



Loosening the belt: unknown diversity of the strangled stinkhorn genus *Staheliomyces* (Phallales, Basidiomycota)

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Abstract

Known to date only from the Neotropics, the genus *Staheliomyces* E. Fisch. (Phallaceae, Phallales) was described from Suriname with only a single species, *S. cinctus* E. Fisch. Nearly 100 years have passed since the original description, and its systematic position and species diversity have not been investigated. Collections from Brazil and Costa Rica were studied, and four new species are described based on morphology, molecular phylogenetic analyses, and nomenclatural review. We provide morphological descriptions together with a key to species, and a proposal to emend the genus. We review the type material of *S. cinctus* located in herbarium of Botanischer Garten der Universität Bern (BERN), and establish the phylogenetic position of the genus which clusters with *Xylophallus* (Schltdl.) E. Fisch. in an exclusively neotropical clade. Our results highlight the importance of studying neglected taxa that impacts knowledge of biodiversity especially for localities that have been poorly collected. The study of herbarium collections can reveal data on forgotten type materials and shed light on the work of pioneering mycologists. This study also brings insights into biogeographical diversity of the phalloid fungi.

Keywords Biodiversity · Gasteroid fungi · Molecular phylogeny · Morphology · Neotropics · 4 new taxa

Introduction

Staheliomyces E. Fisch. was first described in 1921, based on a specimen from Suriname (Fischer 1921). The genus is considered monotypic with the species type *S. cinctus* E. Fisch.,

also known as strangled stinkhorn. Although described from Suriname, Fischer mentioned a first record from Guyana, as a photograph cited as “jungle fungus” on page 74 of Beebe et al. (1917), though there is no taxonomic information associated with the picture. Fischer described the species based on a collection from Gerold Stahel, a botanist who worked for c. 30 years in Paramaribo, Suriname (Reyne 1955). He collected the specimen in 1918 near Coppename River and sent it to E. Fischer (Fischer 1921). *Staheliomyces cinctus* is mainly characterized by the gleba region forming a constricted part of the basidioma above pseudostipe that forms a “belt”, which is the reason for the epithet “*cinctus*.” Fischer presented a very detailed description of the species, but there was little information on the type specimen such as the collection number and herbarium deposition. The lack of a type specimen prevents the development of further comparative taxonomic studies.

Apparently, *S. cinctus* distribution is restricted to the Neotropics, on ombrophilous forests, with formal records for Bolivia (Rocabado et al. 2007), Brazil, specifically in Brazilian Atlantic and Amazon forests (Baseia et al. 2006; Leite et al. 2007; Magnago et al. 2013; Cabral et al. 2014; Trierveiler-Pereira et al. 2019), Costa Rica (Saénz and Nassar 1982), Ecuador (Burr et al. 1996), French Guyana

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(Cheype 2010), Panama (Gube and Piepenbring 2009), South Mexico (Coates et al. 2017), and Suriname (Fischer 1921). In addition, there are 79 occurrences from the neotropics in GBIF (Global Biodiversity Information Facility) that expands the distribution also to Honduras and Colombia (GBIF 2021).

Besides its relatively simple morphology and geographical distribution, as compared to other phalloid fungi, little is known about the ecology of *Staheliomyces*. Facts like the interaction with other species and the usage by humans are yet to be studied. As for phalloids in general, it is well known that insects play major role in spore dispersal. Burr et al. (1996) were able to register this interaction for *S. cinctus* in a specific case of melittophily—dispersion of spores by bees—where they observed the visitation of social bees of the genus *Trigona* on *S. cinctus* basidiomata. In regard to the human usage of species, Trujillo (2009) reported several medicinal, mythological, and ritualistic uses of *S. cinctus* by some indigenous and traditional communities. This species along with other phalloid fungi are also used for medicinal purposes by the Yanomami indigenous people in Brazil, who use it for treatment of leishmaniasis and malaria (Yanomami et al. 2014).

Since the original publication of *S. cinctus*, no other species have been described in the genus. According to Index Fungorum (www.indexfungorum.org) and Flora do Brasil (Cabral 2020), the genus belongs to the family *Phallaceae* (*Phallales*, *Phallomycetidae*) and this systematic position has not been questioned over the years. Several molecular phylogenies for the order *Phallales* have been published (Hosaka et al. 2006; Trierveiler-Pereira et al. 2014; Melanda et al. 2021), but none has included this species, so the phylogenetic position of *Staheliomyces* has not yet been confirmed by DNA analyses. Given the geographical distribution range of the species, it is predicted that some degree of morphological/molecular variation should exist among collections of *S. cinctus*. This type of variation can be seen in the neotropical genus *Xylophallus* (Schltdl.) E. Fisch. (Crous et al. 2018), which is evident when comparing its published descriptions. Collections of *Staheliomyces* have been reported as a single species because no detailed study of the genus has been made. In this study, we have investigated the genus nomenclatural history, morphological/molecular diversity, and phylogenetic placement.

Materials and methods

Morphological analyses and nomenclature

Field trips were made to several areas of Brazilian Amazon rainforest to collect phalloid specimens, especially *Staheliomyces*, following methodologies of Vargas-Isla et al. (2014). Specimens from Brazilian Atlantic Rainforest

deposited at the herbarium of Universidade Federal do Rio Grande do Norte (UFRN-Fungos) were also included for analyses, as well as specimens from a Tropical Wet Forest from Costa Rica (University of Costa Rica Herbarium, USJ). GBIF, which covers data from iNaturalist (<https://www.inaturalist.org>), was consulted to access geographical occurrences of species identified as *Staheliomyces*. Photos from GBIF were downloaded to illustrate the morphological diversity within the genus from South and Central American countries. We followed the Creative Commons (<https://creativecommons.org>) licenses for each downloaded photo, from which we only used the six different licenses that allow the usage of photos for scientific purposes (CC-BY, CC-BY-NC, CC-BY-SA, CC-BY-ND, CC-BY-NC-ND, CC-BY-NC-SA—for licenses' attributions, please check <https://creativecommons.org/licenses/?lang=en>).

Morphological analyses were carried out on fresh and dried material. Macroscopic characters were recorded from field notes and photographs of both collected and herbaria specimens. Fischer named the region where the gleba is located as “belt,” while other authors consider it as “ring” (Saénz and Nassar 1982; Calonge et al. 2005); here, we refer to both glebal region and belt. We considered the portion above the belt as “apical sterile portion,” and the portion below as “pseudostipe.” The constriction—where both pseudostipe and apical sterile portion narrows to the glebal region—measurement was calculated by subtracting the upper part width of pseudostipe from the basal part width of glebal region. The glebal region surface (underneath the gleba) was also described. These measurements were taken using Leica EZ4 stereomicroscope. Color designations follow Küppers (1979). Microscopic details were obtained by mounting slides with dried fragments from different layers and structures of dried basidiomata: apical sterile portion and pseudostipe, belt (glebal region), gleba, volva, and rhizomorphs. Mounts were prepared in 5% potassium hydroxide (KOH) and/or stained with Congo Red dye. Optical Nikon Eclipse Ni (LM) microscope with Nikon DS-Ri1 camera attached, with NIS-Elements AR v.4.51.00 software, was used to make microscopic observations. All illustrations were edited on at GNU Image Manipulation Program (GIMP) version 2.10.24 (<https://www.gimp.org/>).

In order to understand the nomenclatural history, we contacted several herbaria to identify where E. Fischer's collections could have been deposited. After this, we requested information on the existence and conditions of possible type specimens, as well as other collections that could be the original material used to describe *S. cinctus*. We also located the place where several of Fischer's original illustrations are deposited. *Staheliomyces cinctus* is described here based on Fischer's original descriptions, notes, and on the photos of collections deposited at Botanischer Garten der Universität Bern (BERN).

Molecular phylogenetic analyses

Genomic DNA was extracted from a fragment of a basidioma following Hosaka (Hosaka 2009). Three DNA regions were amplified, internal transcribed spacer (ITS), ribosomal large subunit (nc LSU rDNA), and mitochondrial ATPase subunit 6 (*ATP6*), with the primer pairs ITS1/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), and *ATP6-1/ATP6-2* (Kretzer and Bruns 1999), respectively. The amplified regions were visualized on a 1.5% agarose gel stained with GelRed™ (Biotium, Fremont, USA) under UV light. All amplicons were purified with Illustra ExoProStar (GE Healthcare, Chicago, USA) and then sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Waltham, USA) with the same primer pairs, at Federal University of Amazonas (UFAM).

