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Dendrodacrys: a new genus for species with branched hyphidia in *Dacrymyces s.l.*, with the description of four new species

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Abstract: A new genus named *Dendrodacrys* is proposed for a monophyletic group in *Dacrymycetaceae*, containing species with pulvinate to depressed basidiocarps, distinctly branched hymenial hyphidia, and up to 3-septate mature basidiospores. Four taxa in this group, occurring in Europe, are proposed as new species, *viz*. *De. ciprense*, *De. concrescens*, *De. ellipsosporum*, and *De. oblongisporum*, based both on morphological and DNA data (nrDNA, *RPB1*, *RPB2*, *TEF-1α*, 12S). These new species are all described in detail, illustrated, and compared with other published taxa that with which they can be confounded. The new combination *De. paraphysatum* is proposed after revising the type material of *Dacrymyces paraphysatus*, but other combinations or potentially new non-European species descriptions are postponed pending further studies of additional specimens.

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INTRODUCTION

Dacrymyces s.l. is currently treated as a genus of saprotrophic jelly-fungi distributed worldwide, and comprises about half of the species of the class *Dacrymycetes* (McNabb 1973, Reid 1974, Shirouzu *et al.* 2009, 2017). The distinction between genera in *Dacrymycetes* has traditionally been based on the macro- and micromorphological characters of the basidiocarps. Within the series of monographic studies on *Dacrymycetes* carried out by R.F.R. McNabb, the genus *Dacrymyces* seemed to be particularly difficult to circumscribe (McNabb 1973), especially against the genus *Heterotextus* (McNabb 1965). In fact, *Dacrymyces* has frequently been treated as a hotchpotch to include any taxa that could not be properly placed in other, well-characterised genera in the *Dacrymycetes*. As a result, this generic name is often applied to any species producing gelatinous to cartilaginous, cushion-shaped or turbinate basidiocarps, with a rather homogeneous hyphal structure, and either with an amphigenous hymenium or a sterile cortex of cylindrical to moderately differentiated, inflated cells (less so than in *Heterotextus*).

The phylogenetic relationships in *Dacrymycetes* have been re-evaluated with molecular data, and numerous independent studies have shown *Dacrymyces* to be highly polyphyletic (*e.g.* Shirouzu *et al.* 2007, 2013a, 2017, Zamora & Ekman 2020, Savchenko *et al.* 2021). Recent taxonomic revisions have focused on *Dacryonaemataceae* and *Unilacrymaceae* (Zamora & Ekman 2020) and on *Cerinomycetaceae* (Savchenko *et al.* 2021). In these revisions, several species with dacrymyces-like basidiocarps, not closely related to the type of *Dacrymyces*, *Da. stillatus*, have already been clarified and combined into monophyletic genera. On the other hand, the generic boundaries within *Dacrymycetaceae* are far from clear, because phylogenetic relationships among several groups of *Dacrymyces s.l.* and other genera (*e.g*. *Calocera*, which is also polyphyletic) are not currently well-supported, and phenotypic characters distinguishing the different clades overlap considerably. As a result, mycologists studying this class have been very cautious not to make the taxonomy of the group more intricate, avoiding unnecessary splitting and further creation of difficult-to-diagnose genera.

In the course of several sampling campaigns in various European countries during the last 12 yr, we found some specimens of *Dacrymyces s.l.* with conspicuous and often branched hyphidia that turned out to be undescribed species. Our aim is to describe these new species, providing both morphological studies and phylogenetic analyses, as well as a comparison with other morphologically similar species.

Preliminary DNA-based phylogenetic analyses placed them in the same clade as a specimen identified as *Da. dendrocalami*, a species with conspicuously branched hyphidia (Oberwinkler & Tschen 1989). The presence of these branched hyphidia seems to be a rather uncommon character within the family

Dacrymycetaceae, according to Zamora & Ekman (2020). We will therefore evaluate whether this clade merits recognition at generic level, as a further step to solve the polyphyly of *Dacrymyces s.l*.

