



Resolution of eleven reported and five novel *Podaxis* species based on ITS phylogeny, phylogenomics, morphology, ecology, and geographic distribution

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Abstract The genus *Podaxis* was first described from India by Linnaeus in 1771, but several revisions of the genus have left the taxonomy unclear. Forty-four *Podaxis* species names and nine intraspecific varieties are currently accepted, but most fungarium specimens are labelled *Podaxis pistillaris*. Recent molecular analyses based on barcoding genes suggest that the genus comprises several species, but their status is largely unresolved. Here we obtained basidiospores and photographs from 166 fungarium specimens from around the world and generated a phylogeny based on rDNA internal transcribed spacer ITS1, 5.8S and ITS2 (ITS), and a phylogenomic analysis of 3839 BUSCO genes from low-coverage genomes for a subset of the specimens. Combining phylogenetics, phylogenomics, morphology, ecology, and geographical distribution, spanning 250 years of collections, we propose that the genus includes at least 16 unambiguous species. Based on 10 type specimens (holotype, paratype, and syntype), four recorded species were confirmed, *P. carcinomalis*, *P. deflersii*, *P. emerici*, and *P. farlowii*. Comparing phylogenetic analysis with described species, including morphology, ecology, and distribution, we resurrected *P. termitophilus* and designated neotypes, epitypes, or lectotypes for five previously described species, *P. aegyptiacus*, *P. africana*, *P. beringamensis*, *P. calyptatus*, and *P. perraldieri*. Lastly, based on phylogenies and morphology of type material, we synonymized three reported species, *P. algericus*, *P. arabicus*, and *P. rugospora* with *P. pistillaris*, and described five new species that we named *P. desolatus*, *P. inyoensis*, *P. mareebaensis*, *P. namaquensis*, and *P. namibensis*.

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INTRODUCTION

The genus *Podaxis* (*Agaricaceae*, *Basidiomycota*) is distributed in tropical and subtropical areas between N40° and S40° latitude, spanning the continents of Asia, Africa, America, and Australia (Fig. 1a) (Berkeley 1843, Dring 1964, Vellinga 2004, Sharma et al. 2009, Muhsin et al. 2012, Kirk et al. 2013, Conlon et al. 2016, 2019, Medina-Ortiz et al. 2017, He et al. 2019). *Podaxis* has been reported from vastly different environments, ranging from dry, sandy semi-arid grasslands and arid deserts in Africa, Asia, Australia, and the Americas, to mounds of the grass-harvesting termite genera *Trinervitermes* in Africa and *Nasutitermes* in Australia (*Termitidae*, subfamily: *Nasutitermitinae*) (Linnaeus 1771, Masee 1890, Dring & Rayner 1967, Priest & Lenz 1999, Conlon et al. 2016, 2019, 2021). Members of the genus have a long history of use in traditional folk medicine in many countries (Linnaeus f. 1781, Berg 1785, Al-Fatimi

et al. 2006, Diallo et al. 2008), as face paint and to darken hair, and have also been used as food (Hashem & Al-Rahmah 1993, Jiskani 2001, Medina-Ortiz et al. 2017).

The first species of *Podaxis* (as currently defined) to be formally described was *Lycoperdon pistillare* Linnaeus (1771). A decade later, the description of a second species, *Lycoperdon carcinomale*, written by Linnaeus, was published after his death by his son (Linnaeus f. 1781). This was based on a specimen sent to Linnaeus earlier by one of his so-called ‘apostles’, Carl Peter Thunberg, from a termite mound in the southern Cape Province of South Africa (Thunberg 1986). Bosc (1792) described a third species (*Lycoperdon axatum*) from Senegal (Fig. 1b). Desvaux (1809) then erected the genus *Podaxis* to accommodate the latter species, and Fries (1829) added the two Linnaeus species to the genus. During an extensive expedition, three ‘*Podaxon perraldieri*’ specimens from Algeria were collected, as illustrated by Patouillard (1897). One of these specimens is designated in the present study as lectotype for *Podaxis perraldieri* (Fig. 1c).

By the time the first monograph of the genus was published, Masee (1890) recognised seven species and suggested several synonyms. Many new species were subsequently described, but most species were treated as synonymous with *Podaxis pistillaris* in a later monograph (Morse 1933). As a result, specimens collected and stored in museums thereafter were as a result in most cases labelled *P. pistillaris*. Later descriptions of species rarely compared their morphology with the type of the species published prior to Morse’s monograph, and

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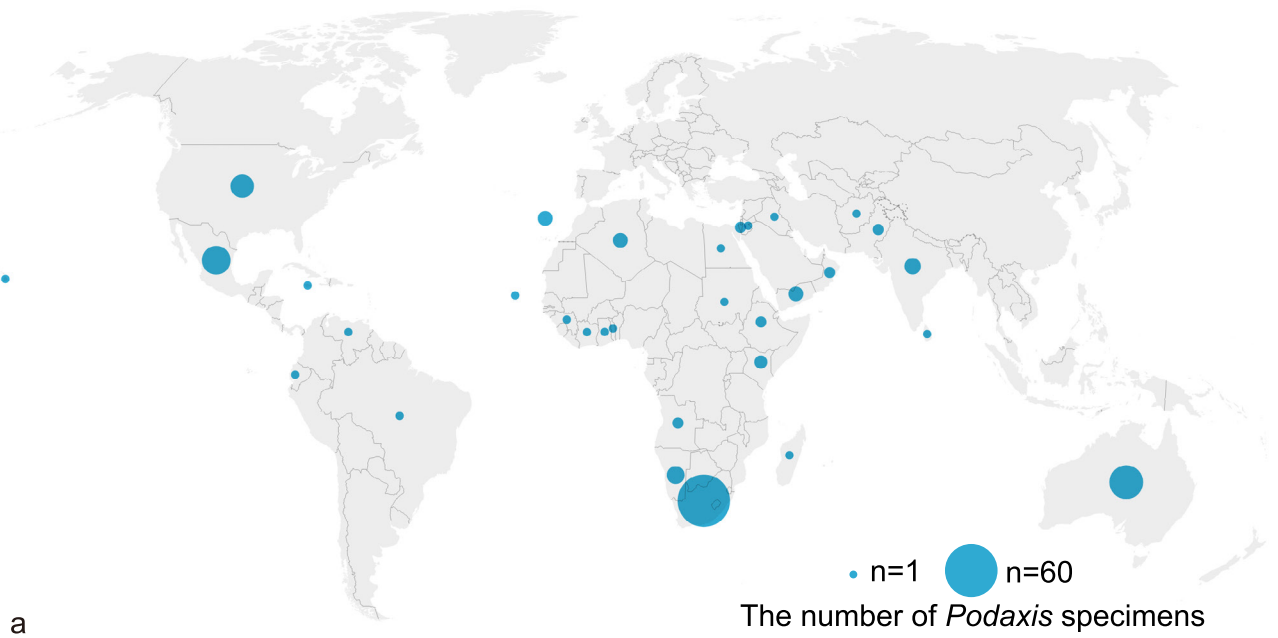
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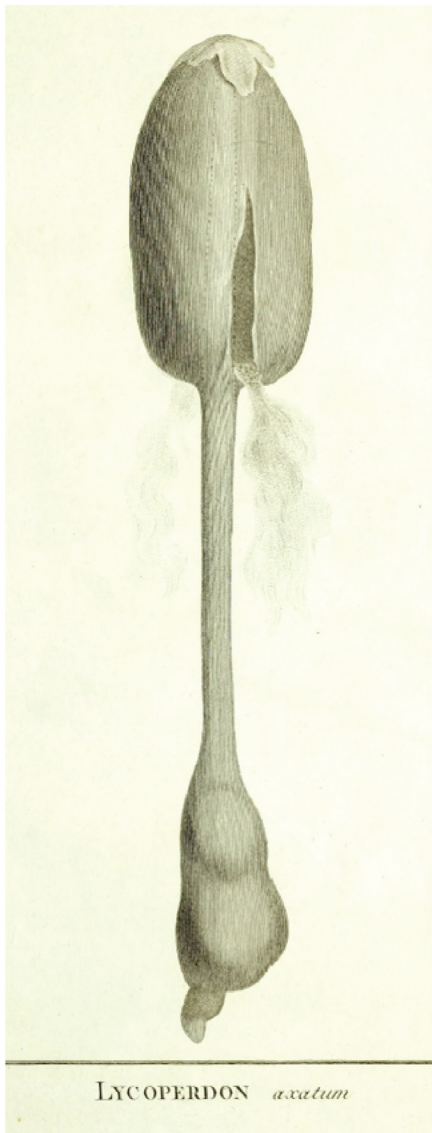
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a



b



c

Fig. 1 The geographic distribution and illustrations of *Podaxis*. a. We obtained *Podaxis* from 32 countries, including two islands, spanning four continents, and the number of specimens per country is indicated by the size of the circles; b. the original illustration of *Lycoperdon axatum* by Bosc (1792); c. three '*Podaxon perraldieri*' specimens from Algeria illustrated by Patouillard (1897).

in some cases did not consider it at all, leading to difficulties in tracking of identities of species and specimens (McKnight 1985, Priest & Lenz 1999, Conlon et al. 2019).

Phylogenetic analyses of the genus have indicated the presence of multiple species (Conlon et al. 2016, Medina-Ortiz et al. 2017), and 44 legitimate species and nine intraspecific varieties are currently listed in Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>). However, only *P. pistillaris* and *Podaxis* sp. are currently recognised in the UNITE database of 53 sequences (<https://unite.ut.ee/index.php>). Conlon et al. (2016) found six well-resolved clades based on sequences of the large subunit (LSU) and the internal transcribed spacer regions (ITS) of ribosomal DNA from 44 fungarium and nine field collected specimens. They used this data to present a phylogeny that was also supported by morphological differences in sporocarps and spore sizes. Interestingly, clades differed in ecology, with some composed exclusively of termite-mound associated specimens, while others were free-living (Fig. 2a, b) (Conlon et al. 2016). Unsurprisingly, geographic origins also impacted phylogenetic placement of specimens, as was also evident from recent work in Mexico (Medina-Ortiz et al. 2017), and documented in a recent phylogenomic study (Conlon et al. 2021).

The phylogenomic study by Conlon et al. (2021) did not consider species designations but suggested that more species exist than the current classification implies. The authors also suggested that existing *Podaxis* species were in need of revision (Conlon et al. 2019). To resolve this, we supplemented an ITS phylogeny with phylogenomic analyses of low-coverage genomes (lc-genomes) from a subset of available samples. We combine phylogenomics with morphology, geographic distribution, and ecology, to obtain an unambiguous taxonomy of *Podaxis* that allowed for revision of reported species and description of novel taxa.

MATERIALS AND METHODS

Specimen materials

We obtained 166 *Podaxis* specimens collected from 32 countries stored in nine fungaria around the world. The fungarium acronyms follow Index Herbariorum (<https://sweetgum.nybg.org/science/ih/>) and specimen information is available in Table S1. Thirty-seven specimens were unnamed (*Podaxis* sp.), and 129 specimens were labelled as 13 reported species with 10 type specimens (Table S1). Seventy-six specimens had ITS sequences available in GenBank (Conlon et al. 2016, 2021, Medina-Ortiz et al. 2017) and 90 were sequenced as part of this study (Fig. 1a; Table S1).

DNA extraction, PCR amplification and sequencing

DNA was extracted from spore samples using a Chelex protocol (200 μ L Chelex 100 (Sigma Aldrich, America), vortexed and incubated at 99.9 °C for 30 min). To generate an ITS phylogeny, we amplified the ITS1-5.8S-ITS2 region for 90 specimens using the ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS4B (5'-CAGGAGACTTGACACGGTCCAG-3') primers (Gardes & Bruns 1993, Conlon et al. 2016, 2021). PCR reactions were prepared in 25 μ L volumes comprising 8.5 μ L sterile distilled water, 1 μ L of each primer, 2 μ L of template, and 12.5 μ L of VWR Red Taq DNA Polymerase Master Mix (VWR International, USA). PCR reactions were run with the following conditions: 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s with a final extension step at 72 °C for 4 min. PCR products were purified using either MSB Spin PCRapace (STRATEC Molecular, Germany) or Exosap-IT (Affymetrix Inc., USA) according to the manufacturer's instruc-

tions and sequenced at Eurofins MWG Operon (Ebersberg, Germany). Forward and reverse sequences were aligned and manually checked using Geneious prime v. 2019.1.1 (Biomatters Ltd., New Zealand).

ITS phylogeny

To generate a phylogeny that revealed the position of *Podaxis* in the *Agaricaceae*, we included 166 *Podaxis* sequences (90 from this study and 76 from GenBank), together with 539 ITS sequences belonging to *Agaricaceae* (Table S1, S2). Sequences were aligned in Mafft v. 7.471 (<http://mafft.cbrc.jp/alignment/software>). A Maximum Likelihood (ML) phylogenetic tree and SH-aLRT test were inferred by using IQ-TREE v. 2.2.0 (Minh et al. 2020). The best model tested by ModelFinder (Kalyaanamoorthy et al. 2017) was TVM+F+R9 and branch support was obtained from bootstrap analysis with 1 000 repetitions.

Low-coverage genome (lc-genome) sequencing

To obtain lc-genomes for as many strains as possible, we quantified DNA concentrations of 166 *Podaxis* spore samples for which DNA extractions were done as described above, using NanoDrop ND-1000 (Thermo Fisher Scientific, Germany). Total amounts of DNA exceeding 500 ng (concentration > 5 ng/ μ L) were selected from 124 samples. Samples were sent to BGI (Shenzhen, China) for 150 bp paired-end sequencing on the BGI DNBSEQ Platform with 10 \times coverage, after a prior whole-genome amplification step due to the low amount of starting DNA.

Phylogenomic analysis

Lc-genome sequences obtained from the 124 specimens were assembled *de novo* using SPAdes v. 3.14.1 (Bankevich et al. 2012), and subsequently subjected to a BUSCO v. 5.1.3 run using the Agaricales_odb10 dataset to obtain single-copy BUSCO genes (Manni et al. 2021). We then evaluated genome completeness by dividing the number of BUSCO genes to the mean BUSCO completeness of ten available genomes (Conlon et al. 2021). We used this score to evaluate the impact of specimen age (collection time) on genome quality, and to guide what lc-genomes were of sufficient quality to be included in subsequent phylogenomic analyses.

Based on completeness scores, 76 specimens were included in the phylogenomic analysis along with 10 *Podaxis* genomes from GenBank (Conlon et al. 2021) and nine other *Agaricaceae* species as outgroup (Table S3). Amino acid sequences were extracted from the BUSCO results and datasets were compiled for each of the orthologous groups using BUSCO_phylogenomics (McGowan & Fitzpatrick 2020, McGowan et al. 2020). 3839 (of 3870 potential) amino acid sequences were aligned in Multiple Sequence Comparison by Log-Expectation (MUSCLE) v. 3.8.3.31 (Madeira et al. 2022) and trimmed using trimAl (Capella-Gutierrez et al. 2009). None of the assemblies contained all 3839 protein sequences, so to minimize the impact of missing genes, and to prevent overrepresentation of relatively large genes, we performed Maximum-Likelihood Species-Tree inference by concatenation and coalescent models separately (Jiang et al. 2020). The concatenation model connected multiple protein sequence alignments, and then inferred Species-tree by IQ-TREE v. 2.2.0 (Minh et al. 2020) with an automatically determined optimal substitution model and 1 000 ultrafast bootstraps (Kalyaanamoorthy et al. 2017, Hoang et al. 2018). The coalescent model first inferred phylogeny of each protein sequence using IQ-TREE v. 2.2.0 (Minh et al. 2020), as does the concatenation model, then constructed the species-tree from the final set of gene trees under the multi-species coalescent model (MSC) using ASTRAL v. 5.7.7 with multi-locus bootstrapping (Seo 2008, Mirarab et al. 2014, Zhang et al. 2020).