Sequences were visualized and assembled on Geneious R6.1 (Biomatters Ltd.), and then submitted to a BLAST search to check for their integrity and for the closest sequences deposited at GenBank (Benson et al. 2015). We retrieved sequences belonging to *Phallales*, mainly from Hosaka et al. (2006), Trierveiler-Pereira et al. (2014), Marincowitz et al. (2015), Melanda et al. (2020). The alignments and manual edition of aligned matrices were done in AliView v. 1.26 (Larsson 2014) using muscle alignment. For molecular phylogenetic analyses, two datasets were prepared: (A) one with all available sequences from *Phallales*, in order to place the genus *Staheliomyces*, using *ATP6*+nc LSU rDNA concatenated; and (B) a second one composed of sequences of *Mutinus* Fr., *Xylophallus*, and *Staheliomyces*, using an ITS+nc LSU rDNA+*ATP6* concatenated matrix, aiming to understand the possible relationships within the genus *Staheliomyces*. The outgroup was chosen based on previous work (Trierveiler-Pereira et al. 2014). To the dataset A, *Hysterangium album* Zeller & C.W. Dodge and *H. cistophilum* (Tul. & C. Tul.) Zeller & C.W. Dodge were chosen as outgroup, and to dataset B, *Mutinus albo truncatus* B.D.B. Silva & Baseia and *Mutinus verrucosus* T.S. Cabral, B.D.B. Silva, K. Hosaka, M.P. Martin & Baseia were used as outgroup. For both datasets, two different approaches were used, Bayesian and maximum likelihood analyses, each using partitioned dataset. The substitution model for each partition (ITS1, 5.8S, ITS2, nc LSU rDNA, *ATP6*) was chosen using MrModelTest (Nylander 2004). MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) was used to run Bayesian analysis (BA), on two parallel runs executed with four incrementally heated simultaneous MCMC simulations over 3 million generations for both datasets, with trees sampled every 1000 generations for both datasets. Maximum likelihood analyses were run in RAxML (Stamatakis 2014) using partitioned dataset, with estimated proportion of invariable sites (GTRGAMMA + I), at CIPRES Science Gateway (Miller et al. 2010). Trees were

visualized and edited in FigTree version 1.4.2. All analyses were submitted to TreeBASE under ID 27667.

Additionally, we explored DNA sequences diversity in order to better understand how species are structured. We performed a sequence-based method of species delimitation, the Bayesian Posterior Tree Poisson (bPTP) analysis, which is indicated to small datasets and especially for species represented by singletons (Zhang et al. 2013). This method infers species boundaries based on a phylogenetic tree. Thus, an unrooted input tree was generated in MrBayes from a concatenated dataset composed only of *Staheliomyces* DNA sequences, and following the parameters: HKY+I +G for ITS partition and GTR+I+G for nc LSU rDNA and *ATP6* partitions; and 2 million generations with tree sampled every 1000 generations. The bPTP analysis was run in a web server (<http://species.h-its.org/ptp/>) with 500,000 MCMC generations and the remaining default settings. Distances matrices were generated based on ITS sequences, calculated both between all specimens, and between one representative of each species defined by bPTP, using *dist.dna* function of the *ape* package (Paradis and Schliep 2019), with default values; the distances were plotted in a heatmap using *heatmapSpp* function of *spider* package (Brown et al. 2012) for R environment.

Results

Phylogenetic analysis

In total, we generated 37 new sequences of phalloid fungi in this study (8 ITS, 17 nc LSU rDNA, and 12 *ATP6*). After DNA sequencing and retrieving sequences from GenBank, in the dataset A, 86 taxa were included (including *Hysterangium album* and *H. cistophilum* as outgroup), with 156 sequences, from which 75 were *ATP6* and 81 were nc LSU rDNA (Online Resource 1 – table). The final aligned matrix had 1444 characters (631 for *ATP6* and 813 for nc LSU rDNA). For the two matrices (nc LSU rDNA and *ATP6*), the GTR+I+G model was selected by MrModelTest. In the phylogenetic trees of *Phallales* obtained either with Bayesian or with maximum likelihood, the genus *Staheliomyces* was placed in family *Phallaceae*, grouping with genus *Xylophallus* (Fig. 1), with high support values (posterior probability, pp = 1; maximum likelihood bootstrap, MLbs = 98).

The dataset B had 13 taxa and 29 sequences, of which 13 were ITS, 12 were nc LSU rDNA and 4 were *ATP6* (Online Resource 1 – table). The final aligned matrix had 2055 positions (276 ITS1, 166 5.8S, 237 ITS2, 815 nc LSU rDNA, and 727 *ATP6*). The following models were chosen for each partition: HKY for ITS1; JC for 5.8S; HKY+I for ITS2; GTR+G for nc LSU rDNA; and F81+I

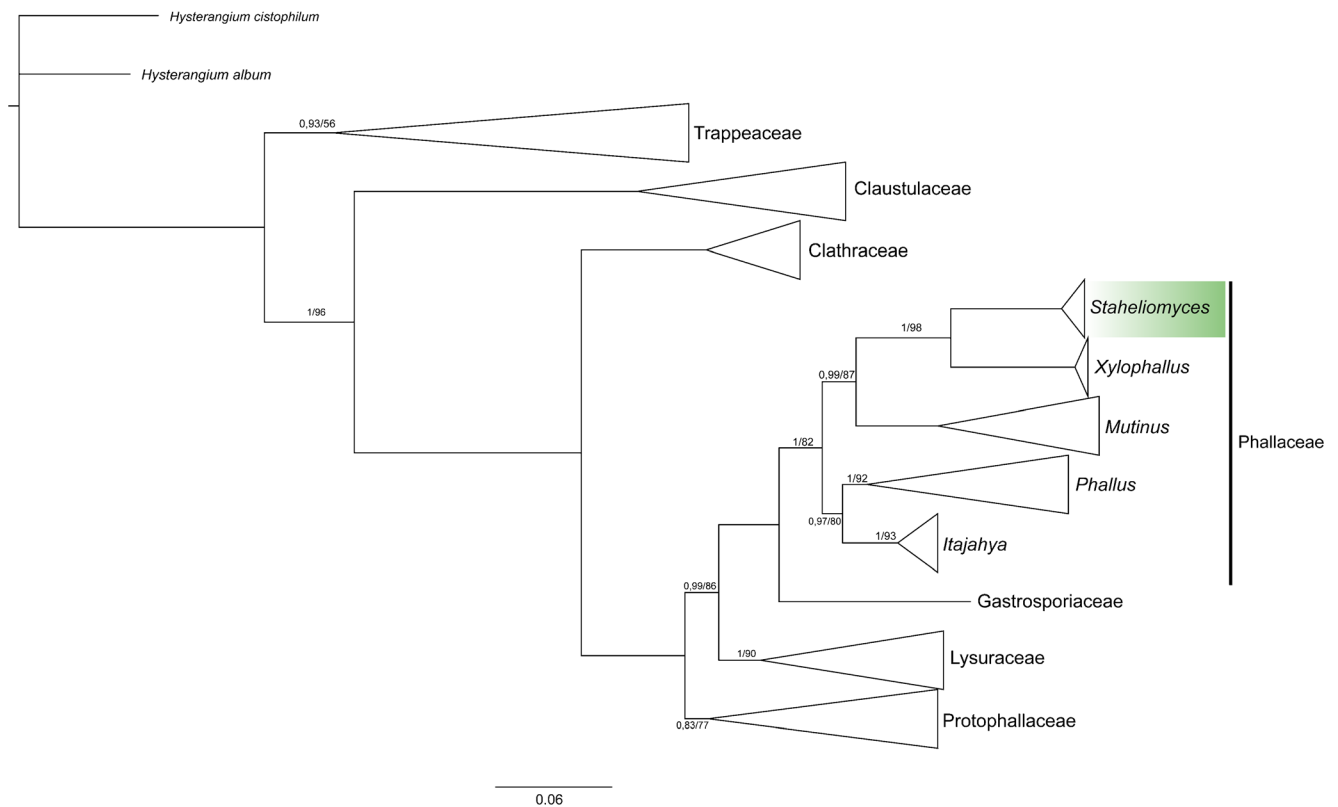


Fig. 1 Consensus phylogenetic tree of order Phallales, obtained by Bayesian inference with dataset A (*ATP6* + nc LSU rDNA). Families clades are collapsed, except for Phallaceae, where genera clades are

collapsed. In green, the phylogenetic placement of *Staheliomyces* genus. Numbers on nodes indicate posterior probabilities (pp, before slash) and maximum likelihood bootstrap values (MLbs, after slash)

for *ATP6*. This dataset resulted in a phylogeny with three different genera of Phallaceae (Fig. 2). In *Staheliomyces*, it is possible to delimit 5 species-level clades, of which four correspond to new species, described and discussed in the next section. We were able to get material for phylogenetic analyses from the specimen INPA 272311 during field trip, but unfortunately, it was not found at herbarium by the time of morphological analyses. Even so, we have decided to include it in the phylogenetic tree because it provides a better node support value, although we could not morphologically describe it.

The bPTP method estimated a mean number of 5 species for the dataset, with acceptance rate of 0.72 (see Online Resource 2), in accordance with phylogenetic tree and morphological analyses, although bPTP clustered some species with relatively low support value (*S. cylindricus* = 0.316 and *S. candeliformis* = 0.368). The pairwise distances of ITS sequences between the 5 species defined by bPTP is shown on Fig. 2b, where distances ranged from 1 to 3%, while between specimens of the same species the minimum genetic distance was 0.2% (Online Resource 3). These analyses also indicate *Staheliomyces* sp. INPA 272311 as part of *S. quadratus*

species; however, we prefer to not maintain it as such, since we do not have enough morphological evidence.

