In Press at Mycologia, preliminary version published on August 3, 2015 as doi:10.3852/14-330

Short title: *Perichaena longipes*

Perichaena longipes, a new myxomycete from the Neotropics

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Abstract: A new species of myxomycete, *Perichaena longipes*, is described from 56 specimens of fruiting bodies that appeared in moist chamber cultures prepared with samples of decaying plant materials collected in Panama, Costa Rica and Brazil. This new species is distinguished from the morphologically similar species *P. pedata* on the basis of the much longer stipe, lighter peridium and the unique ornamentation of the capillitium. The nuc 18S ribosomal DNA sequences obtained from four specimens of *P. longipes* support the distinction of this new taxon and its separation from *P. pedata*. Furthermore, maximum likelihood phylogeny supports earlier evidence that species currently within the genus *Perichaena* do not form a monophyletic clade. Instead they appear to form three separate branches within the bright-spored clade. The first clade includes *P. longipes* together with several species of *Trichia* and *Metatrichia*, the second includes *P. pedata* and *P. chrysosperma*, and the third clade is composed of *P. corticalis*, *P. depressa* and *P. luteola*.

Key words: moist chamber cultures, nuc 18S ribosomal DNA, Panama, paraphyly, phylogeny, Trichiaceae

INTRODUCTION

The genus *Perichaena* (order Trichiales, Myxomycetes) was erected by E.M. Fries in 1817 and currently encompasses 32 species (Lado 2005–2014). The genus is represented by both

sporocarpic and plasmodiocarpic forms generally characterized by a thick, persistent peridium and spores that are yellow to red-brown in mass. Other than a few exceptions, members of the genus have a well-developed, typically branched capillitium, which is roughened, warted or spiny to minutely annulate and lacks spiral bands (Poulain et al. 2011). The capillitial elements are normally irregular and when viewed by scanning electron microscopy (SEM) are sometimes covered by pits (Lado et al. 2009). Some species in the genus appear to have either a very wide or a very restricted distribution, whereas still others are known from only a single type locality. Examples of the latter, described solely on the basis of morphology, are *Perichaena frustrifilaris* Q. Wang, Y. Li & J.K. Bai (Wang et al. 2000), *P. grisea* Q. Wang, Y. Li & J.K. Bai (Wang et al. 2000) and *P. membranacea* Y. Li, Q. Wang & H.Z. Li (Li et al. 1990).

The genus *Perichaena* is currently assigned to the order Trichiales and is usually placed within the family Arcyriaceae, members of which are characterized by a tubular capillitium with no spiral bands (Neubert et al. 1993, Poulain et al. 2011). However, some authors place all forms with a tubular capillitium, including *Perichaena*, within the Trichiaceae, irrespective of capillitial ornamentation (Martin and Alexopoulos 1969, Nannenga-Bremekamp 1991).

A recent phylogeny of the bright-spored myxomycetes, based on a study of the full length nuc 18S ribosomal DNA (18S) and elongation factor (EF1 α) genes, indicated that three species of *Perichaena* (*P. corticalis* [Batsch] Rostaf., *P. depressa* Lib. and *P. luteola* [Kowalski] Gilert) fall into the same cluster as *Trichia* Haller, *Metatrichia* Ing and *Oligonema* Rostaf., whereas species of *Arcyria* F.H. Wigg. represent another phylogenetic branch (Fiore-Donno et al. 2013). Therefore the placement of the genus *Perichaena* in the family Arcyriaceae definitely was not supported by molecular data. However, the validity of the genus *Perichaena* itself did seem to hold because the three species included in that study did form a monophyletic clade (Fiore-Donno et al. 2013, Clark and Haskins 2014). All three species included are characterized by sessile, spherical sporocarps, minutely warted capillitium and similarly ornamented spores. Other

species of *Perichaena* with stipitate or plasmodiocarpic fruiting bodies or other types of capillitium and spore ornamentation hitherto had not been included in molecular phylogenetic analyses.

During a larger project by the first author within the Barro Colorado Nature Monument in Panama, a large series of stipitate specimens of *Perichaena* were collected, initially considered to be unusually long-stiped representatives of *Perichaena pedata* (Lister & G. Lister) G. Lister ex E. Jahn (Jahn 1919) but differing from the latter by the long stipe and distinctive ornamentation of the capillitium and spores. Later additional specimens of the same morphotype were identified from material collected in Costa Rica and Brazil. The unique set of morphological features and their stability between substrates and geographical regions suggested that the specimens probably represented a new species of *Perichaena*. However, it is commonly recognized that some morphological characters of myxomycetes, such as the length of the stipe, may be only the result of phenotypic plasticity (Nannenga-Bremekamp 1991). Therefore we wished to substantiate these taxonomic combinations with a comparison of the genetic variation within the group before erecting a new taxon.

The main purpose of this study was to confirm the distinctness of the proposed new species and also to understand its approximate phylogenetic position within the bright-spored clade of myxomycetes. To this end we sequenced a 5′ region of the 18S gene, recently demonstrated as a useful barcode marker for myxomycetes (Fiore-Donno et al. 2012, 2013). This same locus also was sequenced in two other morphologically distinct species, *Perichaena pedata* (a stipitate form) and *P. chrysosperma* (Curr.) Lister (a plasmodiocarpic form). In addition we generated sequences from four specimens of *Arcyria cinerea* (Bull.) Pers. and one from *A. leiocarpa* (Cooke) Massee to be included in the phylogeny. The publicly available sequences of three other species of *Perichaena* and another 14 species of Trichiales were included in the alignment to generate a more representative phylogeny.

MATERIALS AND METHODS

Field sampling.—The initial (and largest) series of specimens were obtained from moist chamber cultures prepared with samples of dead plant material collected in the Barro Colorado Nature Monument (BCNM) in the Republic of Panama (9°06′31″N, 79°50′37″W). The site is a typical old-growth (> 200 y), lowland (25–61 m) moist tropical forest (Wright et al. 2011) with a 4 mo dry season, an average annual rainfall of approximately 2600 mm and a mean monthly temperature of 26 C (Yavitt et al. 2011). Soils at this locality are Endogleyic Cambisols, which are highly weathered, moderately acidic and have a high clay content (Koehler et al. 2012).