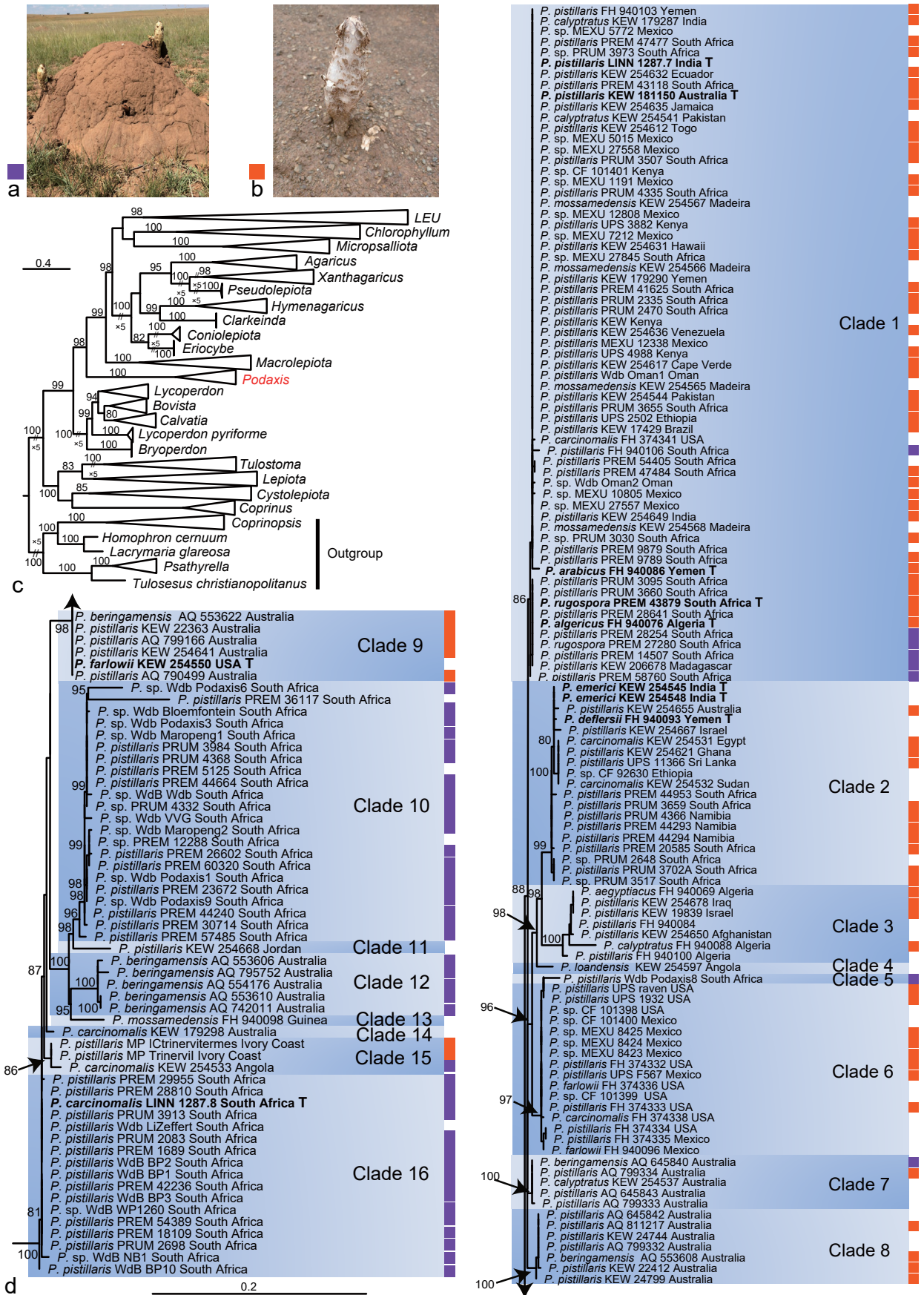


Fig. 2 The ecology and phylogeny of *Podaxis* in the *Agaricaceae*. a, b. *Podaxis* exist in association with termites in the *Nasutitermitinae* (a) or as free-living (b), indicated by a purple or orange box, respectively, in Fig. 2d and 3b; c. a schematic Maximum Likelihood phylogeny based on 704 ITS sequences from the family *Agaricaceae*, including 166 *Podaxis* specimens. ML bootstrap support values > 60 % given above branches and the width of clades represents the number of specimens included (full phylogeny in Fig. S1); d. a detailed Maximum Likelihood phylogenetic ITS tree with bootstrap support values of the sixteen clades of the genus *Podaxis*, labelled based on the results of this work. Distinct clades are indicated with different shades of blue. Type specimens are bold and indicated with a T.

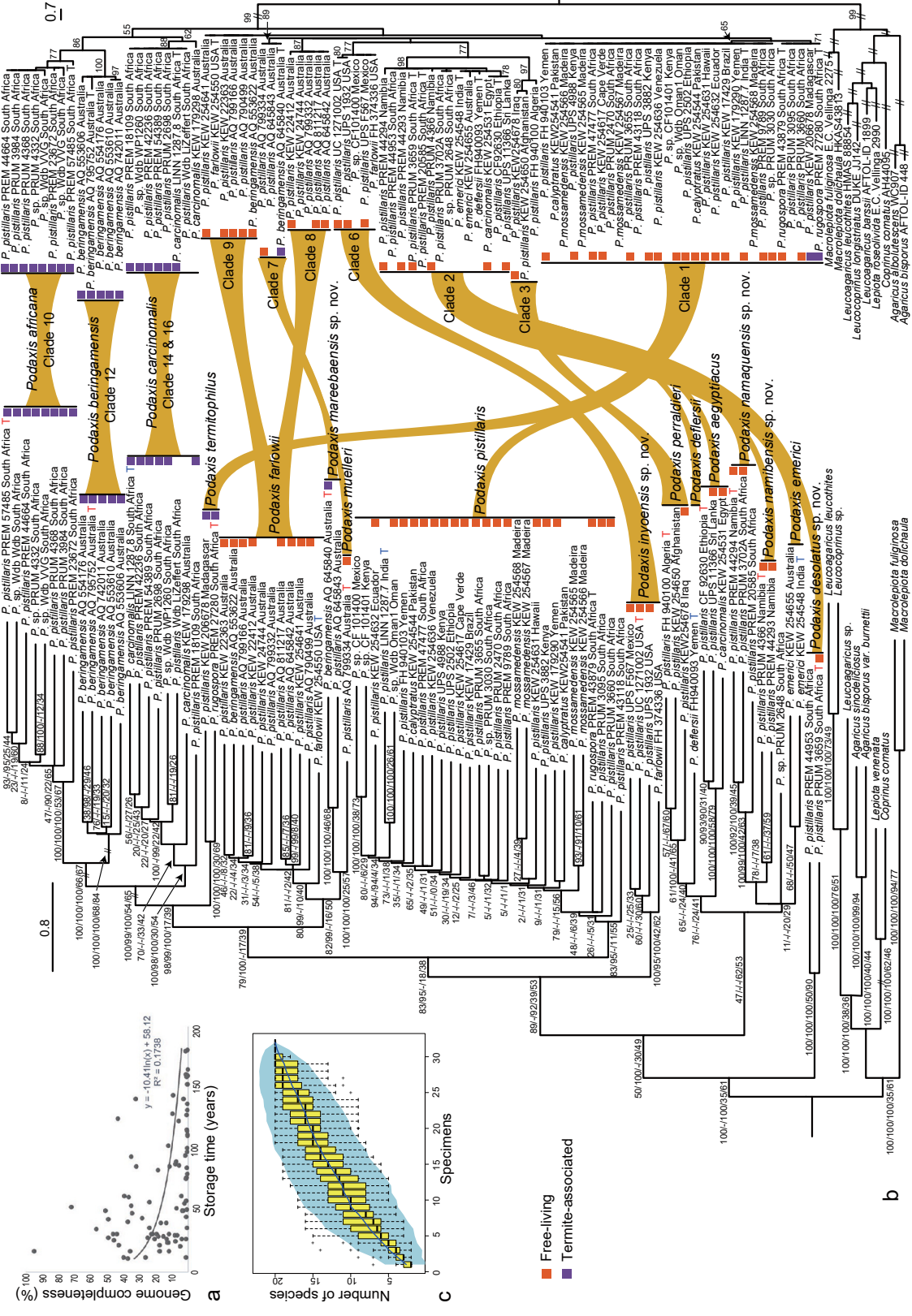


Fig. 3 Phylogenetic analyses of the genus *Podaxis*. a. There was a significant negative association between specimens age (storage time) from all nine herbaria with BUSCO score, estimated as percentage of BUSCO genes obtained per specimen divided by the expected number from high-quality genomes in GenBank; b. comparison of phylogenetic (left) and ITS phylogenetic (right) analysis of *Podaxis* specimens. The phylogenetic analyses were based on 3839 BUSCO genes from 77 low-coverage *Podaxis* genomes, 10 high-quality *Podaxis* genomes and nine outgroup genomes from GenBank identified using the Agaricales_odb10 dataset and BUSCO v. 5.1.3. The phylogenetic tree was inferred based on concatenation model with the adding of Ultrafast ML bootstrap support values in concatenation model. The numbers near branches indicate Local posterior probability/Ultrafast ML bootstrap support values in concatenation model/Ultrafast ML bootstrap support values in coalescent model/Gene-concordance factors (gCF) quantify/Site-concordance factors (sCF). The Maximum Likelihood phylogeny based on ITS of the *Podaxis* specimens included in the phylogenetic analysis with ML bootstrap support values > 60 % indicated above branches. Type specimens are indicated with a black T for old type specimen, a blue T when the new and old type specimen were identical, and a red T when a new type was designated in this paper. For the full list of specimens and outgroups, see Table S3; c. the box plot provides the rate of new species obtained (species accumulation curve) with increased sampling using the exact method (data in Table S4).

Morphological characteristics

Macro-morphological features (basidiocarp, peridium, and stipe size) were measured from photos of herbarium specimens with scale bars. Basidiospores were visualized using a Nikon Eclipse E600 and pictures taken with an Optronics MagnaFire CCD Microscope Camera. The ratio of basidiospore length and width (aspect ratio) were obtained for a minimum of twenty spores per *Podaxis* specimen measured, using ImageJ (Schneider et al. 2012). We analysed the data using one-way ANOVAs with paired Tukey tests in R v. 4.0.0 (R Core Team 2020) and compared the length, width, and the aspect ratio, between specimens belonging to different phylogenetic clades.

Species diversity

To test how sampling efforts by country or region impacted the observed species diversity of *Podaxis*, we used the accumulation function in the 'vegan' library (Oksanen et al. 2020) in R v. 4.0.0 (R Core Team 2020) to generate a rarefaction curve.

RESULTS

Phylogenetics of *Podaxis* using ITS barcode sequencing

Phylogenetic analysis of 166 *Podaxis* sequences among 21 genera in the family *Agaricaceae* confirmed that *Podaxis* formed a well-supported monophyletic genus. Its sister clade was comprised of the mostly mushroom forming genera *Agaricus*, *Chlorophyllum*, *Clarkeinda*, *Coniolepiota*, *Eriocybe*, *Hymenagaricus*, *Leucoagaricus*, *Leucocoprinus*, *Macrolepiota*, *Micropsalliota*, *Pseudolepiota*, and *Xanthagaricus*, as well as other gasteroid genera such as *Bovista*, *Bryoperdon*, *Calvatia*, and *Lycoperdon* (Fig. 2c, S1). The ITS phylogeny revealed 16 *Podaxis* clades with good support, of which ten included more than one specimen with available ecological records (Fig. 2d). The geographic distribution and ecology of seven clades were consistent (Fig. 2d). Ten type specimens, including holotype, paratype, and syntype, of eight described species clustered in four clades in the phylogeny. Two type specimens, LINN 1287.8 (*P. carcinomalis*) and KEW 254550 (*P. farlowii*) were in distinct clades, while the remaining eight type specimens clustered in ITS Clades 1 and 2 (Fig. 2d). Therefore, while the ITS phylogeny demonstrated the need for a taxonomic revision of the genus, it did not provide enough resolution to unambiguously distinguish species.

Phylogenomic analysis of *Podaxis*

We successfully computed genome completeness of 130 genomes (120 lc- and ten high-quality genomes) while contigs from four genomes were too short (Table S3). There was a clear negative association between storage time and genome completeness (Fig. 3a; Table S3), and 44 low-quality genomes had to be excluded from the analyses, including specimens of *P. aegyptiacus*, *P. algericus*, *P. arabicus*, *P. emerici*, *P. loanensis*, and *P. pistillaris*.

The phylogenomic placement of the resulting 77 lc-genomes and 10 high-coverage genomes (> 10×) revealed 16 clades with good support (> 90). Overall, the phylogenomic tree was similar in topology to the ITS phylogeny, confirming the five well-resolved ITS Clades 3, 6, 10, 12, and 16, and improving resolution to six additional ITS Clades 1, 2, 7–9, and 14, while five ITS Clades 4, 5, 11, 13, and 15 lacked genomes (Fig. 2d, 3b). The five well-resolved ITS Clades were consistent with geography and ecology, of which ITS Clades 10 (*P. africana*), 12 (*P. beringamensis*), and 16 (*P. carcinomalis*) were associated with termites and ITS Clades 3 (*P. perraldieri*) and 6 (*P. inyoensis*) were free-living in deserts (Fig. 2d, 3b).

The phylogenomic analysis provided better resolution for ITS Clades 1, 2, 7–9, and 14 where specimens had conflicting ecology or geography in the ITS tree. ITS Clades 1 and 7 included both free-living and termite-associated specimens, Clade 2 included specimens with a geographically wide distribution, and Clade 9 was unresolved based on ITS, potentially due to recombination, incomplete lineage sorting (ILS), or horizontal gene transfer (HGT) (Fig. 2d, 3b). The phylogenomic tree separated ITS Clade 1 into the termite-associated *P. termitophilus* and the free-living *P. pistillaris* and split ITS Clade 7 into the termite-associated *P. mareebaensis* and the free-living *P. muelleri* with strong support (Fig. 3b). Furthermore, *P. termitophilus* now appeared as a sister clade to the three other termite-associated clades (Fig. 3b). The free-living *P. pistillaris* included the type specimens for both *P. pistillaris* (LINN 1287.7) and *P. rugospora* (PREM 43879), implying that they belong to the same species (Fig. 3b). The phylogenomic analysis allowed us to split ITS Clade 2 into six distinct new clades, each of which appeared to be valid species with distinct geographic distribution: *P. emerici* from India and Australia, *P. aegyptiacus* from Egypt, Ghana, Ethiopia, Sudan, and Sri Lanka, *P. namibensis* and *P. namaquensis* from Namibia and eastern South Africa, *P. desolatus* from South Africa, and *P. deflersii* with holotype (FH 940093) from Yemen (Fig. 2d, 3b). In the phylogenomic tree, the unresolved ITS Clade 9 was merged with ITS Clade 8 containing free-living specimens primarily from Australia suggesting that they should be considered a single species (*P. farlowii*) (Fig. 2d, 3b). Lastly, in the phylogenomic analysis, ITS Clade 14, which only included *P. carcinomalis* (KEW 179298), was merged with Clade 16, which included the type of *P. carcinomalis*. The phylogenomic tree was inferred and compared based on both a concatenation and a coalescent model, for which the topology was consistent, and both resolved ecological and geographical inconsistencies, and the ultrafast bootstrap support values of most branches were robust (> 90) (Fig. 3b, S3).