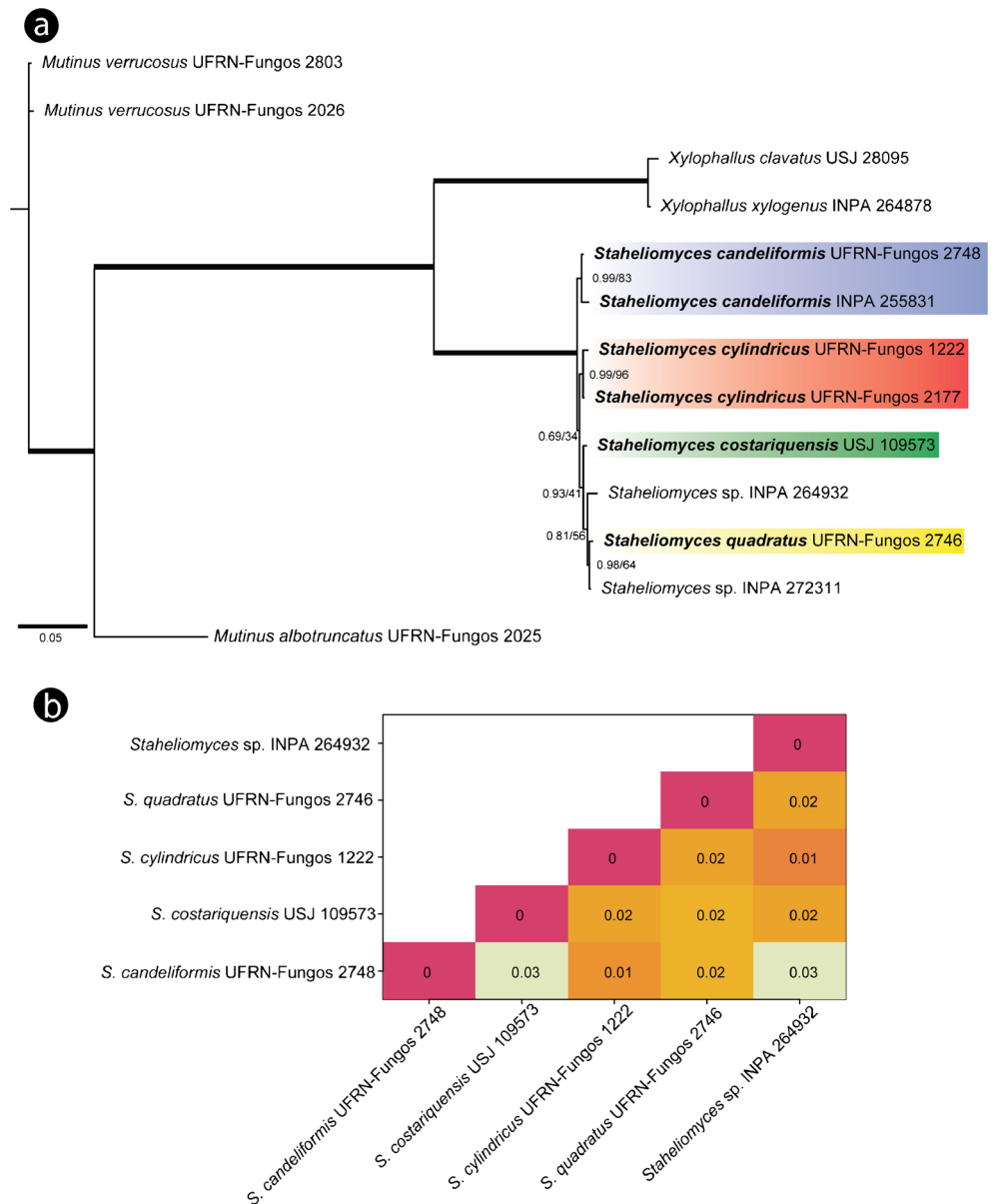
Taxonomy

In this section, we provide a proposal to emend the genus *Staheliomyces*, the translated description from Fischer (1921) and type specimens for *Staheliomyces cinctus* (Fig. 3), as well as descriptions and illustrations (Figs. 4 and 5) for four new species. The main morphological differences among *Staheliomyces* species can be found in Table 1, and the species geographical distribution is in Fig. 6.

Emended description of the genus *Staheliomyces* E. Fisch., Mitt. naturf. Ges. Bern: 142 (1921) [1920] (Fischer 1921) emend. Melanda, N.M. Assis & T.S. Cabral.

Expanded basidioma epigeous, pseudostipitate, pseudostipe white, hollow with lateral perforations, divided in pseudostipe and apical sterile portion. Hypogeous *volva* reddish brown, whitish to white, remaining attached to pseudostipe, with white *rhizomorph* attached to the volva base. Pseudostipe cylindrical constricts to form a belt where the glebal mass is found. *Glebal region* short-cylindrical,

Fig. 2 a Consensus phylogenetic tree of family *Phallaceae*, obtained by Bayesian inference with dataset B (ITS + nc LSU rDNA + *ATP6*), showing the infrageneric phylogenetic relationships in *Staheliomyces*. Colored clades correspond to new species: *S. candeliformis* (blue), *S. costariquensis* (green), *S. quadratus* (yellow), *S. cylindricus* (red). Posterior probabilities values and maximum likelihood (ML) bootstrap values are indicated on nodes (pp/ MLbs). Nodes fully supported are indicated by thicker branches. **b** Heat map representing the distance matrix between species and specimens of *Staheliomyces*, based on pairwise distances from the ITS alignment



doliiform, elongate-doliiform, cylindrical, or almost squared with rounded sides, with rugulose or reticulate texture. *Apical sterile portion* tear-drop, triangular or elongate, with apical aperture present or absent. *Gleba* olive brown, mucilaginous. *Basidiospores* cylindrical to very narrowly elliptic, hyaline, smooth.

The type species: *Staheliomyces cinctus* E. Fisch. (Fischer 1921).

Staheliomyces candeliformis N.M. Assis, Melanda & T.S. Cabral, sp. nov. Figs. 4a–d, 5a–c

Mycobank: 838513

Typification: Brazil, Amazonas, Codajás, 3°47'08.9" S, 62°01'08.8" W, 12 December 2012, leg. Cabral TS 30, holotype (INPA 255831). GenBank accessions: MW546289

(ITS), MW546304 (nc LSU rDNA). Brazil, Amazonas, Parintins, Açaí Community, 2°37'42.7" S, 56°32'49.9" W, 5 November 2015, leg. Cabral TS 234, paratype (UFRN-Fungos 2748). GenBank accessions: MW546288 (ITS), MW546303 (nc LSU rDNA).

Etymology: From the Latin *candela* (candle) referring to the basidiomata shape resembling a lighted candle.

Diagnosis: This species is characterized by its tear-drop shaped apical sterile portion, a pronounced constriction in the pseudostipe that forms the glebal region, reticulate belt surface under the gleba, and reddish brown volva. The reddish brown volva and the reticulate surface under the gleba is also found in *S. quadratus*; however, they differ by the apical sterile portion shape (tear-drop in *S. candeliformis* and elongate with a square tip in *S. quadratus*).

Macromorphological description: *Unexpanded basidiomata* not observed. *Expanded basidiomata* epigeous, 60–130 mm long. Hypogeous *volva* remaining attached to pseudostipe, 25 mm high \times 27 mm diam. (from dried *volva*), reddish brown when dried (Y_{70} , M_{70} , C_{40}). White *rhizomorph* (N_{00} , M_{00} , C_{00}) attached to the *volva* base. *Pseudostipe* 35–87 mm long, widening from the middle to the upper part (8–25 mm diam. basal, 10–27 mm diam. mid-pseudostipe, and 31 mm diam. on upper part), hollow, spongy, cylindrical, with several lateral perforations, smallest (0.5 mm diam.) near the base, larger (4–7 mm diam.) in the middle part, becoming smaller (1–2 mm diam.) near to the constriction, white (N_{00} , M_{00} , C_{00}), upper part of pseudostipe narrowing to 3–11 mm in diam. at the constriction of belt where the glebal mass is spread. *Glebal region* short-cylindrical, 9–11 mm long, basal part of belt widest (7–19 mm diam. basal, 9–17 mm diam. middle, 6–14 mm diam. upper part), belt surface reticulate under gleba. *Apical sterile portion* tear-drop shaped, 14.5–30 mm long, 14 mm diam. base, 22 mm diam. widest part, 7 mm upper part, apex thin, white (N_{00} , M_{00} , C_{00}), hollow, spongy, with several lateral perforations (1–5 mm diam.), apical aperture present. *Gleba* olive brown (N_{99} , A_{50} , M_{10}), mucilaginous; odor neutral.

