the Andes in South America: Argentina, Mendoza province (FIG. 1).

DISCUSSION

The most conspicuous characters of *Perichaena megaspora* are its large spores and their ornamentation in the form of large warts (FIGS. 7, 8, 15–17). In



FIGS. 9–17. *Perichaena megaspora* by SEM. 9. Fragment of the two-layered peridium (Lado 18654). 10. Fragment of the peridium (inner surface) and capillitium (Lado 18626, HOLOTYPE). 11. Inner surface of the inner layer of the peridium with distinct ornamentation (Lado 18501). 12–14. Capillitium details (12: Lado 18626, HOLOTYPE; 13–14: Lado 18654). 15–17. Spores (15: Lado 18654; 16: Lado 18626, HOLOTYPE; 17: Lado 18501). Bars: 9 = 50 µm, 10 = 100 µm, 11–13 = 20 µm, 14–17 = 10 µm.

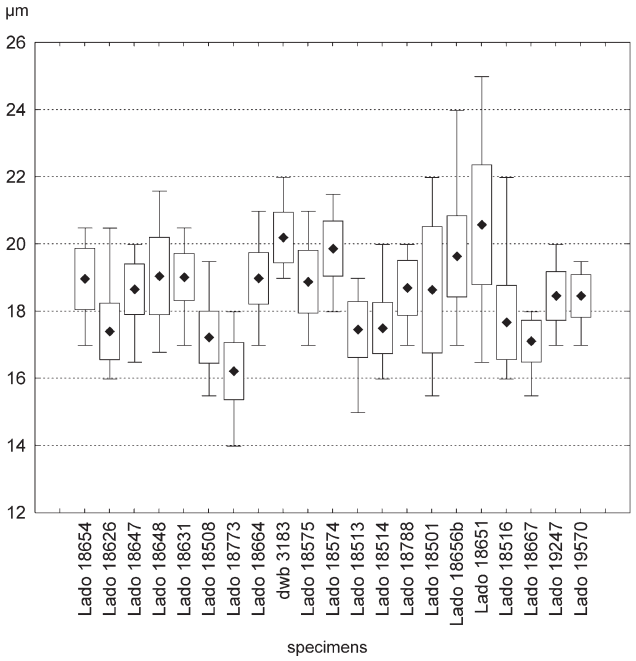


FIG. 18. Box plots for the spore size of all collections of *Perichaena megaspora*, ■ = average, box = standard deviation, vertical line = extreme values.

addition, the capillitial tubes are flattened and variously ornamented from almost smooth to densely warted (FIGS. 5, 6, 10, 12–14). Varied ornamentation of capillitial tubes has been noticed in another species from the genus, described from Argentina (Lado et al. 2009), *P. calongei*. This species however is stalked with a spiny, granular or reticulate ornamentation on the capillitium. In addition, spores of *P. calongei* are smaller, 10–13.5 µm diam, and the warts are more densely arranged.

The large spores characteristic of *P. megaspora* have not been observed in any other species of the genus. The average spore size of the specimens exceeds 16 µm (16.18–20.54 µm; FIG. 18). The most frequently measured value was 19 µm (155 measurements, nearly 15%), and more than 80% of spores were 17 µm or larger. Those 15–15.5 µm diam constituted only 1.9% of the more than 1000 spores examined; one spore was 14 µm. The significant spore variation observed in the new species, (15–)15.5–22(–25) µm, is infrequent in myxomycetes unless a species complex is recognized, such as the nivicolous complex species *Lamproderma atrosporum* Meyl. (Neubert et al. 2000), with spores (12–)13–18(–20) µm diam. However, members of this complex display significant morphological variability as well as variability in spore ornamentation, and therefore several species, varieties and forms have been proposed for *Lamproderma atrosporum* in the newly described genus *Meriderma* (Poulain et al. 2011). In contrast, all the collections of the new *Perichaena* display coherent and uniform morphological characters. Two other nivicolous species that have uniform morphology and significant spore variation are *Physarum andinum,* also described from the Andes, which has spores (7–)9–12.5(–14.5) µm (Ronikier and Lado 2013) and *Trichia alpina* (R.E. Fr.) Meyl., with spores (13–)14–21(–23) µm (Poulain et al. 2011).