As expected, and consistent with previous work, we saw a clear association between geography and ecology with phylogeny, reflecting geographical and ecological diversification of the genus. *Podaxis africana* and *P. carcinomalis* except for KEW 179298 were found in South Africa and associated with termites. *Podaxis beringamensis*, *P. farlowii*, *P. mareebaensis*, and *P. muelleri* were from Australia, with *P. beringamensis* and *P. mareebaensis* being associated with termites. *Podaxis desolates*, *P. namaquensis*, *P. namibensis*, and *P. termitophilus* were from South Africa, Namibia, and Madagascar, and only *P. termitophilus* associated with termites. *Podaxis perraldieri* was free-living in the tropical regions of the cancer desert belt, including Algeria, Afghanistan, Iraq, Israel; and *P. inyoensis* was free-living in the USA and Mexico (Fig. 2d, 3b; Table S1). *Podaxis aegyptiacus* specimens were mainly from Ethiopia, Egypt, and Sri Lanka, while *P. pistillaris* was distributed in countries in Africa, Asia, Australia, and the Americas (Fig. 2d, 3b; Table S1). Notably, there was a clear association between species diversity obtained for a given country and the number of specimens available (Fig. 3c; Table S2).

Morphological characteristics

Morphological comparisons supported the species designations we outlined above (Fig. 4, 5; Table S5). The median and mean height of *P. aegyptiacus*, *P. africana*, and *P. carcinomalis* basidiocarp and peridium were significantly higher than other species, but the three species were distinguishable by the length of the stipe and the basidiospores (Fig. 4). *Podaxis emerici* had the smallest peridium, and the stipe width of *P. carcinomalis* was only narrower than *P. africana* (Fig. 4). The micromorphology varied more than the macromorphology and enabled us to differentiate species with similar macromorphology, such as

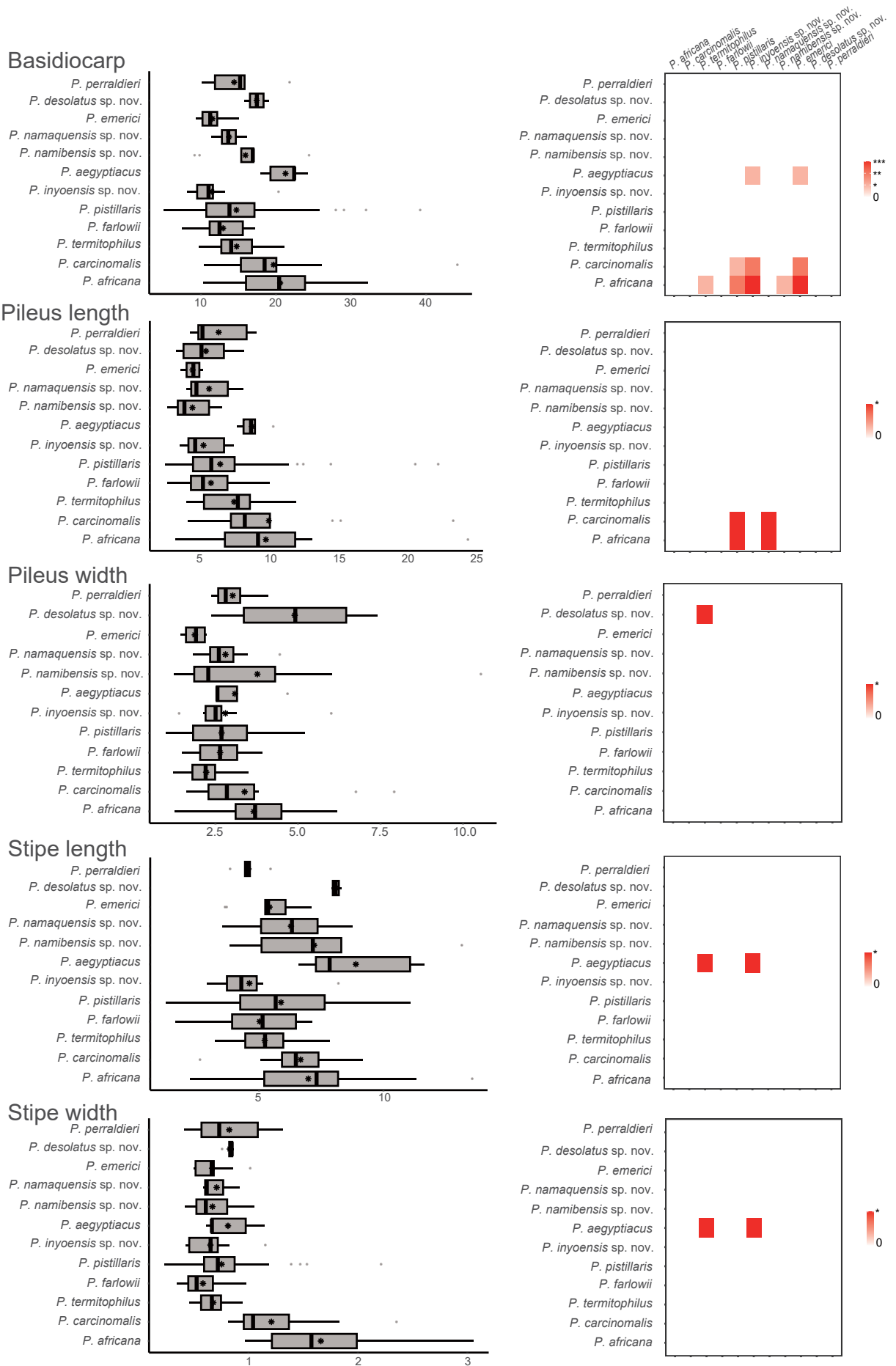


Fig. 4 Boxplots and heatmaps of pairwise Tukey test of macro-morphological measurements of twelve *Podaxis* species, not including *P. beringamensis*, *P. deflersii*, *P. muelleri*, *P. mareebaensis*, and *Podaxis* sp.1–6. Box plots give upper and lower quartiles, vertical bars are medians, stars represent means, whiskers maximum (right) and minimum (left) values, and dots represent outliers. Results of pairwise Tukey tests are provided. Stars indicate significance levels: one star means that $p < 0.05$, two stars mean that $p < 0.01$ and three stars mean that $p < 0.001$. The full results are given in Table S5.

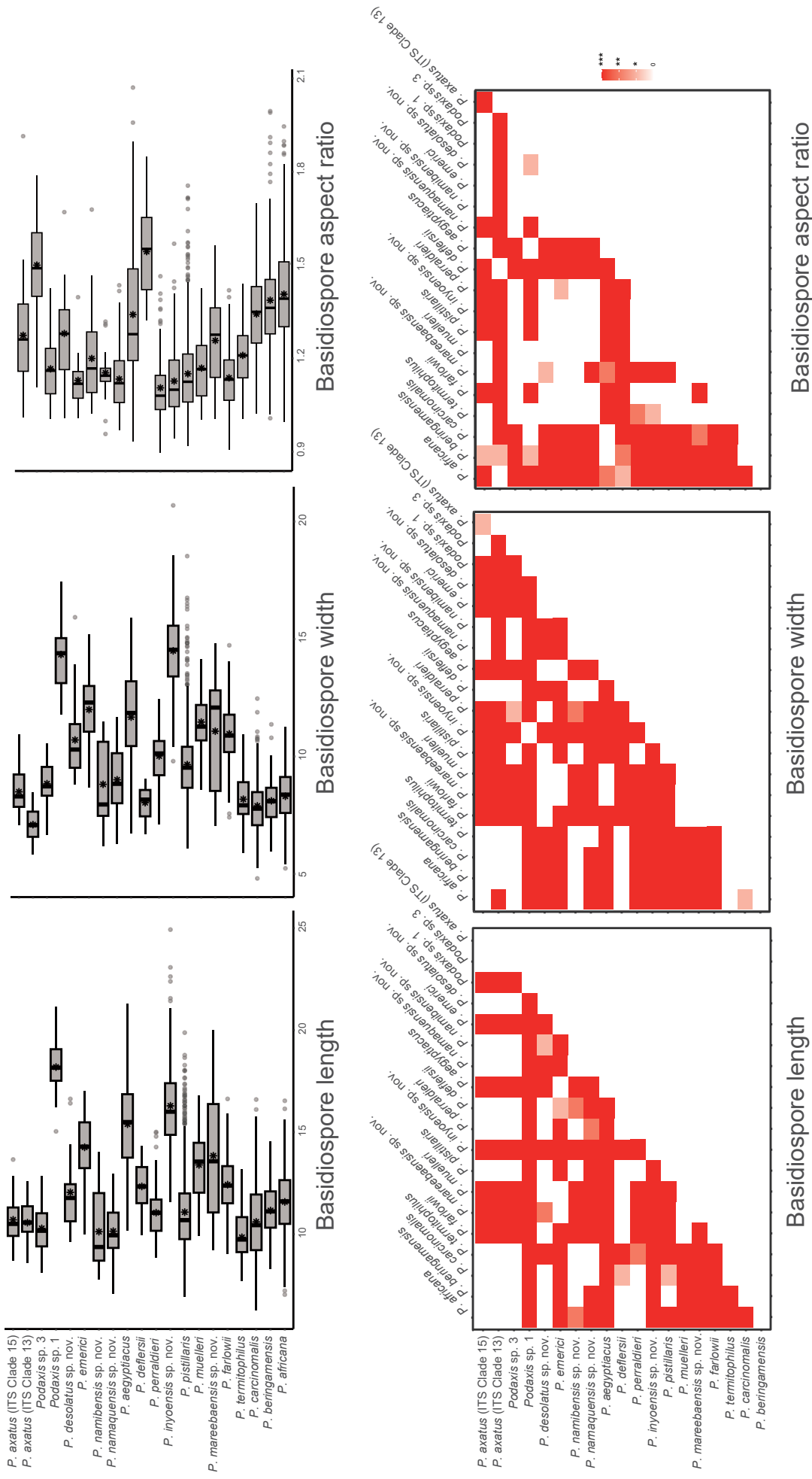


Fig. 5 Boxplots and heatmaps of pairwise Tukey test of micro-morphological measurements of sixteen *Podaxis* species, including with *Podaxis* sp.1, 3, and 6. Box plots give upper and lower quartiles, vertical bars are medians, stars represent means, whiskers represent maximum (right) and minimum (left) values, and dots represent outliers. Results of pairwise Tukey tests are provided. Stars indicate significance levels: one star means that $p < 0.05$, two stars mean that $p < 0.01$ and three stars mean that $p < 0.001$. The full results are given in Table S5.

P. desolates, *P. farlowii*, *P. inyoensis*, *P. namaquensis*, *P. peraldieri*, and *P. pistillaris*, and species without macromorphological data (*P. beringamensis* and *P. muelleri*) (Fig. 4, 5). For example, basidiospores of *P. inyoensis* and *P. aegyptiacus* were longer than other species and basidiospore sizes and aspect ratios allowed us to separate *P. termitophilus* and *P. beringamensis* from other species (Fig. 5; Table S5).

TAXONOMY

Sequence data generated for 166 fungarium specimens in the present study revealed 16 unambiguous species of *Podaxis*. The intention of this study was not to be a complete monograph that includes all species in the genus, but in the end, it provided the opportunity to redefine the genus and reconsider genera listed previously as synonyms of *Podaxis*. Although we did not have a specimen for the type species, *P. axatus*, in the study, we included it in the list below with some suggestions on how it should be dealt with in the future. For the remaining taxa, we only consider species for which we had access to type material. All information for examined specimens is listed in Table S1, so the details of specimens in the 'Additional materials examined' paragraphs have been omitted.

The confusion between *Podaxis* and *Podaxon* is discussed in the notes under *Podaxis*. Some authors considered a taxon that was described under one of these genus names, and later treated under the other, as a new combination. However, according to the Code (Art. 61) (Turland et al. 2018) *Podaxis* and *Podaxon* are orthographic variants, which means that *Podaxon* can be corrected to *Podaxis* in a species name, with the addition of as '*Podaxon*' in the formal listing, without the need for a formal new combination. *Podaxon pistillaris* is, for example, listed here as *Podaxis pistillaris* (L.) Fr. (as '*Podaxon pistillaris*') and thus ascribed to Fries (1829). Thus, although Morse (1933) was the first to treat it as *Podaxis*, the species is not listed as *Podaxis pistillaris* (L.) Morse.

In terms of species synonymies in *Podaxis*, there has been much confusion due to overlapping characteristics between taxa and different authors applying different criteria to distinguish species. In addition, the epithets of some species were changed by different authors (see Notes under *P. axatus* and *P. pistillaris*), which contributed to the confusion. The ultimate taxonomic mayhem was created when Morse (1933) synonymized 25 species with the oldest species in the genus, *P. pistillaris*, even though this was not the type species for the genus. For the sake of completeness, we mention previous synonymies based on morphology in the notes under each species, but we consider all species as distinct until typification issues are resolved, and DNA sequence data can clarify synonymies.

Podaxis Desv., J. Bot. 2: 97. 1809 — MycoBank MB 19269

Etymology. πόδιον (feet) and ἄξος (axis) according to Desvaux (1809) and Saccardo (1888).

Synonyms. *Schweinitzia* Grev., Edinburgh Philos. J. 8: 258. 1823. — MycoBank MB 19307 (*nom. illegit.*, Art. 53.1, later homonym for *Schweinitzia* Elliott ex Nuttall 1818).

Herculea Fr., Syst. Mycol. 2: 278. 1823. — MycoBank MB 848985 (*nom. inval.*, Art. 38.1, no description provided).

Podaxon (unranked) Catachyon Ehrenb. ex Fr., Syst. Mycol. (Lundae) 3(1): 62. 1829. — MycoBank MB 19270.

Cauloglossum Grev. ex Fr., Syst. Mycol. (Lundae) 3(1): 60. 1829. — MycoBank MB 19060.

Chainoderma Masee, in Cooke, Grevillea 19(90): 46. 1890. — MycoBank MB 19062.

Type species. *Podaxis axatus* (Bosc) Masee.

Basidiocarp whitish secotoid, up to 40 cm high, consisting of an ellipsoid oblong. *Pileus* apex bluntly acuminate or rounded, borne on a long woody fibrous *stipe* with a bulbous base. *Peridium* dry and scaly in appearance due to rupture of the exoperidium, dehiscing by splitting away at the base where it is attached to the *stipe*. *Basidiospores* subglobose to ovate, thick-walled, straw-coloured, or reddish brown, with a distinct germ-pore.

Notes — Desvaux (1809) described the genus *Podaxis* to accommodate *Lycoperdon axatum* originally described by Bosc (1792), recognizing its distinction from other *Lycoperdon* species. However, Desvaux (1809) and later Fries (1829), opted for different epithets for the species name without providing any explanations. In the Notes section under *P. axatus* we elucidate the reasons why Bosc's epithet should be conserved. It is worth noting that Desvaux (1809) did not mention *L. carcinomale* and *L. pistillare*, which were previously described by Linnaeus (1771, 1781). This could be attributed to the ongoing hostilities between Sweden and France during the Napoleonic Wars, meant that Desvaux only had available the specimen described by Bosc (who was a French mycologist) to examine.