MATERIAL AND METHODS

Sampling

Specimens were collected in the field in both hydrated and dry states. Some fresh specimens were kept in a refrigerated humid chamber up to 2–3 d in order to study the macro- and micromorphological structures of the living basidiocarps. Otherwise, samples were dried at room temperature and kept as fungarium specimens in CWU, G, H, and UPS (Thiers 2021) for subsequent morphological study. We selected 17 of these newly collected specimens, representing five putative new species, for molecular study.

We chose a subset of representative taxa from all main clades in Zamora & Ekman (2020) to investigate the phylogenetic placement of the target group within the class *Dacrymycetes*. We selected up to two samples per species with at least two unlinked DNA regions available to minimise missing data. For the species delimitation analyses, we restricted the sampling to species in the target clade (putative new genus), and included the only other additional sample (*Da. cf. adpressus*, TNS-21069, AB472729) with DNA data available in GenBank. Nomenclature has been updated following Zamora & Ekman (2020) and Savchenko *et al.* (2021).

Morphology

The morphological methods largely follow Zamora & Ekman (2020) and are thus only briefly summarised below. Basidiocarps were photographed when fresh or after being hydrated, with either a Canon EOS 700D or an Infinity 1 macro camera coupled with a Leica MZ 75 dissecting microscope. The micromorphology was studied with a Zeiss AxioImager A1 compound microscope by mounting hand-cut sections in water and 5 % KOH, and photographs were taken in the latter medium with an AxioCamICc3 digital camera, using differential interference contrast (DIC). Microscopic structures were measured in KOH solution at 630×, either directly or with the aid of Piximètre v. 5.10 (Henriot & Cheype 2016). Hyphidium width was measured in the upper half, basidium length was considered from the apex (excluding sterigmata) to the basal septum, and basidiospore length from the most protuberant part near the hilar appendix (considered subterminal and measured separately) to the opposite pole; the largest perpendicular dimension to these lengths was treated as the width. The basidiospore length/width ratio is expressed as Q. Terminology for the basidium apex follows Van de Put (2014).

General protocols for laboratory work were explained in detail in Zamora & Ekman (2020); Ukrainian samples were processed following Savchenko *et al.* (2021). DNA extractions were always carried out from a single basidiocarp using Chelex 100, following the protocol of Ferencova *et al.* (2017). We amplified fragments of the nrDNA (18S, ITS, nrLSU), *RPB1*, *RPB2*, *TEF-1α*, and 12S (mtSSU) DNA regions using the following primer combinations: The 18S was amplified in two parts with the primer pairs NS1/NS4 (White *et al.* 1990) and NS21UBC/SR6 (Gargas & Taylor 1992, Vilgalys unpubl.). The ITS + nrLSU (D1–D3) region was amplified using ITS1F/LR5 (Gardes & Bruns 1993, Vilgalys & Hester 1990). The *RPB1* was amplified with DacryRPB1-1F/DacryRPB1-2r (Zamora & Ekman 2020). The *RPB2* was amplified either with DacryRPB2-6F/DacryRPB2-11aR, or with DacryRPB2-6.2F/DacryRPB2-11bR (Zamora & Ekman 2020), sometimes using nested PCR. The *TEF-1α* was amplified using EF1-1018F/EF1-2218R (Stielow *et al.* 2015, Rehner & Buckley 2005) and Efdf/EF1-1953R (Rehner unpubl.). Finally, for the 12S we used either the primers DacryMS1 combined with Dacry12S-2r or Dacry12S-4r (Zamora & Ekman 2020), or substituted the forward DacryMS1 with an external newly designed primer, Dacry12S-1F (5' AGGTAGTTGRTAGTGTAA 3'), combined with Dacry12S-2r. PCR programmes followed Zamora & Ekman (2020). Sequencing was done by Macrogen using the amplification primers, except for the *RPB1* for which we mostly used the internal DacryRPB1-A and DacryRPB1-C (Zamora & Ekman 2020).