Later several collections of the new morphotype from nearby Costa Rica were obtained from the UARK herbarium. These specimens from also were recovered from moist chamber cultures of samples of dead plant material 2 y prior, also by the first author. The collection site is in the Sarapiquí region, at the La Selva Biological Research Station (10°25′52″N, 85°59′47″W). Forests at this site are primarily old-growth, lowland, tropical wet forests with an average annual rainfall of approximately 4000 mm and temperatures of 19–31 C.

Finally a single specimen of the new morphotype was obtained from a third locality in Brazil (01°45′N, 61°08′W). This specimen appeared in a moist chamber culture of aerial leaf litter collected by I. L. Coehlo in a tropical wet forest in Caracaraí, Roraima, Brazil, as part of a separate survey underway in the laboratory of the third author.

Moist chamber cultures.—Culture methods are described here in detail only for those samples collected in Panama; equivalent methods were used in the two other surveys that also yielded specimens of this putative new species. The sample materials (forest floor leaf litter and pieces of small woody debris) used to prepare the moist chamber cultures were collected in Jun 2012 and Aug 2013 by the first author. All samples were placed in small paper bags in the field, returned to the laboratory and air-dried. Afterward they were shipped to the University of Arkansas at Fayetteville for processing with the use of the traditional moist chamber culture technique (Stephenson and Stempen 1994). Over 2 y a total of 1008 moist chamber cultures were established and monitored 3–6 mo each. The total number of fructifications was more than 3500, 46 of which represent the putative new species. From Costa Rica we obtained an additional nine herbarium specimens, along with the one from Brazil. Therefore the putative new species is represented by a total of 56 specimens.

Microscopy.—Air-dried specimens were studied with a Zeiss Axioskop 2 Plus dissecting stereomicroscope. Temporary water slides and permanent slides prepared with polyvinyl lactophenol were studied with a Leica MSV226 light microscope (LM) equipped with differential interference contrast. The freeware program CombineZP

(Hadley 2010) was used to create a composite digital image from several stacked images. Microscopic measurements were made with the program Axio Vision 4.8.0.0 (Carl Zeiss Imaging Solutions GmbH). Scanning electron microscopy (SEM) was carried out with an FEI Nova Nanolab 200 FIB/SEM microscope. Air-dried sporocarps were sputter-coated with gold-palladium to form a 5 nm cover and studied at 5–15 kV. All microscopy was carried out at the University of Arkansas.

Twenty-five spores and capillitial threads from five sporocarps were measured to estimate the range of variation. Size of the sporotheca, stipe and hypothallus were measured for 15 sporocarps. The range of variation for these main parameters is given as (minimum–) mean minus standard deviation–mean plus standard deviation (–maximum). Colors are according to the Munsell scale (Munsell 1905).

DNA sequencing.—DNA was extracted from 5–6 adjacent sporocarps with the Invitek Spin Food Kit II (Stratec Molecular GmbH, Germany). Sporocarps were frozen at −80 C in 1.5 mL centrifuge tubes containing acid-washed glass beads 0.7–1.1 mm diam (Sigma Chemicals, USA). Frozen samples were vortexed 1 min at 30 Hz with a Wig-L-Bug grinding mill (Reflex, USA). We followed the protocol recommended by the manufacturer except for the final step, where DNA was eluted in 50 µL buffer (instead of 200 µL).

Partial sequences of the 18S (ca. 550 bp intron-free segment of the 5′ end) were amplified with various primer combinations as proposed by Fiore-Donno et al. (2013). The primary primers used were S1F: AACCTGGTTGATCCTGCC (forward) and SU19R: GACTTGTCCTCTAATTGTTACTCG (reverse) although in some cases, such as if the initial primer pair was not working favorably, other primers and primer combinations were used. All primers and the combinations used for obtaining each sequence can provided (SUPPLEMENTARY MATERIAL I).

The PCR reaction was carried out in 40 cycles (95 C, 2.5 min; 52 C, 30 s; 72 C, 1 min), regardless of primers. Results of the PCR were verified by electrophoresis in an agarose gel in TA buffer stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, California). The amplicons were purified with MSB Spin PCRapace (Stratec Molecular GmbH, Germany) following the manufacturer's protocols except for eluting in 20 µL elution buffer (instead of 10 µL) in the final step. The amplification of the product for sequencing was carried out in 40 cycles (96 C, 70 s; 53 C, 5 s; 60 C, 4 min) with the same primers used for the initial PCR reaction of each specimen. Sequencing was performed on an Applied Biosystems 3130xl Genetic Analyzer at the University of Arkansas DNA Resource Center. Sequences were generated bi-directionally, assembled with the automatic function in Sequencher® 5.2 (Gene Codes Corp., Ann Arbor, Michigan) and manually inspected before alignment.

Four partial 18S sequences were obtained from the alleged new species—two from Panamanian specimens (LMW 2574 [UARK 54115]; LMW 2869 [UARK 54447]) and two from Costa Rican specimens (LMW 26151 [UARK 47993]; LMW 26264 [UARK 48715]). To compare the sequences of the new taxon with those of closely related taxa, we also sequenced the partial 18S of four specimens of *Arcyria cinerea*, one specimen of *A. leiocarpa*, one specimen of *P. chrysosperma* and three specimens of *P. pedata* (SUPPLEMENTARY MATERIAL I). Although a total of 13 sequences were generated, only nine appeared to be unique. The other four sequences were identical to a sequence obtained from the same species, thus representing the same 18S genotype (see below) and were eliminated from analyses. All of the new sequences were deposited in GenBank under accession numbers (GBa) KP241117–KP241129.