Apparently unaware of Bosc's and Desvaux's work, Greville (1823a) placed the two Linnaean species in a new genus, *Schweinitzia*. However, Greville (1823b) soon realised that *Schweinitzia* was already used as a genus name for flowering plants and changed it to *Cauloglossum*. Fries (1829) recognised Desvaux's (1809) concept of *Podaxis*, and he was the first to group Linnaeus's two species, *L. carcinomale* and *L. pistillare*, together with Bosc's (1792) *L. axatum*, within the same genus. Fries (1829) refined the generic description by Desvaux (1809) but suggested that the name '*Podaxon*' should rather be used as he believed '*Podaxis*' was incorrectly derived from Greek. However, we follow the arguments presented by Masee (1890) and Morse (1933) in their monographs, supporting the priority of the original name, *Podaxis*, and consider *Podaxon* an orthographic variant. This practice aligns with the Articles 60 and 61 of the current International Code of Nomenclature for algae, fungi, and plants (ICN) (Turland et al. 2018).

Fries (1829) transferred *L. carcinomale* and *L. pistillare*, the two species used by Greville (1823a, b) to describe *Cauloglossum*, to *Podaxis* (as '*Podaxon*'), but then appropriated *Cauloglossum* for two non-*Podaxis* (as currently defined) species, designating *C. transversarium* as the type species. We follow Johnston (1902) who argued that *Cauloglossum*, as originally defined by Greville, should be treated as a synonym of *Podaxis*, and that *C. transversarium* is unrelated to *Podaxis*. To accommodate *C. transversarium*, Johnston (1902) described a new genus, *Rhopalogaster*. In a previous publication, Fries (1829) referred to the name *Herculea*, which he apparently intended to use for *C. transversarium* and *C. elatum* (Johnston 1902). However, Fries (1829) subsequently placed these two species within Greville's *Cauloglossum*, treating *Herculea* as synonym without providing a description.

Masee established the genus *Chainoderma* to accommodate *Ch. drummondii*, a new species discovered in Australia that exhibited similarities to, but was distinct from, other species of *Podaxis* (Cooke 1890, Masee 1890). Cunningham (1932) treated *Ch. drummondii* as synonym of *P. loandensis*, becoming the first to formally synonymize *Chainoderma* with *Podaxis*, followed by Bottomley (1948). While it is highly likely that *Chainoderma* is a synonym of *Podaxis*, the status of *Ch. drummondii* as distinct from other Australian *Podaxis* species needs to be reassessed, as no material of this species was available for the present study.

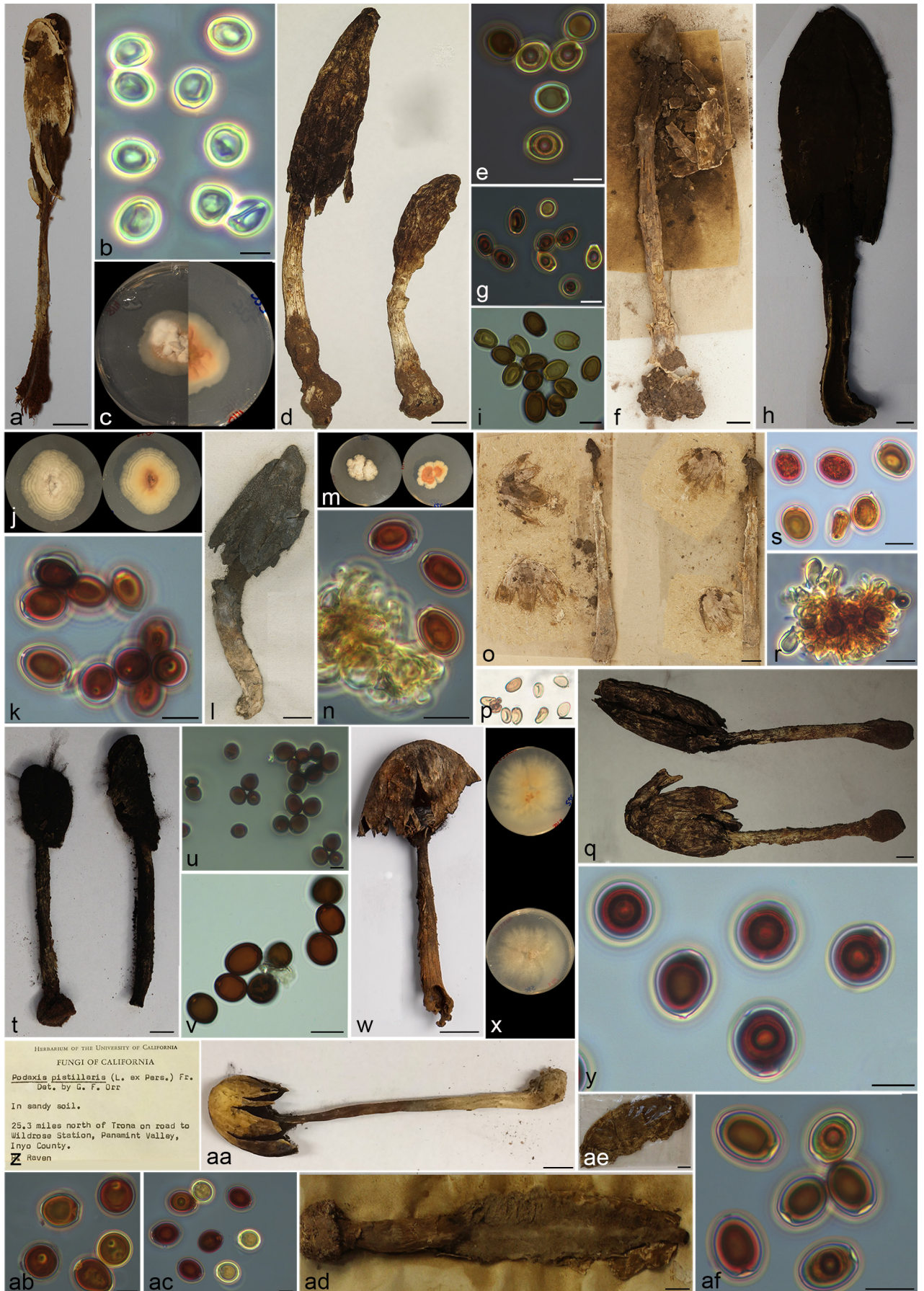


Fig. 6 Photographs of *Podaxis* species. a–c. *Podaxis aegyptiacus*; a. basidiocarp; b. basidiospores; c. culture on PDA. – d, e. *Podaxis africana*; d. basidiocarp; e. basidiospores. – f–i. *Podaxis axatus* (ITS Clade 13 and 15); f. basidiocarp of ITS Clade 13; g. basidiospores of ITS Clade 13; h. basidiocarp of ITS Clade 15; i. basidiospores of ITS Clade 15. – j, k. *Podaxis beringamensis*; j. culture on PDA; k. basidiospores. – l–n. *Podaxis carcinomalis*; l. basidiocarp; m. culture on PDA; n. basidiospores. – o–p. *Podaxis deflersii*; o. basidiocarp; p. basidiospores. – q–s. *Podaxis desolatus* sp. nov.; q. basidiocarp; r. gleba; s. basidiospores. – t–v. *Podaxis emerici*; t. basidiocarp; u, v. basidiospores. – w–y. *Podaxis farlowii*; w. basidiocarp; x. culture on PDA; y. basidiospores. – z–ab. *Podaxis inyoensis* sp. nov.; z. label of holotype (UC 1271002); aa. basidiocarp; ab. basidiospores. – ac. *Podaxis muelleri*; basidiospores. – ad–af. *Podaxis mareebaensis* sp. nov.; ad. basidiocarp; ae. pileus; af. basidiospores. — Scale bar for basidiocarp is 1 cm and for basidiospore is 10 μ m.

Not synonyms of *Podaxis* — The following genera have been treated as synonyms of *Podaxis* by various authors in the past. We provide arguments against these synonymies.

- *Cionium* Link, Mag. Ges. Naturf. Freunde Berlin 3(1): 28. 1809. — MycoBank MB 12037.
- *Mitremyces* Nees, Syst. Pilze (Würzburg): 136. 1816. — MycoBank MB 19218.
- *Catachyon* Ehrenb. ex Fr., Syst. Mycol. (Lundae) 3(1): 62. 1829. — MycoBank MB 19058

Sprengel (1827) classified *P. senegalense* within *Cionium*, a genus considered by Fries (1829) as a synonym of *Cauloglossum*, and a synonym of *Podaxis* by Masee (1890), Patouillard (1890) (as '*Podaxon*') and Cunningham (1932). However, the type species, *Cionium farinaceum* (= *Didymium melanospermum*), is a slime mould (Johnston 1902). *Cionium* is currently recognized as a synonym of *Didymium* Schrad., which is unrelated to *Podaxis* or any other fungus. Therefore, it is not addressed under the ICN.

Podaxis pistillaris was originally described by Sprengel (1827) as *Mitremyces indicus*. *Mitremyces* was later considered a synonym of *Podaxis* by Masee (1890), Cunningham (1932), and Patouillard (1890) (as '*Podaxon*'). However, the type species of *Mitremyces* is *Lycoperdon heterogeneum*, which presumably belongs to the *Geastrales* based on the illustration in the original description (Bosc 1811). *Mitremyces* might be a synonym of *Calostoma*, but the current status of the genus appears to be unresolved (Baseia et al. 2006) (www.indexfungorum.org, www.mycobank.org).

Under the description of *Podaxon*, Fries (1829) mentioned the genus *Catachyon* with a question mark. In a brief note at the bottom of the page, he mentioned a 'nine inch high' *Stemonitis* that was apparently collected by Ehrenberg in Egypt and described by Nees. Fries stated that the species seemed quite distinct from Thunberg's specimens, presumably referring to *P. carcinomalis*. *Catachyon* is not listed as a synonym in either of the two major monographs of the genus (Masee 1890, Morse 1933). However, we could not locate Ehrenberg specimens or any generic description or species names, including a type species, for this genus. Therefore, in our opinion, the name has never been validly published and should be excluded from future synonymies until a specimen from Ehrenberg or a description by Nees is potentially discovered.

***Podaxis aegyptiacus* Mont.** (as '*Podaxon aegyptiacus*'), in Decaisne, Ann. Sci. Nat. Bot., sér. 2 4: 195. 1835 — MycoBank MB 234037; Fig. 6a–c

Etymology. The epithet refers to Egypt, where this species was first recorded.

Synonym. *Cauloglossum aegyptiacum* (Mont.) Corda, in Zobel, Icon. Fung. (Prague) 6: 18. 1854. — MycoBank MB 176908.

Typus. ETHIOPIA, 1998, *Ib Friis*, neotype CF 92630 (C, MBT 10013506, designated here).

Basidiocarp epigeous, stipitate, 18.0–24.2 cm tall, dry, some with clockwise spiral. *Pileus* 7.4–10.1 cm in height × 2.5–4.7 cm diam at maturity, narrowly ellipsoid to paraboloid, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus and base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. *Lamellae* absent, basidiospore-producing tissue stringy-powdery, dark brown to nearly black. *Stipe* from the base to top of pileus, 6.6–11.7 × 0.6–1.1 cm at maturity under the pileus, hollow, stuffed, cylindrical without bulb, dry, hard, and woody, fibrous, striate, white to yellowish brown, some with right hand spiral, swollen at the base. *Basidiospores*

(10.2–)13.7–16.9(–21.3) × (6.7–)10.4–13.2(–15.9) μm, aspect ratio 0.9–2.1 (n = 105 from five specimens), ellipsoid to ovoid, smooth, transparent, central germ pore prominent and hilum inconspicuous, light yellow or yellowish brown to dark brown. The germ pore is not obvious when light yellow, but obvious when brown.

Habitat & Distribution — Growing in sandy soil and distributed in Africa including Ethiopia, Egypt, Ghana, and Sudan and Asia including Sri Lanka.

Additional materials examined. EGYPT, KEW 254531. — GHANA, KEW 254621. — ISRAEL, KEW 254667. — SRI LANKA, UPS 11366. — SUDAN, KEW 254532.

Notes — Montagne described *Podaxis aegyptiacus* in 1835 from a specimen collected in the desert between Suez and Gaza (Audouin et al. 1835, Decaisne 1835). Fischer (1889) reported *P. aegyptiacus* from the Sinai Peninsula and Masee (1890) treated this as a distinct species in his monograph, namely as *Podaxis aegyptica*. Lloyd & Stevenson (1905) and Cleland & Cheel (1923) reported *P. aegyptiacus* based on specimens from Bourke in New South Wales, Australia. However, Cunningham (1932) and Doidge (1950) listed it as synonym of *P. pistillaris*. More recently, Priest & Lenz (1999) examined the Cleland & Cheel (1923) specimen from New South Wales and revised it as *P. pistillaris*.

Podaxis aegyptiacus clusters in a single clade separated from *P. deflersii* and *P. perraldieri* in the phylogenomic analyses (Fig. 3b). The basidiospores of *P. aegyptiacus* are significantly longer than the closely related species, *P. deflersii* (11.5–13.3 × 7.5–8.6 μm, aspect ratio 1.3–1.8) and *P. perraldieri* (10.1–11.7 × 9.2–10.7 μm, aspect ratio 0.9–1.5) (Fig. 5). Four of the five specimens were collected from Israel, Egypt, Ethiopia, and Sudan, near the location of the holotype of *P. aegyptiacus*, the desert between Gaza and Suez (Decaisne 1835). Thus, this species is named *P. aegyptiacus*. Due to the absence of the type from Montagne's description, a neotype (CF 92630) is designated and described in this study based on morphology and phylogenetics.

***Podaxis africana* De Villiers et al., S. African J. Bot. 55(2): 160. 1989** — MycoBank MB 125143; Fig. 6d, e

Etymology. The epithet refers to Africa as the first continent where this species was found.

Typus. SOUTH AFRICA, Gauteng, Pretoria, Walmansthal, collected on a termite heap, 10 Mar. 1965, holotype PREM 42889; Limpopo, Warmbad, Mabalingwe, collected on a termite heap, Jan. 1995, *M. Nel*, epitype PRUM 3984 (PRU, MBT 10013507, designated here).

Basidiocarp epigeous, stipitate, 10.26–32.2 cm tall, dry, dehiscent at maturity. *Pileus* 3.0–24.2 cm in height × 1.3–6.2 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. *Gleba* stringy-powdery, dark brown to nearly black. *Stipe* from the base to top of pileus, 5.2–13.6(–13.6) × 1.0–3.1 cm at maturity under the pileus, stuffed, cylindrical without bulb, dry, hard, and woody, fibrous, striate, white to yellowish brown sometimes staining bright red, slightly swollen at the base. *Basidiospores* (7.0–)10.5–12.6(–16.5) × (5.3–)7.6–9.1(–11.3) μm, aspect ratio 1.0–1.9 (n = 487 from 16 specimens), ellipsoid to ovoid, smooth, transparent, central germ pore prominent and hilum inconspicuous, yellowish brown to dark brown.

Habitat & Distribution — Emerging from termite mounds and distributed in South Africa.

Additional materials examined. SOUTH AFRICA, PREM 5125, PREM 12288, PREM 23672, PREM 26602, PREM 30714, PREM 36117, PREM 44240, PREM 44664, PREM 57485, PREM 60320, PRUM 4332, PRUM 4368, Wdb Bloemfontein, Wdb Maropeng1, Wdb VVG, Wdb Podaxis1, Wdb Podaxis3, Wdb Podaxis6, Wdb Podaxis9, Wdb WdB.