Micromorphological description: *Volva* formed by filamentous hyphae 2.7–5.4 μm ($x = 3.9 \pm 0.5$) diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$), with crystal deposits in globose cells widely distributed among the hyphae. *Rhizomorph* composed of filamentous hyphae 2.3–5.8 μm ($x = 3.9 \pm 0.9$) diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$), with crystal deposits in globose cells widely distributed among the hyphae. *Pseudostipe* pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 27.9–68.7 \times 24.6–56.5 μm ($x = 47.6 \pm 9.0 \times 41.1 \pm 7.8$, $Q_m = 1.16 \pm 0.07$), hyaline, walls thin ($<2.8 \mu\text{m}$). *Glebal region* tissue pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 15.6–44.7 \times 14.1–32.9 μm ($x = 27.5 \pm 5.4 \times 22.9 \pm 4.9$, $Q_m = 1.20 \pm 0.10$), hyaline, walls thin ($<2.5 \mu\text{m}$). *Apical sterile portion* pseudoparenchymatous, composed of globose to ellipsoid cells, 22.0–66.8 \times 18.8–55.1 μm ($x = 42.3 \pm 9.4 \times 36.9 \pm 9.0$, $Q_m = 1.15 \pm 0.07$), hyaline, walls thin ($<2.5 \mu\text{m}$). *Basidiospores* 3.0–3.5 \times 1.5–2.0 μm ($x = 3.3 \pm 0.1 \times 1.7 \pm 0.1$, $Q_m = 1.88 \pm 0.12$, $n = 30$), cylindric to very narrowly elliptic, hyaline, smooth.

Habitat and distribution: On decaying wood in Brazilian Amazon Rainforest domain.

Notes: The tear-drop shaped apical sterile portion, the pronounced constriction of the pseudostipe that forms the belt, reticulate texture of the belt surface beneath the gleba, and reddish brown *volva* are distinguishing features of *S. candeliformis*. This species differs from *S. cinctus* by the belt shape, apical sterile portion shape, as well as by the *volva* color. The reddish brown *volva* and the reticulate texture below the

gleba are also found in *S. quadratus*; however, the latter differs by having an elongate apical sterile portion with a square apex. *S. costaricensis* differs by having a triangular-shaped apical sterile portion with a slightly square apex, rugulose surface texture of the belt, and by the rusty orange outer *volval* surface (Table 1). Phylogenetically, it is the most distinctive clade, formed by specimens from Amazonia, and a sister group of all other *Staheliomyces* species proposed here.

Staheliomyces cinctus E. Fisch., Mitt. naturf. Ges. Bern: 142, Figure 1, p. 138. (1921) [1920]. Fig. 3

Mycobank: 219534

Typification: Suriname, Paramaribo: near Coppename river, in a forest behind a “Caraib” village, 12 June 1918, leg. G. Stahel, lectotype (designated here) deposited at the Wet collection of the Botanical Garden of the University of Bern (BERN), temporarily without voucher or deposit number (!).

Description (loosely translated from Fisher (1921)): “The total height of the basidiome reaches c. 12 cm, the largest diameter reaches 2 cm. Pseudostipe 8.5 cm long, the color was pure white. The spore mass (*gleba*) covers a conspicuously constricted, belt-shaped zone, c. 1 cm high, about 2 cm below the tip, forming a compact greenish mass. The spores are c. 3.5 μm long and have a diameter of 1.5 μm . Above the spore mass follows the approximately 2 cm high spore-free apex (*apical sterile portion*) of the receptaculum. This suddenly widens quite strongly immediately above the spore-covered belt and then tapers conically towards the tip (“mitraeformis,” i.e., in shape of a miter). The wall of this spore-free part (*apical sterile portion*) of the head is essentially the same as the wall of the stem below (*pseudostipe*) the spore-covered belt, only it is extremely delicate and thin and has therefore become quite slack in alcohol. Its chambers are for the most part open to the outside, and one notices several larger openings running through the whole wall. It is also perforated at the top of the head by an opening that appears to have been made in advance. The stem initially expands immediately below the spore-bearing part just like the cap above it, then it gradually decreases in diameter further downwards. Almost all of its chambers are open to the outside in the upper part; In addition, quite a number of large openings penetrating the entire thickness of the wall are striking in the thicker upper part of the stem.”

Habitat and distribution: Suriname.

Specimens examined: Suriname, Calebar creek, on the lower Coppename River, June 1920, leg. G. Stahel. Forest next to the Raleigh Falls on the upper Coppename River (65 km from Paramaribo), 21 August 1920, leg. G. Stahel. “Jodensavanne” at Suriname River, 1921, leg. Junker. Sipaliwini district, “between Tockoemoetoe and Hendrikstop,” 22 March 1922, leg. G. Stahel. Sipaliwini, Mombabasoe, on the Saramacca River,

Fig. 3 *Staheliomyces cinctus* type **a, b, c**; basidiome without identification tag **d**; and “cotypus” **e** determined by E. Fischer (Photos: Katja Rembold).



27 March 1922, leg. G. Stahel. Brokopondo, Brownsberg Nature Park, 4°56'51.6" N 55°10'09.5" W, July 1924, leg. G. Stahel (determined as “cotypus” by Fischer), and 30 January 1925, leg. G. Stahel. All deposited at the wet collection of the Botanical Garden of the University of Bern (BERN), temporarily without voucher or deposit number (!).

Notes: *Staheliomyces cinctus*, the type species of *Staheliomyces*, is characterized by its pseudostipe thickening from the middle to the upper part; several lateral perforations in pseudostipe, smaller in the inferior half of pseudostipe, larger in the middle until near to the constriction; glebal region doliiform; apical sterile portion triangular; volva whitish. The thickened pseudostipe of *S. cinctus* differs from *S. cylindricus*, which has constant width until the belt. The lateral perforations of the pseudostipe of *S. cinctus* differ from *S. costariquensis* and *S. quadratus* in that these two species have several lateral perforations over most of the pseudostipe. The doliiform shape of the glebal region of *S. cinctus* is slightly similar to that of *S. costariquensis*, but the latter differs by having a more elongate upper part to its belt. The triangular apical sterile portion of *S. cinctus* is very different from

S. cylindricus which has a wider rounded tip; from *S. candeliformis* which has a tear-drop like tip; and from *S. quadratus* which has an elongate, squared tip. The triangular slightly squared tip of *S. costariquensis* is somewhat similar to *S. cinctus*, with the tip of the latter not being squared-off.

Staheliomyces costariquensis Ovrebo, Melanda, N.M. Assis & T.S. Cabral, sp. nov. Figs. 4c–h, 5d–f

Mycobank: 838516

Typification: Costa Rica, Heredia Prov., Lindero Occidental, La Selva Biological Station and Reserve, near Puerto Viejo, 17 July 1986, leg. C. Ovrebo 2213, holotype (USJ 109573). GenBank accessions: MW546290 (ITS), MW546305 (nc LSU rDNA), MW543438 (ATP6).

Etymology: With reference to the locality of the collection, Costa Rica.

Diagnosis: This species is characterized by the whitish to rusty orange volval surface, triangular apical sterile portion with a slightly square tip, glebal region elongate, and doliiform, with a rugulose belt surface under gleba. It differs from other

species mostly in that *S. candeliformis* has a tear-drop shaped apical sterile portion; *S. quadratus* has a reddish brown volva; and *S. cinctus* has a doliiform glebal region.

Macromorphological description: *Unexpanded basidiomata* globose to subglobose 20–30 mm diam., whitish (N_{00} , C_{00} , Y_{00}) to rusty orange (Y_{70} , M_{40} , C_{00}). *Expanded basidiomata* epigeous, 150–160 mm long. *Volva* 21–30 mm high \times 26–28 mm diam., whitish (N_{00} , C_{00} , Y_{00}) to rusty orange (Y_{70} , M_{40} , C_{00}), ovoid in fresh material. White *rhizomorph* (N_{00} , M_{00} , C_{00}) attached to the volva base. *Pseudostipe* 70–90 mm long, widening from the middle to the upper part (17–20 mm diam. basal, 26–23 mm diam. mid-pseudostipe, and 25–27 mm diam. on upper part), white (N_{00} , M_{00} , C_{00}), hollow, spongy, cylindrical, with several lateral perforations, smallest (3 mm diam.) near the base, becoming larger (6–8 mm diam.) toward the constriction at the belt, upper part of pseudostipe narrowing to 5–7 mm diam. at the constriction of belt where the glebal mass is spread. *Glebal region* doliiform elongate, 21–29 mm long, thickest in the middle part (20 mm diam. basal, 22.5–23 mm diam. middle, 13 mm upper part), belt surface (under gleba) white (N_{00} , M_{00} , C_{00}), rugulose. *Apical sterile portion* 28 mm long, triangular shape with slightly square tip, 15 mm base, 14 mm medium, and 8 mm upper part, white (N_{00} , M_{00} , C_{00}), hollow, spongy, with several lateral perforations (5–8 mm diam.), apical aperture present. *Gleba* olive brown (N_{99} , A_{50} , M_{10}), mucilaginous.