Sequence alignment.—The nine newly obtained sequences were aligned with 18 sequences of other members of the Trichiales studied by Fiore-Donno et al. (2013). These sequences represented the following taxa: *A. cinerea*, *A. denudata* (L.) Wettst., *A. globosa* Schwein., *A. incarnata* (Pers. ex J.F. Gmel.) Pers., *A. marginoundulata* Nann.-Bremek. & Y. Yamam., *A. stipata* (Schwein.) Lister, *Hemitrichia calyculata* (Speg.) M.L. Farr, *Metatrichia floriformis* (Schwein.) Nann.-Bremek., *M. vesparia* (Batsch) Nann.-Bremek. ex G.W. Martin & Alexop., *P. corticalis*, *P. depressa*, *P. luteola*, *Trichia alpina* (R.E. Fr.) Meyl., *T. decipiens* (Pers.) T. Macbr. (two different genotypes), *T. sordida* Johannesen, *T. persimilis* P. Karst. and *T. varia* (Pers. ex J.F. Gmel.) Pers. Two representatives of the family Reticulariaceae (*Tubifera applanata* Leontyev & Fefelov and *T. ferruginosa* [Batsch] J.F. Gmel.) by Leontyev et al. (2014) were used as outgroup. An alignment of the 29 sequences was generated in MEGA 5.1 (Hall 2011, Tamura et al. 2011) with the automatic procedure implemented in multiple sequence comparison by log-expectation (MUSCLE). Minor changes in the automatic alignment were made by hand in BioEdit (Hall 1999). The alignment is available on TreeBASE, submission 17633 (treebase.org).

Phylogenetic analyses.—We used 434 positions (out of a total of 791) that were aligned unambiguously; most portions of the variable helices did not align. Analyses of the aligned sections were carried out with maximum likelihood (ML) algorithm using MEGA 5.1 (Hall 2011, Tamura et al. 2011). The evolutionary model was chosen in MEGA 5.1 option FIND BEST DNA/PROTEIN MODEL as GTR with gamma-distributed rate variation across sites with a proportion of invariable sites. Branch support was estimated with 1000 bootstrap replicates.

RESULTS

Twenty-seven 18S sequences from members of the Trichiales, including the nine obtained in the present study, were used to construct the ML phylogeny. The topology appeared stable and was

not dependent on (i) the inclusion or exclusion of sequences (ii) the usage of different alignment algorithms (Clustal W, MUSCLE or MAFFT) or (iii) the use of whole length sequences (when available) or only the unambiguously aligned positions. The main purpose of the study was to confirm the genetic distinctiveness of the putative new species and its approximate position within the bright-spored clade. All other conclusions relating to the topology of the tree should be considered as preliminary.

Our phylogeny revealed that all studied representatives of the Trichiaceae form a monophyletic clade with full bootstrap support. This clade is subdivided on three branches. The first branch represents the genera *Trichia* (except for *T. decipiens*), *Metatrichia* and *Perichaena*, which at this time cannot be cannot be clearly held together by any single morphological character(s). The second branch corresponds to the genus *Arcyria*, which can be defined by mostly stalked sporocarps with "cellulite" stalks (stalks filled with spore-like cells), and a net-forming capillitium without spiral bands. The third branch consists of *T. decipiens* and *H. calyculata*, both of which are defined by stalked cellulate sporocarps and a capillitium with spiral bands. This branching pattern within the Trichiaceae corresponds fully to the phylogeny based on full-length sequences of both 18S and EF1αin the bright-spored myxomycetes (Fiore-Donno et al. 2013). This branching pattern indicates that among the main genera of the Trichiaceae only *Arcyria* seems to be monophyletic, with all other genera distributed among different branches and thus presumably characterized as para- or polyphyletic.

The five sequences of *Arcyria cinerea* included in this study (four generated in the lab of the third author and one derived from Fiore-Donno et al. [2013]) did not form a single cluster. Instead three of them clustered with *A. denudata*, *A. leiocarpa* and *A. marginoundulata*; another clustered with *A. stipata* and *A. globosa*; and the last one formed its own sub-basal branch.

The putative new species appeared to be represented by two similar yet unique partial 18S genotypes (p distance 0.08, the calculation is explained below), each of which was

represented by two specimens from the same locality (Panama or Costa Rica). Together these two genotype sequences form a monophyletic group within the *Trichia-Metatrichia-Perichaena* clade. However, the putative new species did not cluster with the other species of *Perichaena*, including the morphologically similar *P. pedata.* Instead the six species of *Perichaena* included in this study formed three independent branches, one consisting of the sessile sporocarpic species (*P. corticalis*, *P. depressa*, *P. luteola*), another by the stipitate *P. pedata* and the (usually) plasmodiocarpic *P. chrysosperma* and the third by the proposed new stipitate species.

TAXONOMY

Perichaena longipes L.M. Walker, Leontyev & S.L. Stephenson, sp. nov. FIG. 1

MycoBank MB810916

Typification: PANAMA. PANAMA: Barro Colorado Nature Monument, Gigante Peninsula, (9°06′31″N, 79°50′37″W), 50 m. Old-growth tropical moist forest, on forest floor leaf litter in moist chamber culture (pH 6.7), 10 Aug 2013, *L.M. Walker LMW 2574* (HOLOTYPE. UARK 54007; GBa KP241126).

Etymology: The name *longipes* (from the Latin *longus* – long, *pes* – leg) refers to the stipe, the most conspicuous feature of the new species.