Sequence alignment

Sequences were assembled and edited in Sequencher v. 4.1.4 (Gene Codes, USA), using IUPAC ambiguity codes for heteromorphic positions. Newly generated sequences are included in Table 1, while information of the remaining sequences can be found in Zamora & Ekman (2020) and in the Joint Genome Institute (Grigoriev *et al.* 2014). We built two alignments, the first one for inferring a general phylogeny to show the phylogenetic position of the new species and to identify the main clades that may deserve generic recognition, and a second alignment to perform species delimitation analyses, containing only the new species and closely related taxa. Most alignments were inferred using MAFFT v. 7 (Katoh & Standley 2013, G-INS-i option) for the ribosomal regions, or manually in the case of the protein coding genes, back-translating them into nucleotides after having excluded introns and aligned the amino acids (introns were not used in subsequent analyses). Two highly variable regions of the 12S, appearing between the conserved motifs AWTTCWTT and GAAMWATGT, and AGGGTTCGYRG and GMTWGAATCW, respectively (some base changes in certain species occur in these motifs) were excluded from the analyses. The 12S region was not used for species delimitation since it was available for only two species in the target clade (*De. concrescens* and *De. ellipsosporum*). The ITS1 and ITS2 were extremely variable across some of the target species and several trials with MAFFT resulted in substantially different alignments; thus, these regions were only included in the species delimitation analysis and after being aligned with BAli-Phy (Suchard & Redelings 2006). We prepared a backbone alignment with up to two samples per species (the ones with the most dissimilar sequences), and executed four runs with 5×10^4 iterations each. ITS1 and ITS2 were treated as two separate partitions, using the GTR + I + Γ model for nucleotide substitutions and the rs07 model for insertion/deletion events. The first 25 % of the runs were discarded as burn-in and the summarised samples showed an average standard deviation of splits frequencies < 0.005, and effective sample sizes > 7 000, verified using Tracer v. 1.7 (Rambaut *et al.* 2018). Alignments are available in TreeBASE (TB2:S29109).

Phylogenetic analyses

We tested congruence among unlinked DNA regions by performing a maximum likelihood (ML) phylogenetic analysis

Table 1. DNA sequences generated in this study, with GenBank accession numbers and voucher information.

Taxon	Country and province	Voucher	GenBank accession numbers					
			18S	ITS + nrLSU	*RPB1*	*RPB2*	*TEF-1α*	12S
Dendrodacrys ciprense	Cyprus, Lemesos	UPS F-946590 (holotype)	OM515350	OM519385	OM502304	OM502321	OM502337	—
	Cyprus, Lemesos	UPS F-946591	OM515351	OM519386	OM502305	OM502322	OM502338	—
	Cyprus, Lemesos	UPS F-946592	OM515352	OM519387	OM502306	OM502323	—	—
Dendrodacrys aff. ciprense	Cyprus, Lemesos	UPS F-946593	OM515353	OM519388	OM502307	OM502324	OM502339	—
Dendrodacrys concrescens	Sweden, Öland	UPS F-946601	OM515354	OM519389	OM502308	OM502325	OM502340	OM677448
	Sweden, Öland	UPS F-946602 (holotype)	OM515355	OM519390	OM502309	OM502326	OM502341	—
	Sweden, Uppland	UPS F-946603	OM515356	OM519391	OM502310	OM502327	OM502342	OM677449
Dendrodacrys ellipsosporum	Spain, Madrid	UPS F-946604 (holotype)	OM515357	OM519392	OM502311	OM502328	OM502343	OM677450
	Spain, Madrid	UPS F-946605	OM515358	OM519393	OM502312	OM502329	OM502344	OM677451
	Spain, Balearic Islands	UPS F-946606	OM515359	OM519394	OM502313	OM502330	OM502345	OM677452
	Spain, Madrid	UPS F-946607	OM515360	OM519395	OM502314	OM502331	OM502346	OM677453
	Spain, Madrid	UPS F-946608	OM515361	OM519396	OM502315	OM502332	OM502347	OM677454
	Ukraine, Crimea	CWU(MYC)4092	OM515362	OM519397	OM502316	—	OM502348	—
	Ukraine, Crimea	CWU(MYC)7560	OM515363	OM519398	OM502317	OM502333	OM502349	—
Dendrodacrys oblongisporum	Norway, Sogn og Fjordane	UPS F-946599	OM515364	OM519399	OM502318	OM502334	OM502350	—
	Spain, Madrid	UPS F-979568 (holotype)	OM515365	OM519400	OM502319	OM502335	OM502351	—
	Spain, Madrid	UPS F-979569	OM515366	OM519401	OM502320	OM502336	OM502352	—