Micromorphological description: *Volva* formed of filamentous hyphae 3–8.6 ($x = 4.5 \pm 1.0$) μm diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin (<1 μm). *Rhizomorph* formed of filamentous hyphae 1.5–6.3 ($x = 3.5 \pm 0.8$) μm diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin (<1 μm), with crystal deposits in globose cells widely distributed among the hyphae. *Pseudostipe* pseudoparenchymatous, composed of globose to elongate cells, 35–70 \times 35–61 μm ($x = 53.5 \pm 6.5 \times 45 \pm 5.4$, $Q_m = 1.18 \pm 0.10$), hyaline, walls thin (<2.5 μm). *Glebal region* tissue pseudoparenchymatous, composed of globose to subglobose cells, 13–36 \times 12–35 μm ($x = 26 \pm 4.8 \times 25 \pm 4.1$, $Q_m = 1.06 \pm 0.06$), hyaline, walls thin (<2.5 μm). *Apical sterile portion* pseudoparenchymatous, composed of globose to elongate cells, 27–58 \times 25–44 μm ($x = 39 \pm 5.8 \times 35 \pm 4.2$, $Q_m = 1.15 \pm 0.13$), hyaline, walls thin (<2 μm). *Basidiospores* 3–3.6 \times 1.5–2 μm ($x = 3.37 \pm 0.1 \times 1.8 \pm 0.1$, $Q_m = 1.84 \pm 0.1$, $n = 30$), cylindrical to very narrowly elliptic, hyaline, smooth.

Habitat and distribution: On decaying wood. Known from Atlantic Lowland tropical rainforest of Costa Rica.

Notes: *Staheliomyces costaricensis* is mainly characterized by the short triangular apical sterile portion pseudostipe with a slightly square tip, relatively long belt with a rugulose surface texture beneath the gleba and a whitish to rusty orange volval surface. This species is comparable to *S. cinctus*, *S. candeliformis*, and *S. quadratus* by the widening of the pseudostipe from the middle to the upper part, and with *S. cylindricus* by the rugulose belt surface. However, these three species differ from *S. costaricensis* by the following: *S. candeliformis* has a tear drop-shaped apical sterile portion; *S. quadratus* has a reddish brown volva; and *S. cinctus* has a doliiform belt. Another morphological characteristic that differentiates *S. cinctus* from *S. costaricensis* is the perforation pattern along the pseudostipe. In *S. cinctus*, perforations are bigger in the central region of the pseudostipe until near to the belt, whereas *S. costaricensis* presents bigger perforations along the entire pseudostipe. Regarding *S. cylindricus*, it has a cylindrical belt shape and elongate apical sterile portion. Other specimens from Costa Rica, according to GBIF data, show morphological variations especially in relation to the apical sterile portion (Fig. 7c, d, e). *Staheliomyces costaricensis* has been found, so far, only in the northeastern region of the country, in a lowland tropical rainforest (Ovrebø and Baroni 1988; Hartshorn and Himmel 1994).

Staheliomyces cylindricus Melanda, N.M. Assis & T.S. Cabral, sp. nov. Figs. 4i–l, 5g–i

Mycobank: 838514

Typification: Brazil, Paraíba, Mamanguape, REBIO Guaribas, 7°10'36.7" S, 35°18'03.3" W, 11 July 2013, leg. Sousa JO, Silva BDB, Rodrigues ACM and Cruz RHFS, holotype (UFRN-Fungos 2177). GenBank accessions: MW546292 (ITS), MW546307 (nc LSU rDNA), MW543439 (ATP6). Brazil, Rio Grande do Norte, Parnamirin, Mata do Jiqui, 6°30'13.3" S, 35°52'56.7" W, 28 August 2008, leg. Fazolino EP and Ali M, paratype (UFRN-Fungos 1222). GenBank accessions: MW546291 (ITS), MW546306 (nc LSU rDNA).

Etymology: From Latin “*cylindricus*” meaning cylindrical, with reference to elongate cylindrical basidiomata.

Diagnosis: This species is characterized by its constant width over the entire length of the pseudostipe, the elongate apical sterile portion with rounded apex, the minimal narrowing of the pseudostipe at the cylindrical belt, rugulose belt surface under gleba, and the white volva. *Staheliomyces*

cinctus, *S. costaricensis*, and *S. candeliformis* differ from *S. cylindricus* by their shorter apical sterile portion, while *S. quadratus* differs by the square apex.

Macromorphological description: *Unexpanded basidiomata* not observed. *Expanded basidiomata* epigeous, 120–140 mm long. Hypogeous *volva* remaining attached to pseudostipe 26–30 mm high \times 17–26 mm diam., white (N_{00} , M_{00} , C_{00}). White *rhizomorph* (N_{00} , M_{00} , C_{00}) attached to the *volva* base. *Pseudostipe* 60–85 mm long \times 11–13 mm diam., constant width over the entire length of the pseudostipe, hollow, spongy, cylindrical, with several perforations smallest (1–3 mm diam.) near the base, larger (3–8 mm diam.) in the middle part, becoming smaller (1–2 mm diam.) until close to the constriction, white (N_{00} , M_{00} , C_{00}), upper part of pseudostipe narrowing to 1 mm in diam. at the constriction of belt where the glebal mass is spread. *Glebal region* cylindrical, 11–25 mm high, slightly tapering at upper part (9–13 mm diam. base, 9–11 mm diam. medium and 6–9 mm diam. upper part), belt surface rugulose under gleba. *Apical sterile portion* elongate, 24–27 mm high, approximate width over the entire length 5–9 mm diam., with wide rounded tip, white (N_{00} , M_{00} , C_{00}), hollow, spongy, with several lateral perforations (1–4 mm diam.), apical aperture absent. *Gleba* olive brown (N_{99} , A_{50} , M_{10}), mucilaginous; odor neutral.

Micromorphological description: *Volva* composed of filamentous hyphae 1.7–3.9 μm ($x = 2.7 \pm 0.4$) diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$), with crystal deposits in globose cells widely distributed among the hyphae. *Rhizomorph* composed of filamentous hyphae 2.6–5 μm ($x = 3.6 \pm 0.6$) diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$), with crystal deposits in globose cells widely distributed among the hyphae. *Pseudostipe* pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 27–64 \times 24–60 μm ($x = 45.4 \pm 8.5 \times 40.1 \pm 6.9$, $Q_m = 1.13 \pm 0.07$), hyaline, walls thin ($<2.0 \mu\text{m}$). *Glebal region* pseudoparenchymatous, composed of globose to ellipsoid cells, 18–43 \times 13–34 μm ($x = 28.5 \pm 5.9 \times 22.5 \pm 4.3$, $Q_m = 1.27 \pm 0.08$), hyaline, walls thin ($<1.5 \mu\text{m}$). *Apical sterile portion* pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 17.5–45 \times 16–42 μm ($x = 30.6 \pm 6.1 \times 26.3 \pm 5.3$, $Q_m = 1.17 \pm 0.08$), hyaline, walls thin ($<2.0 \mu\text{m}$). *Basidiospores* 2.9–3.8 \times 1.7–2.3 μm ($x = 3.4 \pm 0.1 \times 1.9 \pm 0.08$, $Q_m = 1.84 \pm 0.09$, $n=30$), cylindrical to very narrowly elliptic, hyaline, smooth.

Habitat and distribution: On the soil on litter in Atlantic Rainforest domain.

Notes: *Staheliomyces cylindricus* is the most distinctive species among our collections because of its elongate, rounded, apical sterile portion, cylindrical-shaped belt, and white *volva*. The upward narrowing of the pseudostipe toward the belt is less in comparison to all species (Table 1)

so is also a diagnostic feature. This species shares with *S. costaricensis* the rugulose belt surface, rather than reticulated as in *S. quadratus* and *S. candeliformis*. *Staheliomyces cylindricus* has an elongate apical sterile portion while those of *S. cinctus*, *S. costaricensis*, and *S. candeliformis* have a shorter apical sterile portion. The elongate pseudostipe is also found in *S. quadratus* but the apex is square compared to the rounded apex in *S. cylindricus*. Phylogenetically, *S. cylindricus* is in a well-supported group ($pp = 0.99$, $MLbs = 96$), and a sister clade of a group formed by *S. costaricensis*, *S. quadratus*, and *Staheliomyces* sp. INPA 264932 and INPA 272311.

Staheliomyces quadratus N.M. Assis, Melanda, T.S. Cabral, sp. nov. Figs. 4m–p, 5j–l

Mycobank: 838515

Typification: Brazil, Amazonas, Manaus, Reserva Ducke, 2°54'53.3" S, 59°58'39.9" W, 14 January 2014, leg. Cabral TS 84, holotype (UFRN-Fungos 2746). GenBank accessions: MW546286 (ITS), MW546301 (nc LSU rDNA).

Etymology: With reference to square tip apical sterile portion.

Diagnosis: This species is characterized by the square tip of the apical sterile portion that possesses an apical pore, belt having rounded sides, belt surface reticulate under gleba, and by the reddish brown *volva*. *Staheliomyces cinctus* and *S. costaricensis* differ from *S. quadratus* by the shape and texture of belt, while the color of the *volva* is different in *S. cylindricus* (whitish) and *S. costaricensis* (rusty orange).