Notes — Specimens in ITS Clade 10 were collected from Pretoria, South Africa, and associated with termites, which is the same location and ecology reported for *P. africana* by Villiers et al. (1989). The morphology of ITS Clade 10 specimens is similar to the description of *P. africana*, including in the basidiocarp, pileus, stipes, and basidiospores (10.1–13.8 × 8.6–10.1 µm). Based on the distribution, ecology, and morphology, ITS Clade 10 is named *P. africana*. Since PREM 57485 has genomic information available, it is designated as the epitype of *P. africana*. The species is characterised by its association with termites and having the largest spores among the species distributed in Africa.

Podaxis axatus (Bosc) Masee (as '*Podaxis axata*'), J. Bot., Lond. 28: 75. 1890 — MycoBank MB 296666; Fig. 1b, 6f–i

Etymology. The epithet (from ἀξίς = axis) presumably refers to the prominent stipe of this fungus.

Basionym. *Lycoperdon axatum* Bosc, Act. Soc. Hist. Nat. Paris 1: 47. 1792. — MycoBank MB 161251.

Synonyms. *Podaxis senegalensis* Desv., J. Bot. (Desvaux) 2: 97. 1809. — MycoBank MB 431995.

Cionium senegalense (Desv.) Spreng., Syst. Veg., ed. 16 4(1): 529. 1827 (*nom. illegit.*, see Notes under *Podaxon*).

Podaxis calyptratus Fr. (as '*Podaxon*'), Syst. Mycol. (Lundae) 3(1): 63. 1829. — MycoBank MB 497408.

Podaxis loandensis Welw. & Curr. (as '*Podaxon*'), Trans. Linn. Soc. London 26(1): 288. 1858(1870). — MycoBank MB 244750.

Typus. SENEGAL, collected on small island in Senegal river, 1792, *M. Roussilon*, lectotype Act. Soc. Hist. Nat. Paris Plate XI (Bosc 1792) (Fig. 1b, MTB 10013508, designated here). GUINEA, Dubréka, Conakry, 15 June 1899, *J. Dybowski*, epitype (of *Podaxis senegalensis*) FH 940098 (FH, MTB 10013509, designated here). ANGOLA, collected on the sandy soil, 1860, *C. Welwitsch*, epitype (of *Podaxis loandensis*) Iter Angolenseexs. 148 (KEW 254533, K(M), MTB 10013510, designated here).

Descriptions — Bosc (1792: 47; plate XI); Desvaux (1809: 95); Fries (1829: 63); Patouillard (1890: 164).

Habitat & Distribution — Free-living in dry sandy places and distributed in Ivory Coast, Senegal, and Guinea.

Additional materials examined. IVORY COAST, IC *Trinervitermes*, *Trinervil*.

Notes — *Lycoperdon axatum* was originally described by Bosc (1792). When Desvaux (1809) erected the genus *Podaxis* for *L. axatum*, he changed the epithet to *senegalensis*. Sprengel (1827) treated *P. senegalensis* in *Cionium*, but this combination is illegitimate since the type species of *Cionium* is a slime mould (see Notes under *Podaxis*). Fries (1829) again decided to change the epithet of the same species, this time to *calyptratus*, and later Patouillard (1890) treated it as *Podaxon axatum*. In the first monograph of *Podaxis*, Masee (1890) revised the species and incorrectly used *Podaxis axata* (we corrected here to *P. axatus*) and treated *Lycoperdon axatum*, *Cionium senegalense*, *P. calyptratus*, *P. loandensis*, and *P. senegalensis* as synonyms of *P. axata*. Cunningham (1932) treated *P. muelleri* as the synonym of *P. loandensis* based on Lloyd & Stevenson (1905), and did the same for *P. axata*, *P. calyptratus*, and *P. senegalensis*, while Lloyd & Stevenson (1905) was inclined to retain *P. loandensis* as different from *P. muelleri* based on the scantiness of its capillitium. Cunningham (1932) also synonymized *Chainoderma drummondii* with *P. loandensis* without examining specimens, suggesting it could also be a synonym of *P. squarrosus* or *P. gollani*. We agree with Masee's revision

of *P. axatus* in 1890 and do not treat *Chainoderma drummondii* and *P. muelleri* as synonyms of *P. axatus*. Regarding the revision of *P. muelleri*, see below.

Lloyd & Stevenson (1905) and Cunningham (1932) argued that the specimen for *P. axata* was lost, and the descriptions of *P. calyptratus* and *P. senegalensis* were solely based on the illustration by Bosc (1792). Cunningham (1932) pointed out that *P. loandensis* was thus the oldest name with an available specimen, and therefore should have priority. However, the loss of a specimen does not invalidate a name, and the oldest valid name always holds priority. We agree with Masee (1890) and Morse (1933), who argued that there was no justification to create new epithets and that *axatus* should have priority. Avenues to resolve the problem are either to designate a neotype from the same origin (i.e., location and substrate), in this case Senegal, or designate the illustration of Bosc (1792) as a lectotype, supplemented with an epitype specimen from the same geographic region as the original. In the absence of a specimen from the same origin, but a similar one from another locality, a reference specimen can be designated (Ariyawansa et al. 2014). In this study, we propose the designation of Bosc's illustration as the lectotype, and also designate one specimen from Guinea and one from Angola as the epitypes of *P. senegalensis* and *P. loandensis*, respectively. These specimens are chosen based on their location in ITS Clade 13 and 15, respectively, and they are collected near the original collection location (Senegal) and in similar environments.

Although the width and aspect ratio of basidiospores in ITS Clade 13 and 15 are different, the absence of genomes prevents us from determining whether those two clades would cluster into one species in a phylogenomic analysis, similarly to ITS Clade 8 and 9 for *P. farlowii* (Fig. 5; Table S5). Additionally, to achieve further identification and differentiation between the termite-associated and free-living specimens in ITS Clade 15, genome analysis and a larger sample size are required (Fig. 2).

Podaxis beringamensis Priest & M. Lenz, Austral. Syst. Bot. 12(1): 112. 1999 — MycoBank MB 296709; Fig. 6j, k

Etymology. From the Australian aboriginal 'beringama' meaning ant hill.

Typus. AUSTRALIA, Queensland, Cape York Peninsula, collected in termite *Nasutitermes triodeae*, 29 June 1984, *M. Lenz*, *R.A. Barrett* & *L.R. Miller*, holotype DAR 51283 (DAR). AUSTRALIA, Queensland, halfway between ESK and Wivenhoe Dam, -27.275 152.49167, collected in termite mound, 28 Feb. 2003, *Young*, epitype AQ 795752 (BRI, MTB 10013511, designated here).

Description — Priest & Lenz (1999: 111–112; f. 3, 4).

Habitat & Distribution — Emerging from termite mounds in woodland and distributed in Australia.

Additional materials examined. AUSTRALIA, AQ 553606, AQ 553610, AQ 554176, AQ 742011.

Notes — All specimens were collected from termite mounds in Queensland, Australia, and share the same ecology and geographical distribution as the holotype of *P. beringamensis* (Table S1). Basidiospore measurements were the same as the description of *P. beringamensis* (Priest & Lenz 1999). Consequently, the specimens in Clade 12 are determined as *P. beringamensis* and specimen AQ 795752 is designated as epitype due to the availability of both ITS and genome sequences (Fig. 3b). Based on observations of 144 basidiospores from six specimens, the basidiospore width is extended from 9 µm to 11.4 µm, compared to the original description (Priest & Lenz 1999), and the aspect ratio is determined as (1.0–)1.3–1.5(–2.0) (Fig. 5; Table S5).

The species *P. beringamensis*, *P. farlowii*, *P. mareebaensis*, and *P. muelleri* were collected in Australia but they were dis-

tinguishable based on both ITS phylogeny, phylogenomics, ecology, and morphology. The four species cluster in three distinct clades in the ITS phylogeny and showed good resolution in the phylogenomic analysis (Fig. 2d, 3b). *Podaxis muelleri* and *P. mareebaensis* are split in the phylogenomics analysis, which is consistent with their ecology (Fig. 3b). *Podaxis beringamensis* and *P. mareebaensis* are associated with termites, while *P. farlowii* and *P. muelleri* are free-living (Fig. 2d, 3b). All four species are distinct from each other based on morphology. The basidiocarp (14–36 cm) and pileus (up to 15 cm high and 4–6 cm diam) of *P. beringamensis* in the Priest & Lenz (1999) description are larger than *P. farlowii* (7.5–17.2 cm) and basidiospores of *P. beringamensis* (10.3–12.1 × 7.4–8.7 µm, n = 144) are significantly smaller than *P. farlowii* (11.5–13.3 × 10.8–11.8 µm, n = 249), *P. muelleri* (12.0–14.5 × 10.9–12.2 µm, n = 35), and *P. mareebaensis* (11.1–16.4 × 8.5–12.8 µm, n = 68) (Fig. 4, 5).

Podaxis carcinomalis (L.f.) Fr. (as '*Podaxon carcinomalis*'), Syst. Mycol. (Lundae) 3(1): 62. 1829 — MycoBank MB 249571; Fig. 6l–n

Etymology. In the letter that Thunberg (1773) sent with the specimens from South Africa to Linnaeus, he mentioned that it was 'used by the local population to treat cancer'. Based on this, Linnaeus chose the epithet '*carcinomalis*' rather than '*africanum*' that was suggested by Thunberg.

Basionym. *Lycoperdon carcinomale* L. f., Suppl. Pl.: pl. 453. 1781. — MycoBank MB 224210.

Synonyms. *Scleroderma carcinomale* (L.f.) Pers., Syn. Meth. Fung. (Göttingen) 1: 151. 1801. — MycoBank MB 497430.

Schweinitzia carcinomalis (L.f.) Grev., Edinburgh Philos. J. 8(16): 258. 1823 (*nom. illegit.*, Art. 53.1, later homonym for *Schweinitzia* Elliott ex Nuttall, 1818).

Cauloglossum carcinomale (L.) Grev., Scot. Crypt. Fl. 2: 61. 1823. — MycoBank MB 848914.

Typus. SOUTH AFRICA, the Cape of Good Hope, South of Kammanassie Mountains, Western Cape, Ganzekraal river, alongside N9 road, growing in termite mounds, 10 Dec. 1772, C. Thunberg, holotype LINN 1287.8 (LINN).

Description — Fries (1829: 62); Linnaeus f. (1781: 453).

Habitat & Distribution — Emerging from termite mounds and distributed in South Africa and Australia.

Additional materials examined. SOUTH AFRICA, PREM 1689, PREM 18109, PREM 28810, PREM 29955, PREM 42236, PREM 54389, PRUM 2083, PRUM 2698, PRUM 3913, Wdb LiZeffert, Wdb NB1, Wdb WP1260.

Notes — On 5 March 1773, Thunberg, a student of Linnaeus, sent him a specimen collected on 10 December 1772 from 'ant-hills' (termite mounds) at Ganzekraal in the Western Cape, South Africa referring to it as "*Lycoperdon africanum*, used by the local population to treat cancer, No. 343." This event was recorded in their correspondences from 1773 (Linnaeus 1771, 1781, Stover 1794). With the death of Linnaeus in 1778, the unpublished manuscript in which he described *Lycoperdon carcinomalis* (holotype in London with the Linnaean Society) from Thunberg's specimen was edited and published by his son, Linnaeus f., in 1781 (Stover 1794). Linnaeus f. (1781) credited Thunberg for the specimen, but no reason was provided for why he changed the epithet from '*africanum*', as Thunberg suggested, to '*carcinomalis*'. Later, Berkeley & Broome (1886) reported the occurrence of *P. carcinomalis* (L.f.: Pers.) Fr. from 'ant-hills' (termites mounds) in North Queensland, Australia, based on a collection sent by F.M. Bailey. Masee (1890) identified *P. carcinomalis* (including *P. elatus*) in his monograph and described how it was absent from Australia. However, Cooke (1892) recorded three species from Australia, including *P. carcinomalis* from Queensland and South Australia. Hennings (1893) reported *Podaxis* (as '*Podaxon*') *carcinomalis* from

Eritrea and Egypt, while Melchers (1931) found *Podaxis* (as '*Podaxon*') *carcinomalis* in sandy habitats south of the Great Pyramid, Egypt. Morse (1933), Bottomley (1948), and Doidge (1950) listed *P. carcinomalis* as synonym of *P. pistillaris*. More recently, Priest & Lenz (1999) examined the morphology of *P. carcinomalis*, *P. termitophilus*, and *P. pistillaris* based on type specimens, including LINN 1287.8 (holotype of *P. pistillaris*), Herb Thunberg (isotype of *P. carcinomalis*) and PC (holotype of *P. termitophilus*). Based on the narrower basidiospores than *P. pistillaris*, and considering Fischer's (1889) description of *P. carcinomalis* from material collected in southwest Africa, Priest & Lenz (1999) resurrected *P. carcinomalis*. They also synonymized *P. termitophilus* with *P. carcinomalis* based on morphological characteristics.

In this study, we examine 13 specimens that cluster in the phylogenetic Clades 14 and 16, as does the holotype of *P. carcinomale* (LINN 1287.8) based on ITS and genome sequences that included collections from three museums (Fig. 3b). We have access to eight photographs of specimens for measuring sporocarps, and four specimens with a total of 213 basidiospores are available for microscopic examination. It is worth noting that all other specimens of *P. carcinomalis* included in our analysis were also collected from termite mounds, except for the specimen Wdb LiZeffert and KEW 179298, which did not have accompanying ecological records. The ecological characteristics of these specimens are consistent with the original description of *P. carcinomalis* provided by Priest & Lenz (1999) and differ from the characteristics of the free-living species *P. pistillaris*. Based on these findings, we can confidently confirm that this particular clade represents *P. carcinomalis*.

Based on the aspect ratio of its basidiospores, it is possible to distinguish *P. carcinomalis* (1.3–1.5) from *P. pistillaris* (1.1–1.2). Additionally, *P. africana*, *P. beringamensis*, and *P. carcinomalis* are similar in basidiospore size and ecology, appearing on termite mounds. However, they can be differentiated from each other based on their distribution, morphology, and phylogenetic relationships. *Podaxis africana* and *P. carcinomalis* are distributed in South Africa, while *P. beringamensis* is only found in Australia. Furthermore, the pileus of *P. beringamensis* (4–6 cm) is larger than that of *P. carcinomalis* (2.3–3.1 cm) and *P. africana* (2.8–4.1 cm). Phylogenetically, the three species are clearly separated from each other in both the ITS and the phylogenomic analyses (Fig. 2d, 3b).

Podaxis deflersii Pat. (as '*Podaxon deflersii*'), Bull. Soc. Mycol. France 6: 165. 1890 — MycoBank MB 249594; Fig. 6o, p

Etymology. Named after the French botanist Albert Deflers, who collected the type specimen during his botanical exploration of Yemen (Patouillard 1890).

Typus. YEMEN, Pays Fodhli: Wadi el 'Asal (15 km east of E.N.E. Schagra), 22 Mar. 1890, A. Deflers, holotype (of *P. deflersii*) FH 940093 (FH).

Description — Patouillard (1890: 165; pl. XVII, f. 1a–e).

Habitat & Distribution — Emerging from desert soil and distributed in Yemen.

Notes — Patouillard (1890) originally described *P. deflersii* from a material collected in Yemen, and subsequently, Melchers (1931) reported it from the Egyptian desert, although without a description (Melchers 1931, Soliman et al. 2016). Morse (1933) examined the Old and New World *Podaxis* materials and considered basidia, spores and capillitial as the main morphological characters. He observed that these features exhibited similar and progressive development, and any differences observed were attributed to specimen age. Consequently, Morse (1933) treated 25 species, including *P. deflersii*, as a synonym of *P. pistillaris*.