Diagnosis: Sporocarps stipitate, 0.5–0.8 mm tall, solitary or sometimes scattered in small loose groups (FIG. 1a–e). Stipe long, straight or slightly inclined, plicate, dark brown (2.5R1/2) to black, ocher-yellow to yellow-brown (2.5Y6/8) in transmitted light (FIG. 1f), 0.3–0.7 mm long, 25–80 µm diam. Hypothallus discoid, concolorous with the stipe, 0.1–0.4 mm diam (FIG. 1c). Sporotheca globose, 0.15–0.25 mm diam, light yellow to tan (7.5–10Y7/4–6), darker at the base, smooth, sometimes with a weak iridescent shimmer (FIG. 1a, b). Columella absent. Peridium single, tough and persistent, warted on the inner surface (FIG. 1g, h). Capillitium bright yellow in transmitted light (7.5Y8/8–10), tubular, (2.6–)3.2–5.8(–6.2) µm diam, branched and

anastomosed (FIG. 1i, j), densely covered with papillate, branched, coral-like projections (FIG. 1k–n), free ends scanty, short, obtuse, sometimes with a short acuminate tip on a swollen base (FIG. 1j), the small pits (ca. 0.5 µm) sometimes are present between papillae as observed under SEM (FIG. 1l). Spores free, (7.5–)7.8–9.1(–10.7) µm, light yellow to tan in mass (7.5–10Y8/6), almost hyaline in transmitted light (FIG. 1g, i, j), as observed under LM spores appear smooth, whereas under SEM they are verrucose, with a flat cap on the tip of each wart, these caps are star-like as observed from above (FIG. 1p, q).

Habitat and distribution: Sporocarps of *Perichaena longipes* appeared primarily on forest floor leaf litter (47) but also occurred on small pieces of coarse woody debris (7) or on aerial leaf litter (1) in moist chamber cultures. Considering that it was recorded from localities extending from Costa Rica to Brazil, the species seems to occur in similar microhabitats throughout the Neotropics. Sporocarps of *P. longipes* always appeared in moist chamber cultures relatively late, usually after at least 4 wk of continuous culture, and sporocarps were either solitary or scattered (but never gregarious). The average pH of the moist chamber cultures in which *P. longipes* appeared was 6.5(5.0–8.1). These values are fairly typical for the substrates upon which most myxomycetes occur in nature (Stephenson and Stempen 1994).

Other specimens examined: PANAMA: same location, substrate and date as HOLOTYPE, *L.M. Walker LMW 268* (UARK 53971), *LMW 1850* (UARK 53985), *LMW 2007* (UARK 51762), *LMW 2754* (UARK 54115), *LMW 2777* (UARK 54129). COSTA RICA: Sarapiquí, La Selva Biological Research Station, (10°25′52″N, 85°59′47″W), 100 m. Primary tropical wet forest, on leaf litter and pieces of small woody debris in moist chamber culture (pH 6.2), 21 Jan 2012, *L.M. Walker LMW* 26151 (UARK 47993; GBa KP241120); same location, substrate and date as previous, *L.M. Walker LMW* 26264 (UARK 48715; GBa KP241121). BRAZIL: Caracaraí, Roraima, (01°45′N, 61°08′W), 233 m. Primary Amazon forest, on aerial litter in moist chamber culture (pH 5.3), 01 Feb 2014, *I. L. Coelho ILC 30961* (UARK 54507). All of the 47 remaining specimens were also deposited at UARK, although they have not necessarily been examined with the same degree of detail as those listed above.

DISCUSSION

Limitations.—This study may be considered limited by the use of partial gene sequences of only one gene and by the limited number of sequences used in building the phylogeny. However, the topology of the tree presented is stable (see above) and fully corresponds to the topology obtained by Fiore-Donno et al. (2013), who used full-length sequences of two genes (18S, EF1α). This provides additional evidence of the validity of the 5′ domain of 18S rDNA as the molecular barcode for species delimitation in myxomycetes. However, we still consider the phylogeny presented here as preliminary.

Validity of the genus.—Our 18S phylogeny clearly supports the separation of *Perichaena longipes* from all other species of myxomycetes. However, *P. longipes* appeared to be much closer to *Trichia alpina*, *T. varia* and *Metatrichia floriformis* than to other species of *Perichaena*, and this relationship is supported by a high bootstrap value (0.95). This fact calls into question the appropriateness of classifying *P. longipes* as a member of the genus *Perichaena*. However, the new species cannot be assigned to *Trichia* or *Metatrichia* because both genera are characterized by having mostly unbranched capillitial threads that are ornamented with spirals (Martin and Alexopoulos 1969). Our species has a branched and anastomosed capillitium ornamented with coral-like papillae and small pits; a type of ornamentation considered to be typical for the genus *Perichaena* (see above). Therefore we observe here the evident contradiction between morphology and phylogeny. The situation becomes even more complicated when we acknowledge that all studied genera of the Trichiaceae, except *Arcyria*, do not appear to be monophyletic in this phylogeny. Instead *Perichaena* appears to be paraphyletic, represented by three different clades. Members of the genus *Trichia* are found in two of the three main branches of the Trichiales and therefore appear to be polyphyletic, as was indicated by Fiore-Donno et al. (2013). Finally two members of the genus *Metatrichia* reveal varying results, either appearing to be sister or not sister to members of *Trichia* (results of Fiore-Donno et al. [2013] and the phylogeny generated herein, respectively). Therefore, based on the phylogenetic

data, it appears that numerous divisions within the Trichiaceae should be re-evaluated, although not in the context of this study due to the small sampling and the inclusion of only a partial gene sequence from a single gene. Such a revision should only be carried out with a multiple gene phylogeny that includes a significant number of specimens for each species. Instead, with the information available herein, we can only follow the current morphological concept for genera within the Trichiaceae, which assigns our new species to the genus *Perichaena.*