Macromorphological description: *Unexpanded basidiomata* not observed. *Expanded basidiomata* epigeous, 116 mm high. Hypogeous *volva* remaining attached to pseudostipe, 22 mm high \times 20 mm diam. (from dried *volva*), subglobose, reddish brown (Y_{70} , M_{70} , C_{40}) when dry. White *rhizomorph* (N_{00} , M_{00} , C_{00}) attached to the *volva* base. *Pseudostipe* 60 mm long, widening from the middle to the upper part (17 mm diam. basal, 20 mm diam. mid-pseudostipe, and 25 mm diam. on upper part), hollow, spongy, cylindrical, with several lateral perforations, 5–8 mm diam. to the fullest extent, white (N_{00} , M_{00} , C_{00}), upper part of pseudostipe narrowing to 10 mm in diam. at the constriction of belt where the glebal mass is found. *Glebal region* almost squared with rounded sides, 15 mm high, wider in the middle part (15 mm diam. basal and upper parts, 17 mm diam. middle part), with belt surface reticulate under gleba. *Apical sterile portion* 40 mm high, tapered to apex (20 mm diam. basal, 17 mm middle, 14 mm diam. upper part), square at the tip, white (N_{00} , M_{00} , C_{00}), hollow, spongy, with several lateral perforations (1–7 mm diam.), apical aperture present. *Gleba* olive brown (N_{99} , A_{50} , M_{10}), mucilaginous; odor neutral.

Macromorphological description: *Volva* formed of filamentous hyphae 2.8–6.3 ($x = 4.4 \pm 0.7$) μm diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$). *Rhizomorph* formed of filamentous hyphae 2.2–4.6 μm ($x = 3.2 \pm 0.6$) diam., regularly septate, branched, tips inflated, clamp connections absent, hyaline, walls thin ($<1 \mu\text{m}$), with crystal deposits in globose cells widely distributed among the hyphae. *Pseudostipe* pseudoparenchymatous, composed of globose to ellipsoid cells, 20–62 \times 18–49 μm ($x = 40.9 \pm 11.3 \times 32.9 \pm 5.9$, $Q_m = 1.22 \pm 0.14$), hyaline, walls thin ($<2.5 \mu\text{m}$). *Glebal region* pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 17–40 \times 15–42 μm ($x = 26.9 \pm 4.3 \times 23.9 \pm 4.1$, $Q_m = 1.15 \pm 0.05$), hyaline, walls thin ($<1.5 \mu\text{m}$). *Apical sterile portion* pseudoparenchymatous, composed of globose to ellipsoid cells, 25–50 \times 20–40.5 μm ($x = 37.3 \pm 5.6 \times 31.4 \pm 4.9$, $Q_m = 1.19 \pm 0.09$), hyaline, walls thin ($<2.5 \mu\text{m}$). *Basidiospores* 2.9–3.8 \times 1.5–2 μm ($x = 3.4 \pm 0.1 \times 1.8 \pm 0.1$, $Q_m = 1.83 \pm 0.14$, $n = 30$), cylindrical to very narrowly elliptic hyaline, smooth.

Habitat and distribution: On the soil on litter in Brazilian Amazon Rainforest domain.

Notes: *Staheliomyces quadratus* is comparable to *S. cinctus*, *S. costaricensis*, and *S. candeliformis* by the pseudostipe that widens from the middle to the upper part, and by the middle part with big lateral perforations (4–8 mm diam.); however, *S. cinctus* and *S. costaricensis* have a triangular apical sterile portion, and *S. candeliformis* has a tear drop-shaped apical sterile portion. *Staheliomyces quadratus* has a squared tip apical sterile portion. The belt shape differs in these species: *S. cinctus*, *S. costaricensis*, *S. candeliformis*, and *S. quadratus*: doliiform, doliiform elongate, short-cylindrical, and almost squared with rounded sides, respectively. The reticulate texture of belt beneath the gleba in *S. quadratus* is the same of *S. candeliformis*, but *S. cylindricus* and *S. costaricensis* have a rugulose texture. *Staheliomyces quadratus* and *S. candeliformis* also share the same reddish brown volva color, while the volva is whitish in *S. cylindricus* and *S. cinctus*, and rusty orange in *S. costaricensis*.

***Staheliomyces* sp.** Figs. 4q–t, 5m–o

Macromorphological description: *Unexpanded basidiomata* not observed. *Expanded basidiomata* epigeous, 127 mm long. Hypogeous *volva* remaining attached to pseudostipe, 27 mm high \times 22 mm diam., white (N_{00} , M_{00} , C_{00}). White *rhizomorph* (N_{00} , M_{00} , C_{00}) attached to the volva base. *Pseudostipe* 79 mm long, widening from the middle to the upper part (18 mm upper part and 15 mm diam. base), dehydrated material measuring 74 mm long \times 9 mm diam., hollow, spongy, cylindrical, with several lateral perforations, smaller (2 mm diam.) in the lower half, becoming larger (5 mm) from the middle to

the constriction with an abrupt increase in size, white (N_{00} , M_{00} , C_{00}), upper part of pseudostipe narrowing to 7 mm in diam. at the constriction of belt where the glebal mass is spread. *Glebal region* 13 mm high (7 mm diam. upper part, 11 mm diam. middle, 11 mm diam. base), with reticulate surface texture beneath the gleba. *Apical sterile portion* 8 mm long (5 mm diam. upper part, 8 mm diam. middle, 8 mm diam. base), white (N_{00} , M_{00} , C_{00}), hollow, spongy, with several lateral perforations. *Gleba* olive brown (N_{99} , A_{50} , M_{10}), mucilaginous.

Microscopical characters: *Volva* and *rhizomorph* lacking on dried material. *Pseudostipe* pseudoparenchymatous, composed of globose to broadly ellipsoid cells, 26–64 \times 22–56 μm ($x = 40.2 \pm 8.2 \times 35.2 \pm 7.8$, $Q_m = 1.14 \pm 0.05$), hyaline, walls thin ($<2.6 \mu\text{m}$). *Glebal region* pseudoparenchymatous, composed of globose to elongate cells, 16.5–30 \times 13–26 μm ($x = 23.7 \pm 3.0 \times 20.0 \pm 2.4$, $Q_m = 1.18 \pm 0.07$), hyaline, walls thin ($<2.0 \mu\text{m}$). *Apical sterile portion* pseudoparenchymatous, composed of globose to elongate cells, 27–71 \times 24–62 μm ($x = 50.0 \pm 7.6 \times 41.3 \pm 6.6$, $Q_m = 1.21 \pm 0.07$), hyaline, walls thin ($<2.0 \mu\text{m}$). *Basidiospores* 2.9–3.7 \times 1.3–2.2 μm ($x = 3.16 \pm 0.2 \times 1.68 \pm 0.2$, $Q_m = 1.89 \pm 0.19$, $n=30$), cylindrical to very narrowly elliptic, hyaline, smooth.

Habitat and distribution: In soil on litter in Brazilian Amazon Rainforest domain.

Specimen examined: Brazil, Pará, Belterra, Flona Tapajós, Comunidade Maguary, 2°48'12.0" S, 55°00'01.0" W, 30 March 2014, leg. Cabral TS 176 (INPA 264932). GenBank accessions: MW546287 (ITS), MW546302 (nc LSU rDNA), MW543437 (ATP6).

Notes: In *Staheliomyces* sp., distinctive characteristics are the variation in the perforation pattern along the pseudostipe as well as the apical sterile portion that is shorter than the glebal region (8 and 13 mm high, respectively) compared to the other specimens discussed here. Compared to *S. cinctus*, this species has different format of belt (doliiform in *S. cinctus* and tuned a little in the center in *Staheliomyces* sp.) and larger expanded basidioma (150–160 mm high in *S. cinctus*; 127 mm high in *Staheliomyces* sp.). In the materials observed in this study, the shape of the apical sterile portion has been shown to be an important morphological character in species differentiation. However, only one basidioma was collected of *Staheliomyces* sp. Since we did not observe this character (apical sterile portion smaller than belt) in other species analyzed in this study, it was not possible to verify whether it is an individual characteristic of the species or just a failure in the formation of this structure. Therefore, as it was not possible to confirm the authenticity of all morphological characteristics, we add this specimen here as *Staheliomyces* sp. Also, this specimen groups in the clade with *S. quadratus* and *Staheliomyces* sp. (INPA 272311), but with low support value (pp = 0.81; MLbs =

56). Thus, more collections of this fungus are necessary to confirm whether it is a new species.