The holotype (FH 940093) of *P. deflersii* has been successfully sequenced and examined in the laboratory. In the phylogenetic analysis based on ITS sequences, *P. deflersii* clustered with other specimens of other species. However, in the phylogenomic analysis, *P. deflersii* formed a distinct sister clade to *P. perraldieri* and is separated from other species. Basidiospore measurements revealed that *P. deflersii* (11.5–13.3 × 7.5–8.6 µm, n = 15) can be distinguished from *P. perraldieri* (10.1–11.7 × 9.2–10.7 µm, n = 121) by its smaller basidiospores (Fig. 5).

Podaxis desolates G.S. Li, M. Poulsen & Z.W. de Beer, sp. nov. — MycoBank MB 848287; Fig. 6q–s

Diagnosis. Similar to *P. emeric*i but differs by a larger basidiocarp (15.8–19.0 vs 10.0–15.0 cm), pileus (3.0–8.0 vs 3.4–6.3 cm), and stipe (7.9–8.3 vs 3.8–7.6 cm) than *P. emeric*i.

Etymology. From the Latin *desolates* that means desert or desolation, referring to the desolated places where specimens of this species were collected.

Typus. SOUTH AFRICA, Western Cape, Brand se Baai, Lutzville, growing in dunes, Nov. 1992, R. de Villiers, holotype PRUM 3659 (PRU, designated here).

Basidiocarp epigeous, stipitate, 15.8–19.0 cm tall, dry, dehiscent at maturity. **Pileus** 3.0–8.0 cm in height × 2.4–7.4 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Gleba** stringy-powdery, dark brown to nearly black. **Stipe** from the base to top of pileus, 7.9–8.3 × 0.8–0.9 cm at maturity under the pileus, hollow, stuffed, cylindrical without bulb, dry, hard and woody, fibrous, striate, white to yellowish brown sometimes staining bright red, swollen at the base. **Basidiospores** (9.6–)10.6–12.5(–16.6) × (8.8–)9.5–11.4(–15.9) µm, aspect ratio 1.0–1.4 (n = 19 from two collections), ellipsoid to ovoid, smooth, transparent, central germ pore prominent and hilum inconspicuous, light yellow or yellowish brown to dark brown.

Habitat & Distribution — Emerging from dunes and distributed in South Africa.

Additional materials examined. SOUTH AFRICA, PREM 44953 (PREM, Table S1).

Notes — In the phylogenomic analysis (Fig. 3b), the clade containing *P. desolates* is distinct and separated from *P. aegyptiacus*, *P. deflersii*, *P. emeric*i, *P. namaquensis*, *P. namibensis*, and *P. perraldieri*. The basidiospores of *P. desolates* are significantly different from other species (Fig. 5). Specifically, the basidiospores of *P. desolates* are smaller than *P. aegyptiacus* (13.7–16.9 × 10.4–13.2 µm) and *P. emeric*i (13.3–15.5 × 11.1–13.0 µm), but larger than those of *P. namibensis* (8.1–8.9 × 7.2–7.7 µm) and *P. namaquensis* (9.3–11.0 × 8.0–10.0 µm), and wider than *P. perraldieri* (10.1–11.7 × 9.2–10.7 µm) and *P. deflersii* (11.5–13.3 × 7.5–8.6 µm). Furthermore, the basidiocarp, pileus and stipe of *P. desolates* are larger than *P. emeric*i (basidiocarp: 10.3–15.0 cm, pileus: 3.4–4.6 cm in height × 1.4–2.4 cm diam and stipe: 6.1–7.1 cm in height × 0.5–0.9 cm diam) (Fig. 4). Additionally, the distribution of *P. desolates* is limited to South Africa, which is distinct from *P. emeric*i, found in India, Israel, and Australia.

Podaxis emerici Berk. ex Masee (as '*Podaxon*'), J. Bot., Lond. 28: 77. 1890 — MycoBank MB 249407; Fig. 6t–v

Etymology. Named for Captain Emeric Streatfield Berkeley (1834–1898), a British Lieutenant-Major General who was also a botanist and collected the specimen in India.

Basionym. *Xylaria emeric*i Berk. ex. Cooke, Grevillea 11 (59): 86. 1883. — MB 250098.

Typus. INDIA, M.J. Berkeley, holotype KEW 254548 (K(M)).

Description — Cooke (1883: 86; pl. 165, f. 50); Masee (1890: 77).

Habitat & Distribution — Emerging in dunes and distributed in Australia, India, and Israel.

Additional materials examined. AUSTRALIA, KEW 254655. — INDIA, 1866, S.S. Berkeley, Syntype (of *Podaxis emeric*i) KEW 254545 (K(M)).

Notes — The occurrence of *P. emeric*i was reported by Cooke (1883) from Neilgherries, Tamil Nadu, India. However, it was initially misclassified as *Xylaria*. Patouillard (1890) subsequently treated it as *Podaxon* and suggested that the illustrations of Berkeley (1868), previously labelled as *P. carcinomale* from India, actually represented *P. emeric*i. Masee (1890) identified *P. emeric*i along with six other species in the first monograph of *Podaxis*.

The type specimen of *P. emeric*i (KEW 254548, holotype) forms a distinct clade in the phylogenomic tree, along with specimen KEW 254655 collected from Australia. The morphology of the latter specimen is indistinguishable from *P. emeric*i, leading to the designation of this clade as *P. emeric*i (Fig. 2d, 3b). The inclusion of specimen KEW 254655 extends the known distribution of *P. emeric*i from India to Australia. Basidiospores of *P. emeric*i (13.3–15.5 × 11.1–13.0 µm) are significantly larger than the sister species, *P. namaquensis* (9.3–11.0 × 8.0–10.0 µm) and *P. namibensis* (8.1–8.9 × 7.2–7.7 µm) (Fig. 5). See the Notes under *P. desolates* for further details on the differences between *P. desolates* and *P. emeric*i.

Podaxis farlowii Masee (as '*Podaxon*'), J. Bot., Lond. 28: 76. 1890 — MycoBank MB 249237; Fig. 6w–y

Etymology. The epithet refers to W.G. Farlow, mycologist at Harvard University after which the Farlow Herbarium was named, who sent type specimens to K(M) (Masee 1890).

Basionym. *Podaxon farlowii* (Masee) Pat., Bull. Soc. Mycol. France 6: 167. 1890. — MycoBank MB 528493.

Typus. USA, New Mexico, C. Wright, paratype (of *Podaxis farlowii*) 6371 (KEW 254550, K(M)).

Description — Masee (1890: 76, pl. 295, f. 1–3).

Habitat & Distribution — Free-living and emerging in dune, red pindan or sandy soil, and woodland and distributed in Australia and USA.

Additional materials examined. AUSTRALIA, AQ 553608, AQ 553622, AQ 645842, AQ 790499, AQ 799166, AQ 799332, AQ 811217, KEW 22363, KEW 22412, KEW 24799, KEW 254641.

Notes — *Podaxis farlowii* was originally identified and described by Masee (1890) in the first monograph of *Podaxis*, and a variant form *P. farlowii* f. *gracilis*, was later recorded in Sonora, Mexico by Patouillard (1908). However, Morse (1933) concluded that the observed morphological differences were due to the specimen age and, therefore, considered *P. farlowii* and *P. pistillaris* to be the same species, without examining the type specimens. Here we examined the paratype and confirmed the morphology of *P. farlowii*, supporting its distinct status as described by Masee (1890).

The holotype of *P. farlowii* (KEW 254549) failed to be sequenced, and there were insufficient spores available for observations. However, its paratype (KEW 254550) clusters together with other specimens in a distinct clade in the phylogenomic analysis. This clade exhibits the same ecological characteristics and shows similar morphology to the existing description of *P. farlowii*. Thus, this clade is designated as *P. farlowii*. The species has been collected in Australia and the USA (only

KEW 254550) and it is closely related to *P. mareebaensis* and *P. muelleri*, which were also collected in Australia. The three species, *P. farlowii*, *P. muelleri*, and *P. mareebaensis*, can be differentiated based on their phylogenomic placement, ecology, and morphology. *Podaxis farlowii* and *P. muelleri* are recorded as free-living species, whereas *P. mareebaensis* is associated with termites. *Podaxis farlowii* is characterised by having a subequal pileus and stipe, as well as subglobose, brown spores, distinguishing it from *P. mareebaensis* and *P. muelleri* (Saccardo 1925a, b).

Podaxis inyoensis G.S. Li, M. Poulsen & Z.W. de Beer, *sp. nov.* — MycoBank MB 848286; Fig. 6z–ab

Diagnosis. An independent separate clade in phylogeny and phylogenomics and similar to the free-living species *P. perraldieri* and *P. pistillar* in macro-morphology but easily distinguished in having largest basidiospores (14.9–17.4 × 13.4–15.6 µm).

Etymology. Named for Inyo County in California where the type specimen was collected. Inyo means 'dwelling place of the great spirit' in the Mono language.

Typus. USA, California, Inyo County, Panamint Valley, 25.3 miles north of Trona on road to Wildrose station, collected in the sandy soil, *P. Raven*, holotype UC 1271002 (UC, designated here).

Basidiocarp epigeous, stipitate, 8.1–11.6 cm, up to 20.3 cm tall, dry, dehiscent at maturity. **Pileus** 3.3–7.2 cm in height × 1.4–6.0 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Gleba** stringy-powdery, dark brown to nearly black. **Stipe** from the base to top of pileus, 2.9–8.2 × 0.5–1.1 cm at maturity under the pileus, hollow, stuffed, cylindrical without bulb, dry, hard, and woody, fibrous, striate, white to yellowish brown sometimes staining bright red, swollen at the base. **Basidiospores** (11.6–)14.9–17.4(–24.9) × (9.8–)13.4–15.6(–20.7) µm, aspect ratio 0.9–1.6 (n = 246 from 10 specimens), ellipsoid to ovoid, smooth, transparent, central germ pore prominent and hilum inconspicuous, light yellow or yellowish brown to dark brown.

Habitat & Distribution — Free-living and emerging from sandy clay or soil or riverbank, distributed in USA and Mexico.

Additional materials examined. MEXICO, FH 374335; FH 940096; MEXU 8423; MEXU 8424; MEXU 8425; UPS F567. — USA, CF 101398; CF 101399; FH 374332; FH 374333; FH 374334; FH 374336; FH 374338; UPS 1932.

Notes — The specimens belonging to this particular clade form a distinct group in both the ITS and phylogenomic trees. All specimens in this clade were collected from North America, including Mexico and the USA. While this distribution is similar to that of *P. longii*, found in Mexico and Central America, the spores of *P. longii* (6–10 × 6–8 µm) based on McKnight (1985) description are smaller than those of *P. inyoensis*. As a result, this clade represents a new and distinct species, which has been named *P. inyoensis*. Notably, the basidiospores of *P. inyoensis* and *P. aegyptiacus* are the largest among all species in this study. However, they are distinguishable from each other based on the phylogenomic analysis, distribution, macro-morphology, and basidiospore aspect ratio (Fig. 5).

Podaxis muelleri Henn. (as '*Podaxon mülleri*'), Hedwigia 43(3): 187. 1904 — MycoBank MB 244440; Fig. 6ac

Etymology. The epithet refers to Ferdinand Jacob Heinrich von Müller, German-Australian botanist, who collected the specimen.

Typus. AUSTRALIA, Gascogne River, F.V. Müller, holotype Müller s.n.; AUSTRALIA, alongside of Donohue Highway, NW of Boulia, -22.83 139.84, 29 Mar. 2007, Hansen, neotype AQ 799334 (BRI, MBT 10013512, designated here).

Description — Hennings (1904: 187–188).

Basidiospores pale yellow or brown, subolive or elliptical, transparent, germ pore conspicuous, (9.9–)11.9–14.4(–16.6) × (8.6–)10.7–12.1(–14.2) µm, aspect ratio 1.1–1.3 (n = 35 from two specimens).

Habitat & Distribution — Growing in soil and distributed in Australia.

Additional materials examined. AUSTRALIA, AQ 799333.

Notes — *Podaxis muelleri* was proposed by Hennings (1904) based on a specimen from Australia, and later treated by Cunningham (1932) as a synonym of *P. loandensis*. However, we agree with the revision made by Masee (1890) (see the Notes in *P. axatus*) that *P. muelleri* is not a synonym of *P. axatus*. The specimens in this clade, along with *P. mareebaensis*, form a distinct clade based on ITS, but they are separated in the phylogenomic analysis, and by ecological association and morphology (Fig. 2d, 3b). The morphology and distribution of this clade closely match the description of *P. muelleri* from the Gascogne River in Western Australia, particularly in terms of basidiospore size (10–16 × 10–12 µm). Therefore, this species has been named *Podaxis muelleri*. Because the holotype (Müller s.n.) was lost, we designate a new specimen from the same region and with the same morphology (AQ 799334) as a neotype, and the specimen has both genome and ITS sequence available. The basidiospores of *P. muelleri* (11.9–14.4 × 10.7–12.1 µm, n = 35) are significantly larger than the two other species found in Australia: the free-living *P. farlowii* (11.5–13.3 × 10.8–11.8 µm, n = 249) and the termite-associated *P. beringamensis* (10.3–12.1 × 7.4–8.7 µm, n = 144) (Fig. 5). The aspect ratio of *P. muelleri* (1.1–1.3) is smaller than that of *P. mareebaensis* (1.1–1.6), clearly distinguishing it from *P. mareebaensis* (Fig. 5).

Podaxis mareebaensis G.S. Li, M. Poulsen & Z.W. de Beer, *sp. nov.* — MycoBank MB 848285; Fig. 6ad–af

Diagnosis. Similar to free-living *P. muelleri* collected from Australia but different in ecology and morphology. *Podaxis mareebaensis* emerging in termite mounds and easily distinguished by its basidiospores aspect ratio (1.1–1.6) from *P. muelleri* (1.1–1.3).

Etymology. Named for the town of Mareeba where the type specimen was collected. The town's name is derived from an Aboriginal word that means 'the meeting of the waters'.

Typus. AUSTRALIA, Queensland, on Cairns-Mareeba Rd, 30 km E (W) of Kuranda Near Mareeba, -16.92 145.42, emerging in termite mound, 16 Mar. 2001, Ramsden, holotype AQ 645840 (BRI, designated here).

Basidiocarp epigeous, stipitate, up to 15.2 cm tall, dry, indehiscent at maturity. **Pileus** up to 8.9 cm in height × 4.1 cm diam at maturity, narrowly ellipsoid to paraboloid, white pale cream with light brown bottom, dry, brittle, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Gleba** taupe filamentous. **Stipe** up to 4.6 cm long under the pileus and 1.3 mm thick at maturity, cylindrical with bulb, dry, hard and woody, fibrous, striate, white to greenish white. **Basidiospores** light yellow or brown, subolive or elliptical, transparent, germ pore conspicuous, (9.2–)11.1–16.4(–20.0) × (7.0–)8.5–12.8(–14.8) µm, aspect ratio 1.1–1.6 (n = 68 from three specimens).

Habitat & Distribution — Growing in termite mounds and distributed in Australia.

Additional materials examined. AUSTRALIA, AQ 645843, KEW 254537.