One or two species?—Sequences of the 18S were generated from four different specimens of *P. longipes.* The two specimens collected in Panama appear to have an identical 18S genotype, as do the two specimens collected in Costa Rica; however, there is an 8% sequence divergence between the two genotypes. This brings us to question whether these two 18S genotypes represent one or two different species. To answer similar questions in another group of Lucosporideans, Leontyev et al. (2015) proposed an approach for calculating the p distances between all 18S genotypes. This distance index is calculated as the proportion (p) of nucleotide sites at which two sequences are different and varies from 0 (sequences are identical) to 1 (sequences share no common nucleotides) (Hall 2011, Tamura et al. 2011). It was shown that there exists a natural gap between p values when comparing specimens of the same species (low p values) to those of different species (high p values) (Leontyev et al. 2015). We could not carry out these calculations in the present study because most of the species are represented only by one specimen. We, however, can compare our data with the natural threshold values proposed by Leontyev et al. (2015) to distinguish among species within the bright-spored myxomycetes. The value proposed for distinguishing species within the bright-spored myxomycetes is p = 0.11–0.15, indicating that if the difference between two partial 18S genotypes is lower than 0.11 then they both belong to the same species whereas if the calculated p value is higher than 0.15 they belong to different species (Leontyev et al. 2015). Therefore the value p = 0.08 obtained by comparing sequences of *Perichaena longipes* from Panama and Costa Rica corresponds to only

intraspecies genetic diversity and does not suggest the separation into separate species. This conclusion is supported by analyses reported herein that showed no morphological differences between specimens from Panama and Costa Rica.

When the same analysis is applied to the morphospecies *A. cinerea* the results are different. Herein *A. cinerea* is represented by five specimens, each of which corresponds to its own 18S genotype (see FIG. 2). All of them have p distances much higher than mentioned species threshold (p 0.37–0.62). This in addition to the morphological diversity often seen in this morphospecies (not examined in great detail here) is further evidence to a proposed idea that *A. cinerea* not a single species but instead may be a species complex (Clark et al. 2002).

Morphological analysis.—The distinctive set of morphological characters together with the unique 18S sequences indicate that *P. longipes* is a species of myxomycete new to science. It is assigned to the genus *Perichaena* on the basis of the thick and persistent peridium along with the presence of a well-developed, irregular, branching and ornamented capillitium lacking spiral bands, the surface of which is covered by pits when viewed by SEM (Novozhilov et al. 2008, Lado et al. 2009, Poulain et al. 2011). The bright yellow spores also validate the placement of *P. longipes* into the higher-level, bright-spored clade of the myxomycetes (Fiore-Donno et al. 2013).

As noted in the taxonomic diagnosis, the single most notable feature of *P. longipes* is the long stipe of the sporocarp. Although a stipitate sporocarp is not the most common expression for species of *Perichaena*, it is by no means unusual. At least six other species of *Perichaena* are characterized by a well-developed stipe. These are *P. heterospinospora* Novozhilov, Zemlyanskaya, Schnittler & S.L. Stephenson (Novozhilov et al. 2008), *P. papulosa* C.H. Liu & J.H. Chang (Liu et al. 2007), *P. pedata*, *P. polygonospora* Novozhilov, Zemlyanskaya, Schnittler & S.L. Stephenson (Novozhilov et al. 2008), *P. pulcherrima* Petch (Petch 1909) and *P. reticulospora* H.W. Keller & D.R. Reynolds (Keller and Reynolds 1971). Four other species—*P.*

areolata Rammeloo (Rammeloo 1984), *P. calongei* Lado, D. Wrigley & Estrada (Lado et al. 2009), *P. chrysosperma,* and *P. stipitata* Lado, Estrada & D. Wrigley (Estrada-Torres et al. 2009)—have reduced or short stipes. However, it should be noted that the latter are not always stipitate but instead may display a variety of sporocarp types, even within a single fruiting. In contrast, *P. longipes* appears to form only erect, stipitate sporocarps, because there was never any evidence of sessile or plasmodiocarpic forms in our collections. Therefore in many instances *P. longipes* may be distinguished from other species of *Perichaena* simply on the basis of the much greater length of the stipe. When comparing stipe lengths to the two most morphologically similar species (*P. pedata*, *P. stipitata*), the the stipe in *P. longipes* is commonly more than twice the length found in either of these other species. Moreover, the stipe of *P. stipitata* usually has a frosting of lime and thus appears white (Estrada-Torres et al. 2009). It is noteworthy that in the wet tropics, a number of species of myxomycetes have been observed to possess longer stipes when compared to the same species found in temperate regions of the world, presumably because the greater height above the substrate may aid in more effective drying of spores (Schnittler and Stephenson 2000). This is another reason that multiple characters should be evaluated to support the status of a separate species for *P. longipes*. Because of the general morphological similarities between *P. longipes, P. pedata* and *P. stipitata* although to a lesser extent in the second instance. A quick reference (TABLE I) is included herein for morphological comparison between the three species.

The sporotheca of *P. longipes* is similar to those of many other stipitate species of *Perichaena*, excluding the sporocarp of *P. polygonospora*, which is much smaller (0.05–0.1 mm), lacks a capillitium and has a unique polygonal spore shape (Novozhilov et al. 2008). The sporocarp of *P. longipes* is light yellow to tan, darker at the base, and the peridium is smooth, sometimes with a weak iridescence (FIG. 1a, b). Most other stipitate species of *Perichaena* are darker and/or do not have a smooth peridia surface. For instance, the sporotheca of *P. papulosa*

is brownish orange and has an apical wart (Liu et al. 2007), *P. polygonospora* is buff or buff-yellow and covered with orange-brown protuberances (Novozhilov et al. 2008) and *P. calongei* is yellow to dark brown, with dark lines marking the edges of the peridia plates of dehiscence (Lado et al. 2009). Another stipitate species, *P. areolata*, is not light yellow similar to *P. longipes*; however, the peridium has a mottled appearance and consists of two layers versus the single layer in *P. longipes* (Lado et al. 2009).

A peridium with a single layer also occurs in *P. pedata*, *P. stipitata*, *P. polygonospora*, *P. heterospinospora* and *P. papulosa*. However, in *P. longipes* the peridium is relatively tough, persistent and the inner surface is densely and irregularly verrucose. None of the other stipitate species of *Perichaena* have a similar peridium. *Perichaena pedata* and *P. stipitata* do have a peridium in which the inner surface is ornamented, but the ornamentation is different in all three instances (TABLE I). The inner peridium of *P. pedata* consists of a few low, rounded ridges, whereas in *P. stipitata* the ornamentation is composed of large ocellate elements (Estrada-Torres et al. 2009). *Perichaena areolata*, *P. chrysosperma and P. polygonospora* also have a verrucose inner surface of the peridium, but they are distinctly different in overall morphology. The published descriptions of *P. papulosa*, *P. reticulospora*, *P. heterospinospora* and *P. pulcherrima* do not include any information on the structure of the inner peridial surface.