There are four *Perichaena* species whose spores reach or slightly exceed 15 µm: *P. brevifila* T.E. Brooks & H.W. Keller, *P. frustifilaris* Q. Wang, Yu Li & J.K. Bai, *P. pulcherrima* Petch and *P. vermicularis*. The first differs from *P. megaspora* in having a circumscissile mode of dehiscence, a darker, black to dark brown peridium and minutely warted spores (Keller and Brooks 1971). According to the original description (Wang et al. 2000), *Perichaena frustifilaris* forms smaller sporophores, 0.2–0.5 mm diam, that are in the form of globose or subglobose sporocarps and it has different capillitium, with ornamentation forming an irregular reticulum, and coarsely spiny spores. *Perichaena pulcherrima* forms stalked sporocarps (stalk up to 3 mm high) and is reddish purple so it can be readily distinguished from *P. megaspora* macroscopically. It also has differently ornamented spores that are covered by spines connected in a reticulum by SEM (Petch 1909, Poulain et al. 2011, Wrigley de Basanta et al. 2013). Finally, *Perichaena vermicularis* differs from the new species in color and shape of sporophores (pale yellow to ochraceous, vermiform to reticulate plasmodiocarps) and has minutely ornamented spores.

Macroscopically the new *Perichaena* is somewhat similar to *P. chrysosperma*, but the latter usually forms longer or circular plasmodiocarps. It also differs microscopically by having a densely spiny capillitium. Other species from the genus, apart from their much smaller spores, either have a different-colored peridium, form much smaller sporophores, have circumscissile dehiscence or are stipitate, so they are unlikely to be mistaken for the new species.

From other genera, some sessile species of *Trichia*, such as *T. alpina* or *T. contorta*, resemble *P. megaspora* macroscopically, but they have capillitial tubules of uniform diameter, ornamented with true spiral bands. *Trichia alpina* was found together with *P. megaspora* (e.g. coll. Lado 18656b) and when it produces brown sporophores it could be confused with the new species until microscopic characters are checked.

Mature sporocarps of *Perichaena megaspora* took 7–34 d to appear in moist chamber culture. Plant litter used for the culture at 24 h was pH 6.7. The collection was produced on one of three replicates of the same substrate. It is not known whether the sporophores

developed from sclerotia in the substrate or from spores or microcysts. Because the first sporophores appeared in a few days after wetting the substrate and the last ones more than a month later, it is possible that latent sclerotia formed the first and microcysts or spores the later ones. Whichever it was, because the substrate material was collected 16 mo before it was put into culture, the stage in the life cycle shows a certain longevity.

Perichaena megaspora was collected during the austral summer (Feb–Mar), but always at higher elevations (2257–3479 m) where the season is delayed compared to lowland areas. Also the material for moist chamber culture was obtained above 2700 m. High elevation alone is not sufficient to recognize a species as nivicolous, in that snowbank myxomycetes are not alpine, occurring more frequently in the montane belt (Ronikier and Ronikier 2009). The presence of melting snow cover is generally a good criterion for this ecological group, although the sporophores may persist in the field for some time after snowmelt. Several localities of the new species, including the type locality, were in the vicinity of melting snow, so we consider the newly described species to be nivicolous. Other localities where the species was collected represented similar habitats, although no melting snow was noticed in the vicinity at the time of collection. At most localities *P. megaspora* was found together with other nivicolous species, such as *Trichia alpina* (in one case on the same piece of substrate) or some nivicolous species from the genera *Lamproderma* and *Meriderma*. Sporophores of *P. megaspora* in most cases were well developed and seemed to be relatively fresh.

The ecology of *P. megaspora* suggests two possibilities: either the species is strictly nivicolous, but due to the relatively thick peridium its sporophores are more resistant to climatic conditions than those of other nivicolous myxomycetes such as *Lamproderma* that have a delicate, thin peridium, or the species appears soon after snowmelt, not during the period of melting snow. It is also possible that *P. megaspora* may not be strictly nivicolous. Its co-occurrence with *Trichia alpina,* which also may not be strictly nivicolous (see Meylan 1929, Novozhilov and Schnittler 1996, Ronikier et al. 2008) together with the demonstrated ability to grow in moist chamber cultures, seems to support this hypothesis. Although few strictly nivicolous species have been successfully cultured to date and fruiting of these species has been achieved only under culture conditions at low temperatures (Marx 1998, Ronikier et al. 2010), the new species was cultured at room temperature. Thus it seems possible that some nivicolous myxomycetes can be cultured if the correct conditions for the particular species are met.

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