Notes — In the phylogeny based on ITS, *P. mareebaensis* clustered with *P. muelleri*, but they were separated by phylogenomics and exhibited different ecological associations (emerging from soil or termite mounds) (Fig. 2d, 3b). The morphology of *P. mareebaensis* is distinguishable from the description of the



Fig. 7 Photographs of *Podaxis* species. a–c. *Podaxis namaquensis* sp. nov.; a. basidiocarp; b, c. basidiospores. – d–f. *Podaxis namibensis* sp. nov.; d. label of holotype (PRUM 4366); e. basidiospores; f. basidiocarp. – g, h. *Podaxis perraldieri*; g. basidiocarp; h. basidiospores. – i–l. *Podaxis pistillaris*; i. basidiocarp; j. basidiospores; k. gleba; l. culture on PDA. – m–o. *Podaxis termitophilus*; m. basidiocarp; n. label of epitype (PREM 27280); o. basidiospores. – p, r. *Podaxis* sp. 1 (ITS Clade 4); p. basidiocarp; r. basidiospores. – q. *Podaxis* sp. 3 (ITS Clade 11); basidiocarp. — Scale bar for basidiocarp is 1 cm and for basidiospore is 10 μ m.

holotype of *P. muelleri* from Gascogne River, western Australia, based on its larger basidiospores (11.1–16.4 × 8.5–12.8 vs 11.9–14.4 × 10.7–12.1 µm) and higher aspect ratio (1.1–1.6 vs 1.1–1.3) (Fig. 5). The basidiospores of *P. mareebaensis* are also significantly larger than those of the free-living *P. farlowii* (11.5–13.3 × 10.8–11.8 µm, n = 249) and the termite-associated *P. beringamensis* (10.3–12.1 × 7.4–8.7 µm, n = 144) (Fig. 2, 5). The pileus of *P. farlowii* (6.5–7.0 cm in height × 1.9–2.8 cm diam) is smaller than that of *P. mareebaensis* (8.9 cm in height × 4.1 cm diam), and *P. beringamensis* (up to 15 cm high × 4–6 cm diam). The apex of the narrowest part of the *P. mareebaensis* stipe (1.3 cm) is thicker than that of *P. farlowii* (0.7–0.9 cm) (Fig. 4). The basidiospore size of *P. mareebaensis* is similar to *P. loandensis* (synonym of *P. axatus*) (10.16–15.24 µm long), but it can be distinguished by habitat and geographic distribution. *Podaxis loandensis* occurs in grass and sandy habitats along the coast of the Angolan ocean. Based on these findings, this clade represents a novel species that we named *Podaxis mareebaensis*, with the holotype collected in Queensland, Australia.

Podaxis namaquensis G.S. Li, M. Poulsen & Z.W. de Beer, *sp. nov.* — MycoBank MB 848284; Fig. 7a–c

Diagnosis. Similar to *P. emerici* and *P. namibensis* but differs by its basidiospores (9.3–11.0 × 8.0–10.0 µm), larger than *P. namibensis* (8.1–8.9 × 7.2–7.7 µm) and smaller than *P. emerici* (13.3–15.5 × 11.1–13.0 µm).

Etymology. From Namaqualand, traditionally home of the Nama people (in older sources also called the Namaqua), an African ethnic group who lived in the arid region extending along the west coast that now forms part of the IlKaras Region in Namibia and the Northern Cape Province of South Africa.

Typus. SOUTH AFRICA, Northern Cape, Kgalagadi Transfrontier Park, Bitterpan, collected in dune, 25 Apr. 1992, *M. Joubert*, holotype PRUM 3702A (PRU, designated here).

Basidiocarp epigeous, stipitate, (9.1–)16.9–24.4 cm tall, dry, dehiscent at maturity. **Pileus** 2.4–6.4 cm in height × 1.2–10.5 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Gleba** stringy-powdery, dark brown to nearly black. **Stipe** from the base to top of pileus, 3.8–13.2 × 0.4–1.1 cm at maturity under the pileus, hollow, stuffed, cylindrical without bulb, dry, hard and woody, fibrous, striate, or sometime with right hand spiral, white to yellowish brown sometimes staining bright red, swollen at the base. **Basidiospores** (7.0–)9.3–11.0(–12.9) × (6.2–)8.0–10.0(–11.7) µm, aspect ratio 1.0–1.4 (n = 115 from five specimens), ellipsoid to ovoid, smooth, transparent, central germ pore prominent, light yellow or yellow greenish.

Habitat & Distribution — Emerging from dune near the desert, free-living and distributed in Namibia and eastern South Africa.

Additional materials examined. NAMIBIA, PREM 44294. — SOUTH AFRICA, PREM 20585, PRUM 2648, PRUM 3517.

Notes — *Podaxis namaquensis* was collected in South Africa and Namibia and formed a distinct clade in the phylogenomic analysis, closely related to *P. namibensis* but different in distribution from other free-living species in this study (Fig. 2d, 3b). The basidiospores of *P. namaquensis* (9.3–11.0 × 8.0–10.0 µm) are larger than those of *P. namibensis* (8.1–8.9 × 7.2–7.7 µm) and smaller than those of *P. emerici* (13.3–15.5 × 11.1–13.0 µm).

Podaxis namibensis G.S. Li, M. Poulsen & Z.W. de Beer, *sp. nov.* — MycoBank MB 848283; Fig. 7d–f

Diagnosis. Similar to *P. namaquensis* but differs by its smaller basidiospores (8.1–8.9 × 7.2–7.7 µm) than *P. namaquensis* (9.3–11.0 × 8.0–10.0 µm).

Etymology. Name for Namib, the world's oldest desert, from where the type was collected.

Typus. NAMIBIA, Namib-Naukluft National Park, half a mile East of Gobabeb, collected in dune, 10 Sept. 1997, *C. Bemy*, holotype PRUM 4366 (PRU, designated here).

Basidiocarp epigeous, stipitate, 11.3–16.1 cm tall, dry, dehiscent at maturity. **Pileus** 3.8–7.9 cm in height × 1.8–4.4 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical, white to pale cream with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Gleba** stringy-powdery, dark brown to nearly black. **Stipe** from the base to top of pileus, 3.5–8.7 × 0.6–0.9 cm at maturity under the pileus, hollow, stuffed, cylindrical without bulb, dry, hard and woody, fibrous, striate, or sometime with right hand spiral, white to yellowish brown sometimes staining bright red, swollen at the base. **Basidiospores** (7.8–)8.1–8.9(–9.8) × (6.2–)7.2–7.7(–8.9) µm, aspect ratio 1.0–1.3 (n = 24 from 2 specimens), ellipsoid to ovoid, smooth, transparent, central germ pore prominent, light yellow or yellow greenish.

Habitat & Distribution — Emerging from dune near the desert, free-living and distributed in Namibia.

Additional materials examined. NAMIBIA, PREM 44293.

Notes — *Podaxis namaquensis* and *P. namibensis* form independent clades and are separated from each other in the phylogenomic analysis, which is consistent with their ITS similarity of less than 97 % (611 bp) (Fig. 3b). The distribution of the two species are also different, with *P. namibensis* being found only in Namibia and the distribution of *P. namaquensis* extending from Namibia to South Africa (Fig. 3b). The basidiocarp and basidiospores of *P. namibensis* (11.3–16.1 cm; 8.1–8.9 × 7.2–7.7 µm) are distinguishable and smaller than *P. namaquensis* (16.9–24.4 cm; 9.3–11.1 × 8.3–10.1 µm) (Fig. 5). The basidiospores of *P. namibensis* are nearly spherical and differ from the elliptical basidiospores of *P. elatus* (Welwitsch & Currey 1870), while smaller than *P. emerici* (13.3–15.5 × 11.1–13.0 µm) and with an aspect ratio that is distinct from *P. aegyptiacus* (1.2–1.4) (Fig. 5).

Podaxis perraldieri Pat. (as '*Podaxon perraldieri*'), *Cat. Pl. Cel. Tunisie* (Paris): 68. 1897 — MycoBank MB 147577; Fig. 7g, h

Etymology. Named for the French botanist Henri-René le Tourneux de la Perraudière, who collected the type specimen during a botanical exploration of Algeria (Cosson 1861). Patouillard latinized the French name Perraudière to *Perralderius* as was custom at the time (Stearn 1983).

Typus. ALGERIA, Mansourat pr. de Constantine, 20 Mar. 1858, *L.T. Perraudière*, lectotype FH 940100 (FH, MBT 10013513, designated here).

Basidiocarp epigeous, stipitate, 10.1–21.8 cm tall, dry, dehiscent at maturity. **Pileus** obtuse ovoid, 4.0–8.1 cm in height, 2.4–3.0 cm thick at maturity, white, barely spotted with brownish, thin, papery, smooth, or slightly scaly at the top, opening upwards in irregular lobes. **Gleba** stringy-powdery, dark brown to nearly black. **Stipe** cylindrical, thickened below, smooth, greenish white, left-hand helix, under pileus 3.8–5.5 cm long, 0.4–1.3 mm thick, bearing at the top some white flaps of the pileus; the interior was hollow, with the tissue greenish white throughout. **Basidiospores** first colourless, small, and rounded, then straw-like and yellow or yellowish brown, broadly elliptical,

transparent; germ pore and hilum inconspicuous; dimensions (8.9–)10.1–11.7(–15.0) × (7.1–)9.2–10.7(–12.4) μm, aspect ratio 0.9–1.5 (n = 121 from six specimens).

Habitat & Distribution — Emerging from sand and gravel, free-living and distributed in the tropic of the cancer desert belt, including Afghanistan, Algeria, Iraq, and Israel.

Additional materials examined. AFGHANISTAN, KEW 254650. — ALGERIA, FH 940069, FH 940088. — IRAQ, KEW 254678. — ISRAEL, KEW 19839.

Notes — Patouillard (1897) included '*Podaxon aegyptiacus*' in his catalogue of fungi and plants from Tunisia and, as a footnote under that species, he described '*Podaxon perraldieri*' from Algeria, based on the argument that 'it might also occur in Tunisia'. He also mentioned '*Podaxon perraldieri*' in his illustration of three specimens 'of different ages' of the same fungus from an earlier publication compiled solely of illustrations of the Tunisian species (Patouillard 1897). However, he did not designate a type specimen for *P. perraldieri* in either publication. In the protologue he mentioned that the fungus was collected by La Perraudière at Mansourah near Constantine in Algeria in March 1858. The collection details of a specimen (FH 940100) that we obtained from the Farlow Herbarium at Harvard University, labelled as *Podaxis pistillaris*, stated that it was collected by 'H.R.L.T. Perraudière' at 'Mansourat pr. de Constantine, Algeria' on 20 March 1858. This collection expedition was mentioned by Cosson (1861) in an obituary for La Perraudière, who succumbed in 1861 at the age of 30 during an expedition in Algeria. In the obituary, the La Perraudière's family was thanked for donating his 'herbarium' to the Société Botanique de France. Although it is not clear exactly when, it appears that the fungal specimens in this collection were given to Patouillard, who was a prominent mycologist in Paris during the late 19th century. Patouillard's specimen collection was incorporated into the Farlow Herbarium (<https://huh.harvard.edu/pages/farlow-herbarium-fh>).

The collection information provided by Patouillard (1897) for *P. perraldieri* corresponds in all respects with the current data for FH 940100, apart from the locality that was given as 'Mansourah' by Patouillard, and 'Mansourat' in the FH record. According to Google maps (www.google.com/maps) there is a modern-day suburb called Mansourah about 1 km east of the old town of Constantine in Algeria, while a search for 'Mansourat' gave no records in Google Maps, and only a locality named Douar Mansourat, Morocco, exists in Mapcarta (www.mapcarta.com). Therefore, it is evident that the FH specimen most likely originated from La Perraudière's original collection. However, the specimen was neither designated as 'type' nor bears the species name '*P. perraldieri*'. Cunningham (1932) and Morse (1933) listed *P. perraldieri* as a synonym of *P. pistillaris* based on published descriptions without studying the original specimen. That is possibly why the FH specimen was labelled as *P. pistillaris*. We examined the morphology of the specimen (FH 940100) which closely matched the description in the protologue and corresponded to the illustration by Patouillard (1897) (Fig. 1c). Therefore, we designated this specimen as lectotype for *P. perraldieri* in accordance with Article 9 of the ICN (Turland et al. 2018).

Additional DNA sequences obtained from specimens collected in various desert regions, including Afghanistan, Algeria, Egypt, Iraq, and Israel, grouped together with the examined specimen of *P. perraldieri* (Fig. 2d, 3b; Table S1). In the phylogenomic analyses, the closest relative to *P. perraldieri* is *P. deflersii*; however, these two species are distinctly different in terms of the aspect ratio of basidiospores (0.9–1.5 vs 1.3–1.8) and the similarity of their ITS sequences, which is 96.6 % (611 bp) (Fig. 3b, S2).

***Podaxis pistillaris* (L.) Fr.** (as '*Podaxon pistillaris*'), Syst. Mycol. (Lundae) 3(1): 63. 1829 — MycoBank MB 356687; Fig. 7i–l

Etymology. Linnaeus (1771) seemingly derived the epithet from the description of Boccone's (1697) illustration of the fungus, that was labelled as *Pistillori species crepitus Lupi*. The term presumably refers to the *pistillum* (pestle) shape of the basidiome.

Basionym. *Lycoperdon pistillare* L., Mant. Pl. Altera: 313. 1771. — MycoBank MB 222263.

Sanctioning citation. *Podaxon pistillaris* (L.) Fr., Syst. Mycol. 3(1): 63. 1829. — MycoBank MB 496300.

Synonyms. *Scleroderma pistillare* (L.) Pers., Syn. Meth. Fung. (Göttingen) 1: 150. 1801. — MycoBank MB 496365.

Schweinitzia pistillaris Grev., Edinburgh Philos. J. 8: 258. 1823 (*nom. illegit.*, Art. 53.1, later homonym for *Schweinitzia* Elliott ex Nuttall, 1818).

Cauloglossum pistillare (L.) Grev., Scot. Crypt. Fl. 1: 60. 1823. — MycoBank MB 848913.

Mitremyces indicus Spreng., Syst. Veg., ed. 16 4(1): 518. 1827. — MycoBank MB 177845.

Podaxis indicus (Spreng.) Masee (as '*Podaxon indica*'), J. Bot., Lond. 28: 74. 1890. — MycoBank MB 296767.

Podaxis arabicus Pat. (as '*Podaxon arabicus*'), Bull. Soc. Mycol. France 3(2): 122. 1887. — MycoBank MB 234333.

Podaxis algericus Pat. (as '*Podaxon algericus*'), Bull. Soc. Mycol. France 20(2): 53. 1904. — MycoBank MB 584711.

Podaxis rugospora De Villiers, Eicker & Van der Westh., S. African J. Bot. 55(2): 160. 1989. — MycoBank MB 125144.

Intraspecific forms of *P. pistillaris*;

Podaxis pistillaris var. *africana* Henn. (as '*Podaxon pistillaris* var. *africanus*'), Bull. Herb. Boissier 1: 98. 1893. — MycoBank MB 622921 from Ethiopia.

Podaxis pistillaris f. *macrosporus* G. Cunn. (as '*macrospora*'), Proc. Linn. Soc. New South Wales 57: 32. 1932. — MycoBank MB 293195 from Australia.

Podaxis pistillaris var. *paurospora* Deam. & Morse, Mycologia 33(6): 610. 1941. — MycoBank MB 346348 from Brownsville, Texas.

Typus. INDIA, J.G. König, holotype LINN 1287.7 (LINN).

Description — Patouillard (as '*P. arabicus*') (1887: 122; pl. XI, f. 1, 1a); Masee (as '*P. indica*') (1890: 74; pl. 294, f. 1–9); Patouillard (as '*P. algericus*') (1904: 53; pl. 5); De Villiers et al. (as '*P. rugospora*') (1989: 160; f. 9–16).