The capillitium of *P. longipes*, av. 3.2–5.8 µm diam, is among the largest found in any of the stipitate species of *Perichaena*. The relatively large size is explained in part by the well-developed ornamentation, which cannot be confidently excluded from consideration when the diameter is measured. In addition to the large size of the capillitium, the ornamentation is unlike that of any other described species in the Trichiaceae. The surface of the capillitium in *P. pedata* is irregularly and densely ornamented with papillate, branched, coral-like projections that are separated by pits (ca. 0.5 µm diam) visible only by SEM (FIG. 1k–n). In both of the species (*P.*

pedata, *P. stipitata*) that are the most morphologically similar to *P. longipes* (TABLE I), the ornamentation is very different. In *P. pedata* the capillitium is ornamented with regularly and sparsely distributed spines and there are no pits between them (Estrada-Torres et al. 2009). *Perichaena stipitata* has a capillitium characterized by ornamentation consisting of large craters (3.8–6.8 μm) when viewed by SEM (Estrada-Torres et al. 2009) and there are no spines or other projections.

The difference between *P. longipes* and the other stipitate species with respect to spore size and ornamentation is not strong. However, for each pair of species being compared it is easy to find at least one distinguishing character. The spores of *P. pedata* are only sparsely warted, whereas those of *P. stipitata* and *P. longipes* are densely warted. This causes the spores of *P. stipitata* to be similar to those of *P. longipes*; however, they are considerably larger, 12.0–15.0 μm vs. 7.8–9.1 μm< in *P. longipes* (Poulain et al. 2011).

The morphology of the warts covering the spores of the new species is a noticeable feature because these structures have flattened, star-shaped tips when viewed by SEM (FIG. 1p, q). Flat tips like this are unknown in other stipitate species except for *P. papulosa*, *P. chrysosperma* and *P. calongei*. However, they are common in the genus *Trichia* (*T. contorta*, *T. munda* [Lister] Meyl., *T. sordida*, *T. varia*), a taxon to which our new species seem to be closely related.

The stipitate species of *Perichaena* discussed in this paper are included in the key.

KEY TO THE STIPITATE AND SUBSESSILE SPECIES OF *PERICHAENA*

1	Spores polygonal	*P. polygonospora*
1′	Spores globose or subglobose to ovate	2
2	Spores banded-reticulate	*P. reticulospora*
2′	Spores not banded-reticulate but with ornamentation	3
3	Spores ornamented with scattered tall pyramidal spines	*P. heterospinospora*

3′ Spores densely ornamented with warts or spines, but less than 0.5 µm high 4

4 Spore diam 12–18 µm 5

4′ Spore diam 7–12 µm 6

5 Stipe calcareous, grayish or brown to black, spore diam 12–15 µm ..... *P. stipitata*

5′ Stipe not calcareous, brown or red brown, spore diam 14.5–18 µm ..... *P. pulcherrima*

6 Peridium single 7

6′ Peridium double 9

7 Sporocarp with apical protuberance, dehiscence leaving a cup-like base ..... *P. papulosa*

7′ Sporocarp without an apical protuberance..... 8

8 Peridium with irregular dehiscence, capillitial tubules less than 3.5 µm diam with scattered small spines ..... *P. pedata*

8′ Peridium persistent, capillitial tubules between 3.2 and 5.8 µm and densely ornamented with papillate, branched, coral-like projections …..... *P. longipes*

9 Sporocarp subsessile to sessile or plasmodiocarpic, capillitial tubules with long spines, 2.9–5.5 µm long ..... *P. chrysosperma*

9′ Sporocarp subsessile to sessile but not plasmodiocarpic, capillitial tubules with spines less than 3 µm long 10

10 Peridium marked with dark lines along edges of the plates of dehiscence, capillitial tubules with spines, granules, or pits ..... *P. calongei*

10′ Peridium not marked with dark lines, dehiscence not along plates, capillitial tubules with regularly distributed spines ..... *P. areolata*

ACKNOWLEDGMENTS

This research was supported in part by the Smithsonian Tropical Research Institute, with direct support from staff scientists Allen Herre, Benjamin L. Turner and S. Joseph Wright. The molecular study was partially supported by a scholarship from the Fulbright Scholar Program (grant 68130017) to the second author. Additional support was provided by the Department of Biological Sciences at the University of Arkansas. We thank Jeff Silberman at the University of Arkansas for his support and the use of his laboratory for all molecular work. We acknowledge Isadora Coelho for providing her specimen of *Perichaena longipes* from Brazil. We also acknowledge Isadora for the three 18S sequences obtained from her specimens of *Arcyria cinerea* and Adam Rollins for his specimens of *P. pedata*.

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LEGENDS

FIG. 1. *Perichaena longipes*. a–e. Sporocarps. f. Stipe and the base of sporotheca in transmitted light. g. Peridium and spores in transmitted light. h. Inner surface of the peridium. i–j. Capillitium and spores. k. Optical section of a capillitial thread. l–n. Details of the ornamentation of capillitial threads. o. Spores and capillitium under SEM. p. Spore. q. Detail of spore ornamentation; bar. Specimens: a, b, h, j–q. *LMW 2574* (UARK 54007); c–g, i. *LMW 1850* (UARK 53985). Bars: a–e = 200 µm; f = 500 µm; g = 30 µm; h = 5 µm; i = 50 µm; j = 10 µm; k = 2 µm; l, n = 2 µm; o = 20 µm; p = 2 µm; q = 0.5 µm.