Key to *Staheliomyces* species

1. Volva white/whitish.....2
- 1'. Volva not white/whitish3
2. Apical sterile portion triangular; belt shape doliiform..... *S. cinctus*
- 2'. Apical sterile portion elongate with wide rounded apex; belt shape cylindrical.....*S. cylindricus*
3. Volva rusty orange with white areas; belt surface (under gleba) rugulose; apical sterile portion with triangular, slightly squared apex; belt shape elongate-doliiform.....*S. costaricensis*
- 3'. Volva reddish brown; belt surface (under gleba) reticulate; apical sterile portion and belt shape different.....4
4. Apical sterile portion tear-drop shaped; belt shape short-cylindric.....*S. candeliformis*
- 4'. Apical sterile portion elongate with a square apex; belt shape nearly square with rounded sides.....*S. quadratus*

Discussion

It has been a hundred years since the publication of the genus *Staheliomyces*, and so far, only one species has been described, namely *S. cinctus* (Fischer 1921). The only information about the type specimen in the protologue is that Gerold Stahel collected it in 1918, in Paramaribo, Suriname. Stahel made great contribution to the knowledge of neotropical fungi, by sending several collections to known mycologists. Eduard Fischer was a Swiss botanist and mycologist, from University of Bern, and later from Botanic Garden and Botanical Institute in Bern, who described several species of gasteroid fungi, from which many were sent by G. Stahel (Fischer 1933a).

Originally, Eduard Fischer's collection was deposited at the Botanical Garden of University of BERN, but part of his collection was moved to ETH Zurich (Eidgenössische Technische Hochschule) fungal collection. We contacted both curators of fungal collections and Dr. Katja Rembold, curator of the herbarium BERN, found Fischer' *Staheliomyces* collection, collected and sent by Stahel during the years of 1918–1925. She kindly provided pictures and information about this collection, which allowed us to define type material for *S. cinctus*. Several collections of *Staheliomyces* were found at BERN, also numerous original illustrations made by Fischer for different species are currently stored in the archive of the Library Plant Sciences (a sub-section of the University Library of Bern).

The type material found at BERN (Fig. 3a and b) is now apparently composed only of an immature basidiome, but Fischer's illustration of the expanded basidiome cited in protologue is represented here in Fig. 3c. Among Fischer's collection there is also an expanded basidiome without an identification tag (Fig. 3d). Since Fischer mentions that the original material sent by Stahel is composed of both mature and immature basidiome, we believe this unidentified material can be part of the original material, and therefore a possible syntype. We designate here as lectotype the material composed of immature basidiome, since it has the original identification tag with information on date and locality of collection, as well as the collector, indicated by Fischer in his publication—all in accordance to the International Code of Nomenclature for algae, fungi, and plants (Section 2, Art. 9.12) (Turland et al. 2018). We also found a material designated by Fischer himself as "cotypus" on the herbarium tag (Fig. 3e), which corresponds to the illustration on Fischer (1933b), page 98 figure 72A. All these findings are especially outstanding, since it is very difficult to find well-preserved specimens of phalloid fungi from such a long period of time since the original description (c. 100 years), specifically the original material.

Morphological and phylogenetic molecular analyses from the collected material showed some expected diversity within *Staheliomyces*. We expected that there would be some degree of infrageneric diversity because this genus has never been analyzed in detail. Recently, several examples of hidden diversity have been found in monospecific genera that have been published, especially when studying Neotropical species, as in *Myriostoma* Desv. and *Xylophallus* (Sousa et al. 2017; Crous et al. 2018). This highlights the importance of studying neglected taxa especially in geographic regions that are underexplored or hard to reach.

In the phylogenetic analysis A (Fig. 1), *Staheliomyces* belongs to *Phallaceae* in an exclusively Neotropical clade that includes *Staheliomyces* and *Xylophallus*. This is particularly interesting and brings insights into future biogeographical studies of phalloid fungi, since the species from this clade appear to have diversified both recently and rapidly, as it is reflected by the short branch lengths in phylogenies. Morphologically, these two genera are very distinct, but they do share a common character, which is also found in *Mutinus*. These three genera do not have a differentiation between the receptacle and the pseudostipe, as it is in *Phallus* Junius and *Itajahya* Möller species—where the receptacle forms a bell-like structure at the apex of basidioma. Studies aiming to reconstruct ancestral character states would be necessary to understand the dynamic of character evolution in phalloid fungi.

Fig. 4 *Staheliomyces* spp. macro-morphology. **a, e, i, m, q** Basidiomata in field. **b, f, j, n, r** Close-up in belt and apical sterile portion. **c, g, k, o, s** Belt texture of dehydrated basidiomata. **d, h, l, p, t** Volva close-up. **a, b, d** *S. candeliformis* (UFRN-Fungos 2748, paratype): (d) Part of dehydrated volva. **c** *S. candeliformis* (INPA 255831, holotype). **e, f, g, h** *S. costaricensis* (USJ 109573, holotype). **i, j, l** *S. cylindricus* (UFRN-Fungos 2177, holotype), (Photo: Julieth Souza). **k** *S. cylindricus* (UFRN-Fungos 1222, paratype). **m, n, o, p** *S. quadratus* (UFRN-Fungos 2746, holotype). **q, r, s, t** *Staheliomyces* sp. (INPA 264932). Scale bars: (a, b, e, f, h, i, j, l, m, n, p, q, r, t) = 10 mm, (k) = 2mm, (c, d, g, o, s) = 1 mm

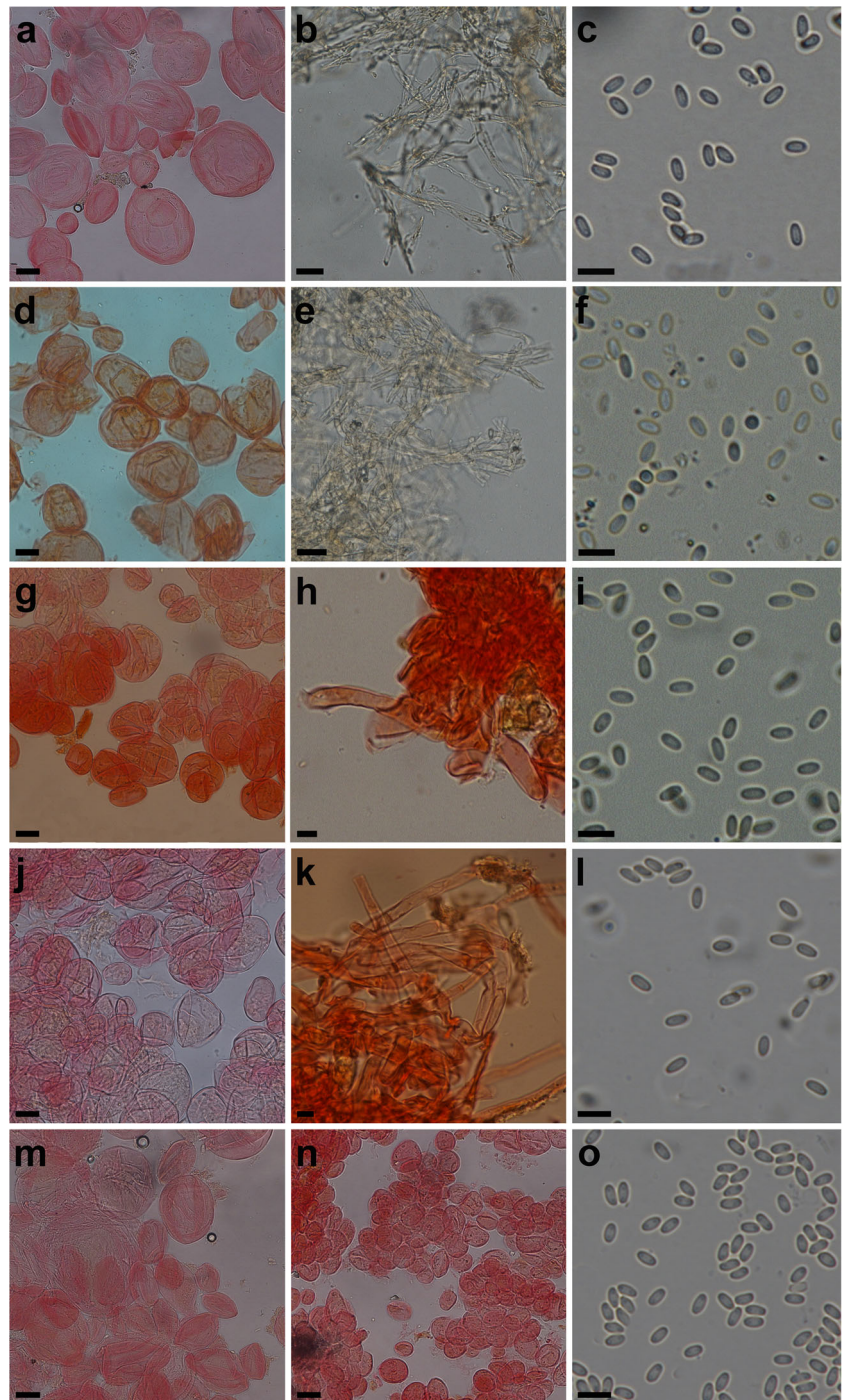


In South America, *Staheliomyces* and *Xylophallus* currently have a disjunct distribution in Amazonia and Brazilian Atlantic rainforest, which is a pattern also found in several groups of Neotropical plant and animal species. For plants and animals, this pattern is usually related to the last glacial cycle (21 Mya), which together with Andean uplift in Neogene, led to climate and geographical changes that drastically modified the landscape (Hoorn et al. 2010; Sobral-Souza et al. 2015; Ledo and Colli 2017). There has been an expansion of dry forests and retraction of humid forests, where the dry forests functioned as a corridor between these biomes—now called the South American dry diagonal (Sobral-Souza et al. 2015). The same disjunct distribution pattern is also found in several groups of gasteroid fungi including phalloid species (da Silva et al. 2013; Accioly et al. 2019; Cabral et al. 2019), which might also indicate a signature of possible connections between the two forests in the past. Similarly,

Sánchez-Ramírez et al. (2015) evaluated the effects of past glaciation in diversification of *Amanita caesarea* (Scop.) Pers. species complex in North America, and revealed a cryptic diversity and high speciation rates in refugia. In spite of suggestions on the emergence of the order *Phallales* to 77 Mya (He et al. 2019), it is still not known when phalloid species colonized Neotropical areas. For this reason, biogeographical and dating studies need to be developed in order to better understand the past processes that influenced the speciation in this group. Additionally, a better sampling effort is needed in specific Neotropical areas, as between Amazonia and Atlantic Rainforest or Central America, to confirm the current geographical distribution of Neotropical species.