Basidiocarp epigeous, stipitate, 5.1–39.2 cm in tall, dry, indehiscent at maturity. **Pileus** 2.5–22.1 cm in height × 1.0–5.2 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical throughout development, white to pale cream, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. **Lamellae** absent, basidiospore-producing tissue stringy-powdery, reddish brown or yellowish ochre-brown to dark brown to nearly black. **Stipe** 1.2–11.1 × 0.6–2.2 cm at maturity under the pileus, cylindrical with a basal bulb, dry, hard and woody, spiral, fibrous, striate, white to yellowish brown sometimes staining bright red. **Annulus** and **volva** absent. **Basidiospores** (6.9–)9.8–12.0(–19.9) × (6.1–)9.0–10.4(–18.5) μm, aspect ratio 0.9–1.8 (n = 978 from 39 specimens), broadly ellipsoid to ovoid, nearly globose, rarely narrowly ellipsoid, or submitriform, smooth, central germ pore prominent, olivaceous, greenish olivaceous to yellowish earth brown, without apiculus.

Habitat & Distribution — Emerging from dunes near the desert, free-living and distributed in tropical and subtropical Africa, Asia, and America.

Additional materials examined. ALGERIA, Laghouat, collected underneath *Tamarix* tree, 1903, *Cap. Sem.*, syntype (of *Podaxis algericus*) FH 940076. — AUSTRALIA, KEW 181150. — BRAZIL, KEW 17429. — CAPE VERDE, KEW 254617. — ECUADOR, KEW 254632. — ETHIOPIA, UPS 2502. — INDIA, KEW 179287, KEW 254649. — JAMAICA, KEW 254635. — KENYA, SNM CF101401, UPS 3882, UPS 4988. — MADEIRA, KEW 254565, KEW 254566, KEW 254567, KEW 254568. — MEXICO, MEXU 1191, MEXU 5015, MEXU 5772, MEXU 7212, MEXU 10805, MEXU 12338, MEXU 12808, MEXU 27557, MEXU 27558, MEXU 27845, SNM CF101400. — NAMIBIA, PREM 41625, PREM 47484, PRUM 3095. — OMAN, Wdb Oman 1, Wdb Oman 2. — PAKISTAN, KEW 254541, KEW

254544. – SOUTH AFRICA, Northwest Province, Rustenburg district, 20 miles West of Thabazimbi, 16 November 1966, *D. Edwards*, holotype (of *Podaxis rugospora*) PREM 43879, PREM 9789, PREM 28641, PREM 34405, PREM 43118, PREM 47477, PRUM 2335, PRUM 2470, PRUM 3030, PRUM 3507, PRUM 3655, PRUM 3660, PRUM 3973, PRUM 4335. – TOGO, KEW 254612. – USA, California, FH 374341, Hawaii, KEW 254631. – VENEZUELA, KEW 254636. – YEMEN, Aden, collected in Sandy plains, 28 Mar. 1887, *A. Deffers*, holotype (of *Podaxis arabicus*) FH 940086, FH 940103, KEW 179290.

Notes — *Podaxis pistillare* was described by Linnaeus (1771), in the genus *Lycoperdon*. It was later sanctioned by Persoon (1801) and transferred to the genus *Scleroderma*. Desvaux (1809) introduced the genus *Podaxis* based on the description of *L. axatus* by Bosc (1792), designating *L. axatus* as the type species and renaming it *Podaxis senegalensis*. However, Desvaux (1809) did not mention *L. pistillaris* and *L. carcinomale*, possibly due to the hostilities between Sweden and France precluded that he could examine specimens (see Notes under *Podaxis*). Apparently unaware of the studies of Bosc and Desvaux, Greville (1823a) created a new genus called *Schweinitzia* for *L. pistillare* and *L. carcinomale*, which was illegitimate as it was a later homonym for another genus. Sprengel (1827) placed *P. pistillaris* in the genus *Mitremyces* and provided it with a new epithet, *M. indica*. Although Sprengel (1827) listed *Mitremyces* as synonym of *Podaxis*, the type species of *Mitremyces*, *Lycoperdon heterogeneum*, was unrelated to *Podaxis*. Sprengel (1827) also classified *P. senegalense* in the genus *Cionium*, however, the type species of *Cionium*, *Cionium farinaceum*, is a slime mould (see Notes under *Podaxis*). Berkeley (1843) described a specimen of '*P. pistillaris*' from Cape Verde Islands with Figure, but the specimen is not present in K(M). In his monograph, Masee (1890) used the epithet of *P. indica* for *P. pistillaris* and *P. arabicus* considering them synonyms. Cunningham (1932) synonymized *Podaxon aegyptiacus* and *P. arabicus* with *P. pistillaris*. Morse (1933) and subsequent authors, such as Bottomley (1948), Doidge (1950), Pilat (1959), and Keirle et al. (2004), synonymized numerous *Podaxis* species (25, 27, 5, 26 and 25 species, respectively) with *P. pistillaris*.

In ITS Clade 1, there are a total of 56 specimens with two ecological records. This clade includes the types of *P. pistillaris*, *P. arabicus*, *P. algericus*, and *P. rugospora* (Fig. 2d). Among them, 31 specimens were subjected to lc-genome sequencing, and the phylogenomic analysis reveals two distinct clades (Fig. 2d, 3b), corresponding to the ecological records of the specimens. Notably, only one specimen (PREM 34405) from the clade of *P. pistillaris* was collected from a termite mound, but unfortunately, its genome sequencing failed. The morphology and distribution of the two clades are also different, further supporting their distinction as two species (Fig. 2d, 3b). Therefore, the two clades are designated as *P. termitophilus* and *P. pistillaris*, respectively.

Based on the ITS phylogeny and phylogenomic analysis, *P. pistillaris*, *P. arabicus*, *P. algericus*, and *P. rugospora* belong to the same clade. As the morphology of these four species is also similar, we synonymize the previously reported species *P. arabicus*, *P. algericus* and *P. rugospora* with *P. pistillaris*.

Podaxis termitophilus Jum. & H. Perrier (as '*Podaxon termitophilum*'), C. r. hebdom. Séanc. Acad. Sci., Paris 145: 275. 1907 — MycoBank MB 147319; Fig. 7m–o

Etymology. The epithet refers to the fact that this species grows on termite mounds.

Typus. MADAGASCAR, 1915, *Jumelle*, holotype *Jumelle* PC (DAR0070241, DAR). – SOUTH AFRICA, Gauteng, Pretoria, alongside road to Swing Bridge, growing in the termite nest, 18 Nov. 1933, *H.E. Schwikert*, epitype PREM 27280 (PREM, MBT 10013514, designated here).

Basidiocarp epigeous, stipitate, 9.7–21.1 cm in tall, dry, dehiscent at maturity. *Pileus* 3.8–11.4 cm in height × 1.2–3.5 cm diam at maturity, narrowly ellipsoid to paraboloid or obtusely conical throughout development, white to pale cream and some with brown patches, dry, shiny, brittle, veil persistent on pileus surface, base of pileus remaining adhered to the stipe, pileus breaking apart into fibrous scales to release basidiospores. *Gleba* stringy-powdery, reddish brown or dark brown to nearly black. *Stipe* 3.2–7.8 × 0.5–0.9 cm at maturity under the pileus, cylindrical with a basal bulb, dry, hard and woody, spiral, fibrous, striate, white to yellowish brown sometimes staining bright red. *Basidiospores* (7.7–)9.0–11.0(–13.2) × (5.9–)7.6–8.9(–10.9) μm, aspect ratio 1.0–1.4 (n = 47 from three specimen), broadly ellipsoid to ovoid, nearly globose, rarely narrowly ellipsoid, or subtrifurciform, smooth, central germ pore prominent, yellowish earth brown.

Habitat & Distribution — Emerging from termite mounds and distributed in South Africa and Madagascar.

Additional materials examined. MADAGASCAR, KEW 206678. – SOUTH AFRICA, PREM 14507, PREM 28254, PREM 58760.

Notes — Jumelle & Perrier de la Bathe (1907) provided a species description with a detailed account of termite-fungus associations of *Termitomyces* and *Podaxis* in Madagascar. Pilat (1959) listed several synonyms of *P. pistillaris*, including *P. termitophilus*, without providing any explanation. Priest & Lenz (1999) later synonymized *P. termitophilus* with *P. carcinomalis* based on the morphology of the holotype (*Jumelle* PC) from Madagascar, although it had already been noted that the basidiospores of *P. termitophilus* were slightly shorter and more globose than those of *P. carcinomalis*. Additionally, Priest & Lenz (1999) found that the collection of *P. termitophilus* labelled as the type specimen at PC was actually dated 1915, which was after the original description published in 1907 (Priest & Lenz 1999).

In our study, two out of five specimens with the same ecology, belonging to ITS Clade 1, cluster with *P. termitophilus* in the phylogenomic analysis (Fig. 2d, 3b). The morphological characteristics of this clade are similar to the description of *P. termitophilus*, and the measurements of the pileus, stipe and basidiospores are within the ranges reported for the holotype (Jumelle & Perrier de la Bathe 1907, Priest & Lenz 1999). The clade also includes a specimen collected in Madagascar (KEW 206678) (i.e., the same location as the original *P. termitophilus* description) and the ecology of the specimens in this clade also agree with the original description. Based on morphology, ecology, and distribution, this clade is designated as *P. termitophilus*. The phylogenetic analysis based on ITS and genome sequences separate *P. termitophilus* and *P. carcinomalis* (Fig. 2d, 3b), and morphological differences, including for basidiocarp, peridium, stipe, basidiospore size, and aspect ratio, support their distinction (Fig. 4, 5). With combined evidence from phylogeny, morphology, ecology, and geographic distribution, we resurrect the species *P. termitophilus* and designate the specimen PREM 27280, which possesses ITS and genome sequence data, as the epitype.

DISCUSSION

Strong support for species revisions of the genus *Podaxis*

Despite more than two hundred years of research into the taxonomy of *Podaxis*, proper species status of most specimens has remained largely unresolved, with consequences for our understanding of the biology of the genus (Linnaeus 1771, Masee 1890, Priest & Lenz 1999, Conlon et al. 2016, 2019, 2021). Combining robust phylogenomic analyses with morphological characteristics allowed us to assign a large number of specimens in the genus to 16 species including 11 reported and five

novel species. We confirmed four published species *P. emerici*, *P. farlowii*, *P. carcinomalis*, and *P. deflersii* according to their types, resurrected *P. termitophilus*, and designated neotypes, epitypes, or lectotypes to five reported species, *P. aegyptiacus*, *P. africana*, *P. beringamensis*, *P. calyptratus*, *P. perraldieri*, and *P. termitophilus*. We further synonymized three species, *P. arabicus*, *P. algericus*, and *P. rugospora* with *P. pistillaris*, and described five new species, *P. desolatus*, *P. inyoensis*, *P. namibensis*, *P. namaquensis*, and *P. mareebaensis*. We also designated the illustration of Bosc (1792) as lectotype of the type species *P. axatus* and supplemented this with an epitype specimen from the same geographic origin as the original. Three additional species likely exist, inferred from Clades 4, 5, and 11 in the ITS phylogeny, and distinct differences in the geographical distribution and basidiospore sizes from the revised species in this study (named *Podaxis* sp. 1, *Podaxis* sp. 2, and *Podaxis* sp. 3) (Fig. 2d, 5). However, since they lack lc-genomes, more specimens and molecular data are needed to resolve their phylogenomic relationships and species status.

The rarefaction analysis revealed that the number of species continues to increase with the addition of more specimens worldwide, and the absence of a smooth trend also implies that there are still potentially unknown species to be discovered (Fig. 3c; Table S4). This is plausible, as most specimens we could include were sampled from South Africa (60) and Australia (24), which were also the most species-rich countries (seven and six species, respectively), in contrast to the typically one or two species present in other countries. While our rarefaction analysis indicated that sampling coverage was insufficient to capture true biological diversity, our approach provides a strong foundation for future work, as it is based on robust species designations of the 86 specimens with sequenced genomes and takes into account the association between species richness and sampling effort.

Phylogenomics powerfully resolves species boundaries and evolution

Phylogenomic analysis has revolutionized knowledge of fungal evolution and revealed robust deep nodes of the fungal tree of life (Fitzpatrick et al. 2006, Kuramae et al. 2006, Yang 2011). Through the application of phylogenomic analysis to the genus *Podaxis* we were able to delineate species boundaries with consistency in morphology, geography, and ecology, and resolved several clades that were previously unresolved using barcode gene or showed inconsistencies in geography or ecology. The phylogenomic tree split up ITS Clades 1 and 7 that previously included both free-living and termite-associated specimens respectively, divided the ITS Clade 2 with a wide geographic distribution and heterogeneous basidiospore sizes into different species, and showed a clear and convincing phylogenomic placement of ITS Clade 9 with robust support. The coalescent model was more suitable for rapidly evolving clades, for instance ITS Clade 2, as the concatenation model appeared to lead to long-branch attraction (LBA) (Fig. S2, S3). Consequently, the concatenation model provided better resolution to infer phylogeny at the inter-species level while the coalescent model appeared to better resolve the placement of specimens within species (Fig. 3b, S3). In summary, phylogenomic analysis based on concatenation and coalescent model both resolve incomplete lineage sorting (ILS) and the origins of associations with termites. The phylogenomic analysis further indicated that the origin of termite association in the genus was independent in Australia (*P. mareebaensis*) and Africa in the last common ancestor of *P. africana*, *P. beringamensis*, *P. carcinomalis*, and *P. termitophilus*, and further elucidated that association with termites in Australian *Podaxis* has had two independent origins,

one from a migration from Africa and one from species already present and free-living in Australia.

Limitations for museomics – omics on museum specimens – of *Podaxis*

We were able to use historic *Podaxis* samples collected over a 250-year time span, which greatly improved our current understanding of the species diversity of the genus. Low coverage genomes derived from minute spore samples helped resolve some of these inconsistencies to allow delineation of several clades with consistent morphology, geography, and ecology. It was clear from our genome completeness analysis that increased storage time negatively impacts genome integrity, so that significant DNA degradation occurs with time. However, basidiospores remained a satisfactory source for sufficient BUSCO genes for a large number of specimens, allowing for omics to be carried out on centuries-old museum specimens (museomics). Specimen age is more challenging for morphological analyses as it was not possible to observe important structures, such as the pileipellis / exoperidium covered with hyphae (De Villiers et al. 1989, Vellinga et al. 2003). Similarly, the exoperidium, endoperidium, gleba, and capillitium could not be observed without destroying the specimens. While these limitations impacted our analyses of, e.g., *P. linnaea*, *P. beringamensis*, and *P. emerici*, combining genomics with geography, ecology, and basidiospore measurements, allowed us to nevertheless achieve confident species delimitation.

Data availability

ITS sequences are available from GenBank with accessions OP739101–OP739205. Low-coverage genome sequences available from GenBank with BioProject ID: PRJNA896504.

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Supplementary material

Table S1 List of *Podaxis* specimens included in the phylogenetic reconstruction.

Table S2 Taxa included in the ITS phylogenetic analysis, including voucher number, origin, GenBank numbers and publication sources.

Table S3 The completeness of lc-genome assembly by BUSCO, specimen age and GenBank accession.

Note: An asterisk (*) means that the specimen was used for analysing the relationship between genome completeness and specimen age. A plus (+) means that the specimen was included in the phylogenomic analysis.

Table S4 The number of specimens of *Podaxis* across species by country, as well as the total number of species per country (far right).

Table S5 Morphological measurements of specimens: basidiocarp, peridium, and stipe measurements are based on photographs. Basidiospores were observed in the microscope.