FIG. 2. Phylogenetic position of *Perichaena longipes* within the Reticulariaceae. The tree is based on partial 18S sequences (791 bp, 434 aligned positions retained) constructed with MEGA 5.1 and rooted with genus *Tubifera*. ML bootstrap replicates are shown for each branch. Each species name is accompanied by the GBa number of the 18S rDNA gene sequence used in the phylogeny (one representative for each genotype if more than one). Green circles mark representatives of the genus *Perichaena* and blue the morphospecies *Arcyria cinerea*. Sequences obtained in this study are in boldface. The number of identical 18S genotypes (obtained in this study) are in parentheses (if present).

FOOTNOTES

Submitted 10 Dec 2014; accepted for publication 15 May 2015.

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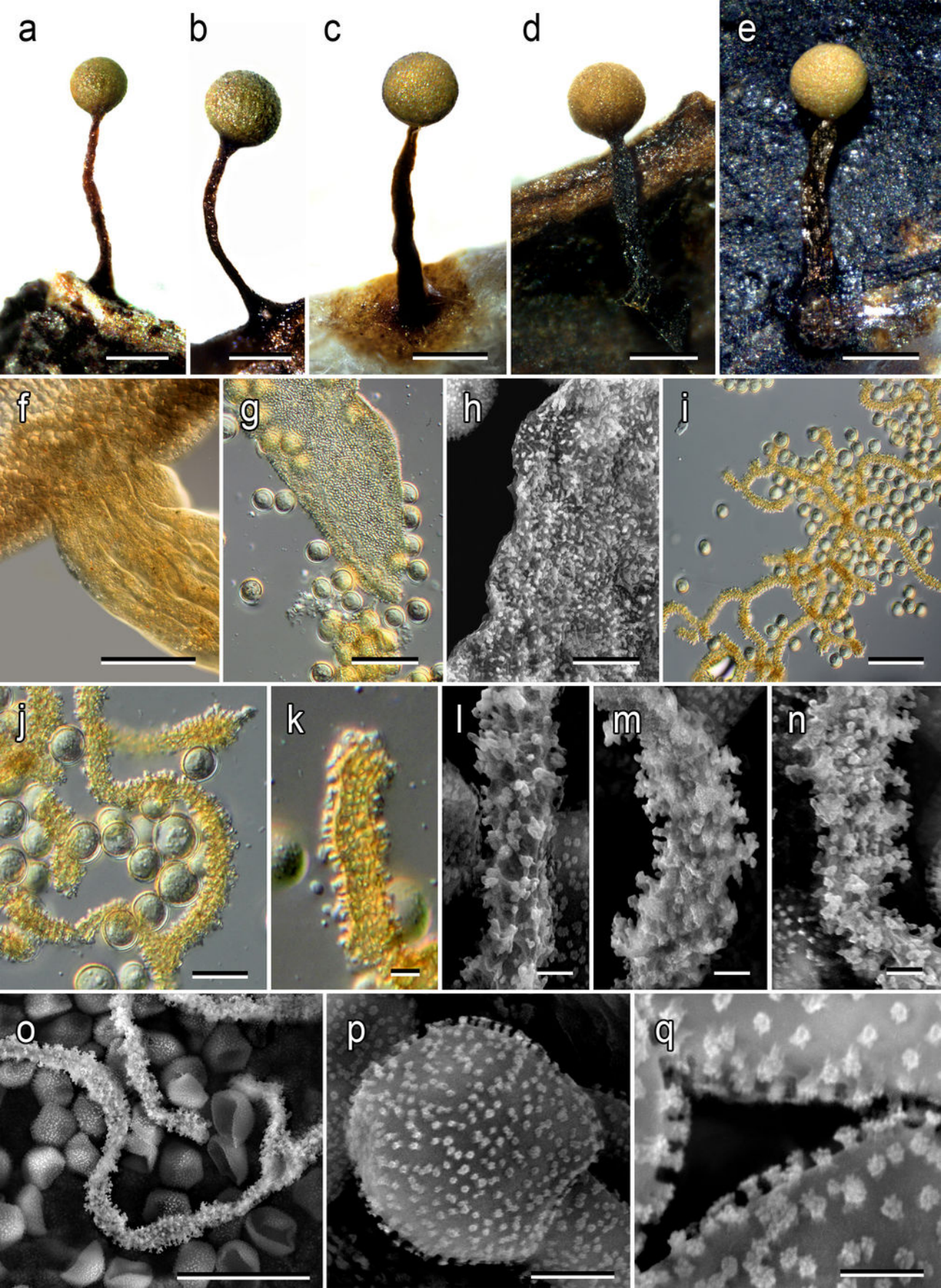
TABLE I. Morphological characters of three stipitate species of *Perichaena*[a]

	Perichaena longipes	*Perichaena pedata*	*Perichaena stipitata*
Total height (mm)	0.5–0.8	0.2–0.8	0.08–0.38
Diam of sporocarp (mm)	0.15–0.25	0.2–0.5	0.1–0.5
Color of sporotheca	Bright yellow to ochraceous	Ochraceous or fawn	Orange yellow to dark brown
Shape of sporotheca	Globose to subglobose	Subglobulose	Subglobose to subhemispheric
Color of stipe	Dark brown to blackish	Dark brown to blackish	Calcareous and white, or brown to black without calcium
Length of stipe (mm)	0.3–0.7	0.45–0.60	0.1–0.5
Diam of stipe (mm)	0.25–0.80	1/2 to twice the diameter of the sporophore	0.05–0.38
Structure of stipe	Plicate	Stout and roughened	Filled with crystalline deposits and refuse matter, sometimes striated
General structure of capillitium	Branching, tubular, free ends are scanty and obtuse	Profuse and branching	Scanty, branching, tubular, few free ends
Color of capillitium	Yellow	Yellow	Yellow
Ornamentation of capillitium	Densely ornamented with irregular spines and warts	Small, regular, scattered spines	Irregular with large holes (3.8–6.8 μm)
Ornamentation of capillitium by SEM (μm)	Pits (~ 0.5 μm)	Not reticulate or pitted	Holes (3.8–6.8)
Diameter of capillitium (μm)	3.2–5.8	1.5–3.5	1.4–3.6
Spore size (μm)	7.8–9.1	9.0–11.0	12.0–15.0
Color of spore mass	Bright yellow	Bright yellow	Orange yellow
Spore ornamentation	Prominent and abundant warts, flattened at apex and resembling a star shape	Minutely warted	Very flattened warts
Peridium	Single, thick, persistent	Single, thick, persistent	Single, membranous
Inner peridial ornamentation	Densely verrucate	Short, rounded ridges and various sparce verrucate elements	Ocellate and weakly wrinkled