of each dataset using IQ-TREE v. 1.6.12 (Nguyen *et al.* 2015), running 500 standard bootstrap (bs) replicates. We considered a conflict among topologies when a strongly supported (bs ≥ 75 %) clade from one phylogeny was contradicted by another strongly supported clade in another phylogeny (Mason-Gamer & Kellogg 1996). The partitioning scheme and model parameters were calculated based on the Bayesian information criterion with the version of ModelFinder (Kalyaanamoorthy *et al.* 2017) integrated into IQ-TREE. We used five potential subsets for the nrDNA dataset (18S, ITS1, 5.8S, ITS2, and nrLSU), three for each protein coding gene alignment (codon positions), and left the 12S dataset unpartitioned. Since no incongruence was detected, the datasets were concatenated and analysed using ML and Bayesian inference. In a previous study (Zamora & Ekman 2020) the use of the much more computationally intense coalescence analyses did not show any substantial improvements or topological changes. Therefore, the trees obtained here through the analyses of the concatenated dataset are considered representative of the species tree.

Maximum Likelihood analyses were performed as indicated above for each single-region alignment, repeating the analyses five times starting from random trees. Brach support was assessed by standard bootstrapping, performing 500 replicates in total. Bayesian analyses were done with MrBayes v. 3.2.6 (Ronquist *et al.* 2012), using the same partitioning scheme obtained in the ML analysis, with model parameters but not tree topology unlinked across subsets, and using model jumping to sample across models in each subset (Huelsenbeck *et al.* 2004). We allowed a gamma distributed rate heterogeneity across sites (approximated by four categories) and a proportion of invariant sites. We used the following priors: a (1, 1, 1, 1, 1, 1) Dirichlet prior for the substitution rates, a (1, 1, 1, 1) Dirichlet prior on the state frequencies, and a uniform (0, 1) prior for the proportion of invariable sites. Branch lengths were linked and proportional across partitions, and we used the compound Dirichlet prior Unconstrained:GammaDir (1, 0.158, 1, 1), based on the tree length estimates from the best replicate of the ML analysis. Mixing was considered adequate with the temperature parameter set to 0.2. We executed four runs starting from random trees, each with four chains, for up to 1×10^8 generations and sampling every 1 000th tree. The analyses were automatically stopped when the average standard deviation of split frequencies (ASDSF) dropped below 0.01. The first half of the analysis was discarded as burn-in, and the 50 % majority-rule tree with posterior probabilities (pp, considered significant when ≥ 0.95) and average branch lengths was calculated from the post-burn-in trees. We checked with Tracer v. 1.7 (Rambaut *et al.* 2018) that effective sample size (ESS) for each parameter was above 200. Trees were visualised in FigTree v. 1.4 (Rambaut 2016) and rooted based on the results from Zamora & Ekman (2020).

Species delimitation

Specimens were assigned to putative species using the multispecies coalescent approach implemented in STACEY v.