By comparing our material to published descriptions, we noticed that *S. cinctus* is not represented in the collections that we have made in this study, although we do believe that Fig. 7j from GBIF data may well represent that

Fig. 5 *Staheliomyces* spp. micro-morphology.
a, d, g, j, m Pseudoparenchymatous hyphae of apical sterile portion.
b, e, h, k Hyphae from volva.
n Hyphae from belt.
c, f, i, l, o Basidiospores.
a, b, c *S. candeliformis* (INPA 255831, holotype).
d, e, f *S. costaricensis* (USJ 109573, holotype).
g, h *S. cylindricus* (UFRN-Fungos 1222, paratype).
i *S. cylindricus* (UFRN-Fungos 2177, holotype).
j, k, l *S. quadratus* (UFRN-Fungos 2746, holotype).
m, n, o *Staheliomyces* sp. (INPA 264932). Scale bars: (a, b, d, e, g, j, m, n) = 20 μ m, (c, f, h, i, k, l, o) = 5 μ m



species. It is a specimen from French Guyana, macro-morphologically very similar to Fischer's description, and geographically very close to the type locality (Suriname)—found specifically on the border with Suriname. It is also similar to some of the material found at BERN, described by Fischer (data not shown). On the other hand, based on the phylogenetic analysis (Fig. 2a) which is allied to the morphological analysis, at least four new species could be identified and described here. There is a clear

morphological delimitation of species, even though some phylogenetic clades do not have high support values.

Staheliomyces cylindricus is the most distinct species in our study and the most phylogenetic consistent clade, characterized by the elongate apical sterile portion, and known only by specimens from Brazilian Atlantic Rainforest. *Staheliomyces costaricensis* is an intriguing species characterized mainly by its long belt and very short apical sterile portion, consistently different from

Table 1 Comparative morphological table with *Staheliomyces* species

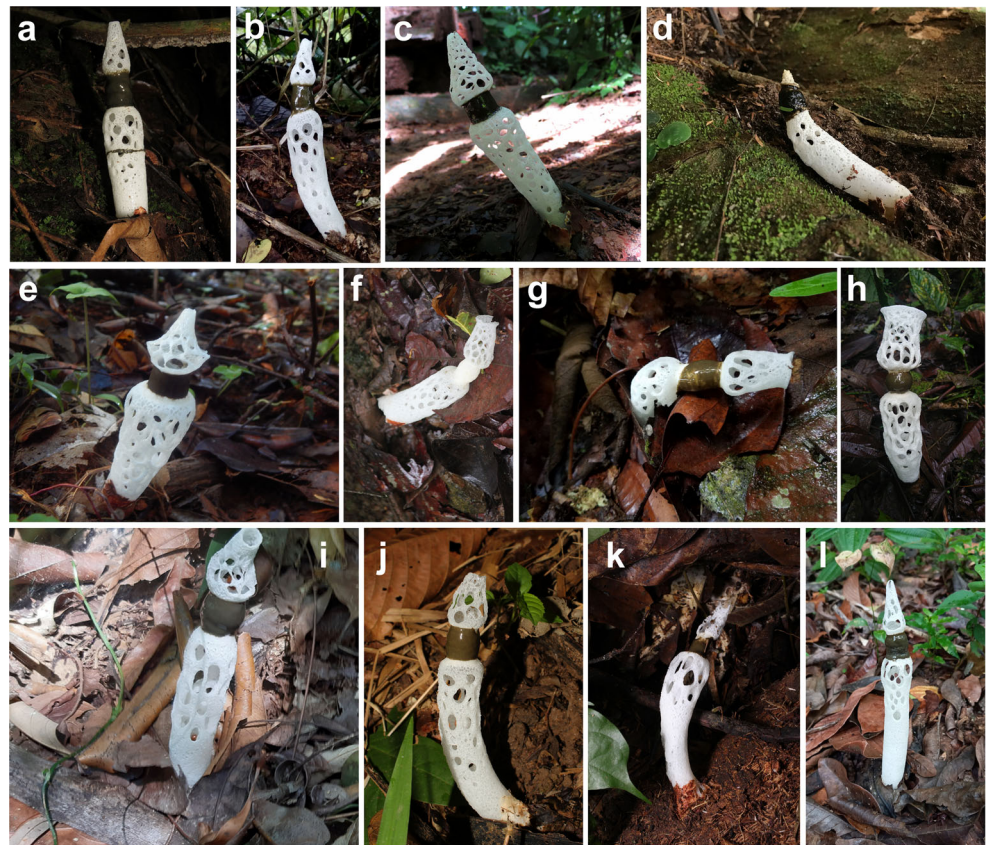
Character	<i>S. candeliformis</i>	<i>S. cinctus</i>	<i>S. costaricensis</i>	<i>S. cylindricus</i>	<i>S. quadratus</i>
Volva color	Reddish brown	Whitish*	Rust orange with white areas	White	Reddish brown
Pseudostipe width (shape)	Widening from the middle to the upper part	Widening from the middle to the upper part	Widening from the middle to the upper part	Constant width over the entire length	Widening from the middle to the upper part
Constriction in diam. (mm)	3–11	5	5–7	1	10
Glebal region shape	Short-cylindrical	Doliiform	Doliiform elongate	Cylindrical	Almost squared with rounded sides
Belt texture under gleba	Reticulate	**	Rugulose	Rugulose	Reticulate
Apical sterile portion shape	Tear-drop	Triangular	Triangular, slightly square tip	Elongate, rounded tip	Elongate, square tip

*Data analyzed in Fig. 3d (basidiomata in alcohol). ** Not analyzed in original description

Fig. 6 Geographical distribution of *Staheliomyces* spp.



Fig. 7 Morphological diversity in *Staheliomyces* genus. Data from GBIF/iNaturalist. **a** Brazil. **b** Colombia. **c, d, e** Costa Rica. **f, g, h** Ecuador. **i** Honduras. **j** French Guiana. **k** Panama. **l** Peru. Photo credits: **a** ©Rich Hoyer, **b** ©Tefa Duarthe, **c** ©Katja Schulz, **d** ©Josh Vandermeulen, **e** ©Jacob Ashton, **f** ©Mike Ellis, **g** ©Jeremiah Leach, **h** ©Julia Patiño, **i** ©Oliver Komar, **j** ©Sébastien SANT, **k** ©keesgroenendijk, **l** ©Lindsey Cathcart



other specimens. Apparently, at least two morphospecies can be identified in Costa Rica (Fig. 7c and d) according to our data and the data obtained in GBIF (GBIF 2021). *Staheliomyces costariquensis* occurs in the North portion, while the other one is found mainly on South Costa Rica, showing differences mainly in the shape of apical sterile portion (Fig. 7c–e). *Staheliomyces candeliformis*, in turn, grouped in an Amazonian clade, and has a tear-drop apical sterile portion, while *S. quadratus* has a square apical sterile portion. We conclude that the most distinguishable characters for species in *Staheliomyces* are the shape and size of apical sterile portion, shape and surface of the belt, and the color of the volva. This is clearly shown in Fig. 7 where it is possible to see different shapes for the apical sterile portion which are different from those described in this study; for example, the cup shape seen in some of the Ecuadorian specimens (Fig. 7f–h).

The expansion of basidiomata sampling in the known geographical occurrence range of *Staheliomyces*, together with the detailed study of species morphology and phylogenetic or phylogenomics analyses, will ameliorate our understanding of the diversity found within the genus. These further studies may also indicate the

biogeographical drivers of such a diversity as well as may clarify the speciation events in the order *Phallales*.

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Authors contribution Material preparation and data collection and analyses were performed by Tiara S. Cabral, Gislaïne C.S. Melanda, and Nathalia M. de Assis. The first draft of the manuscript was written by T.S.C., G.C.S.M, and N.M.A, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article.

Declarations

Conflict of interest The authors declare no competing interests.

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