[a] Characters of *P. longipes* are according to available herbarium material, whereas characters of *P. pedata* and *P. stipitata* are according to Estrada-Torres et al. (2009) in conjunction with Poulain et al. (2011).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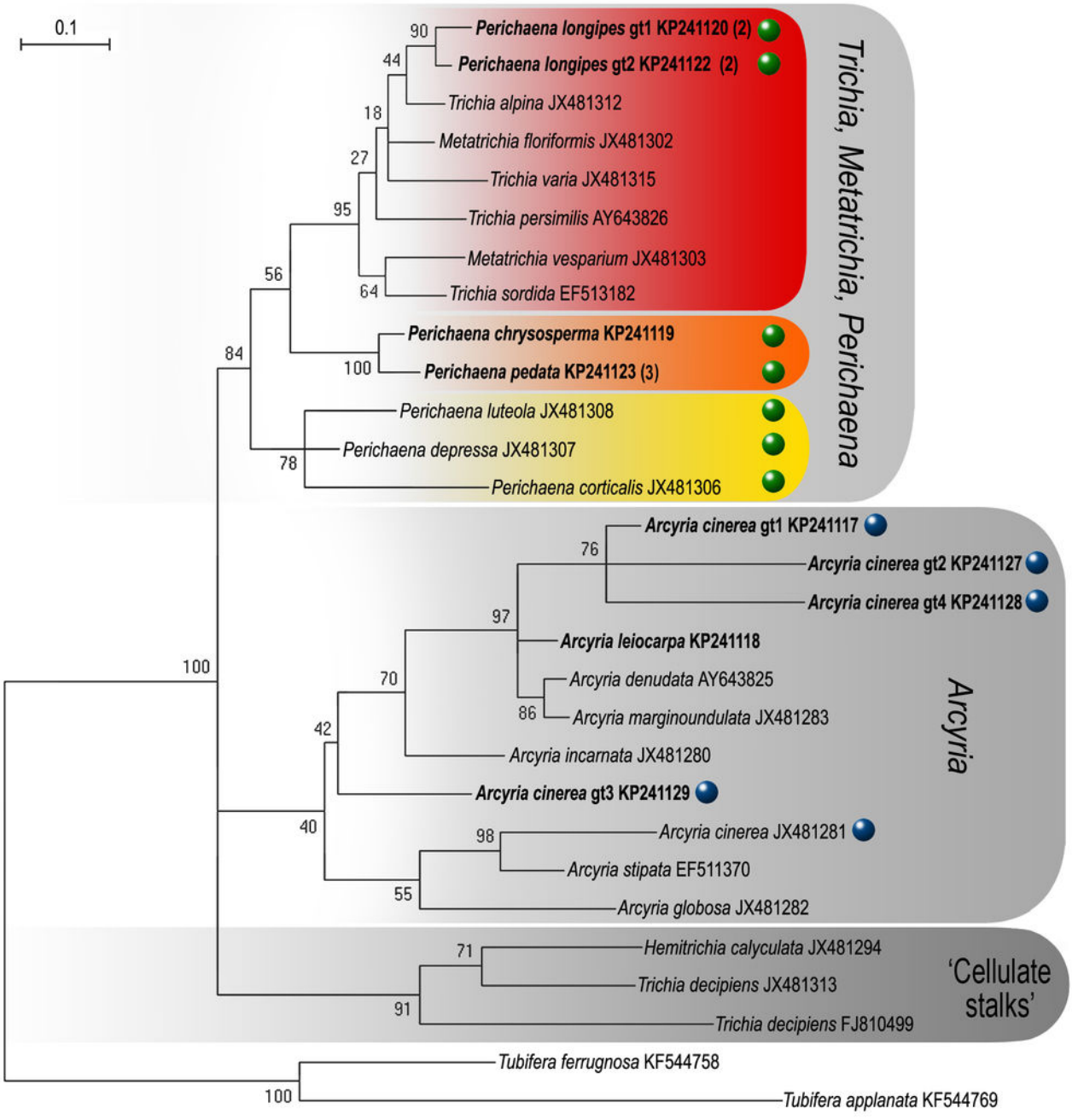
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Perichaena longipes gt1 KP241120 (2)
Perichaena longipes gt2 KP241122 (2)
Trichia alpina JX481312
Metatrichia floriformis JX481302
Trichia varia JX481315
Trichia persimilis AY643826
Metatrichia vesparium JX481303
Trichia sordida EF513182
Perichaena chrysosperma KP241119
Perichaena pedata KP241123 (3)
Perichaena luteola JX481308
Perichaena depressa JX481307
Perichaena corticalis JX481306
Trichia, Metatrichia, Perichaena
Arcyria cinerea gt1 KP241117
Arcyria cinerea gt2 KP241127
Arcyria cinerea gt4 KP241128
Arcyria leiocarpa KP241118
Arcyria denudata AY643825
Arcyria marginoundulata JX481283
Arcyria incarnata JX481280
Arcyria cinerea gt3 KP241129
Arcyria cinerea JX481281
Arcyria stipata EF511370
Arcyria globosa JX481282
Arcyria
Hemitrichia calyculata JX481294
Trichia decipiens JX481313
Trichia decipiens FJ810499
'Cellulate stalks'
Tubifera ferrugnosa KF544758
Tubifera applanata KF544769
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