1.2.4 (Jones 2017) as part of the BEAST2 platform (Bouckaert *et al.* 2014). Clock and tree model parameters were estimated independently for each of the four unlinked DNA regions. An uncorrelated lognormal relaxed clock model (Drummond *et al.* 2006) was used. The dataset was divided into eight subsets (two for each non-recombining DNA region, one with lower and the other with higher substitution rates), as follows: (i) 18S + 5.8S + nrLSU, (ii) ITS1 + ITS2, (iii–v) 1st + 2nd codon positions of protein coding regions, (vi-viii) 3rd codon position of protein coding regions. Model parameters were estimated for each DNA subset with bModelTest (Bouckaert & Drummond 2017), allowing all transition/transversion split models. We ran four MCMC parallel analyses for 2×10^8 generations, sampling every $1 \times 10^{4\text{th}}$ tree. The collapse height parameter was set as $\varepsilon = 10^{-4}$, and we used the Beta (1,1) prior on the collapse weight parameter (ω). We noted some convergence problems in one of the runs for one of the partitions (1st + 2nd codon positions of *TEF-1α*) and excluded this run for subsequent analyses. The first half of the other three runs was discarded as burn-in. The most likely number of clusters (*i.e.* putative species) was calculated from the remaining sample using SpeciesDelimitationAnalyzer (Jones *et al*. 2015). The similarity matrix of pairwise posterior probabilities was visualised and plotted in R (R Core Team 2021) following Jones *et al.* (2015).

RESULTS

Phylogeny

The best partitioning scheme and models for each subset in the concatenated ML analysis were: (i) 18S, TN + F + I + Γ4, (ii) 5.8S + 12S, GTR + F + I + Γ4, (iii) nrLSU, TN + F + I + Γ4, (iv) *RPB1* 1st + *RPB2* 1st, TIM2 + F + I + Γ4, (v) *RPB1* 2nd + *RPB2* 2nd, TIM3 + F + I + Γ4, (vi) *RPB1* 3rd + *RPB2* 3rd, GTR + F + I + Γ4, (vii) *TEF-1α* 1st, F81 + F + I + Γ4, (viii) *TEF-1α* 2nd, JC + I + Γ4, and (ix) *TEF-1α* 3rd, GTR + F + Γ4. All ML tree replicates had a similar topology, and the likelihood score for the best one was lnL = -73248.971. The concatenated Bayesian analysis halted after 5×10^6 generations (ASDSF < 0.01). All parameters had an ESS exceeding 800 in the posterior sample, and all PSRF values were in the range 0.998–1.005. The topologies of the 50 % majority-rule Bayesian consensus tree and of the ML trees were similar, and thus only the best ML tree with bs and pp values is shown in Fig. 1.

The overall topology of the *Dacrymycetes* tree (Fig. 1) is highly consistent with that reported by Zamora & Ekman (2020). The four families recognized received bs = 100 % and pp = 1.00 support. Within *Dacrymycetaceae*, we have identified the same 8 main groups (D1–D8), plus *Dacrymyces fennicus* as sister to D6 (*Femsjonia*) with high support (bs = 93 %, pp = 1.00). Clades D1, D2, D4–D7 received bs = 100 % and pp = 1.00 support, clade D3 was represented by a single sample, and clade D8 was well-supported (bs = 77 %, pp = 1.00). The target group (clade D5) was sister to clade D8 (clampless species) with partial support (bs = 58 %, pp = 1.00). Within clade D5, relationships were generally highly supported. *Dacrymyces cf*. *dendrocalami* and *Da. cf*. *adpressus* were resolved as sister to each other with bs = 100 % and pp = 1.00 support. Four putative new species (see below), named *Dendrodacrys ciprense*, *De. concrescens*, *De. ellipsosporum*, and *De. oblongisporum*, also received bs = 100 % and pp = 1.00 support. In addition, *De. ciprense* and *De. oblongisporum*, together with an isolated sample (*De. aff*. *ciprense*) formed a well-circumscribed clade with bs = 100 % and pp = 1.00 support, but the relationships among these three groups only received partial support (bs = 72 %, pp = 0.97).

Species delimitation

SpeciesDelimitationAnalyzer yielded two main species delimitation schemes, one with seven putative species (45.1 % posterior probability), and the other with eight putative species (34.7 % posterior probability). All other delimitation schemes had < 5 % posterior probability. All relevant model parameters in the STACEY analysis had an ESS exceeding 500 in the posterior sample. The topology of the STACEY chronogram is almost fully supported above the species level (Fig. 2). From the root, two main clades can be distinguished; the first is fully supported and includes *Da. cf*. *dendrocalami* (one sample estimated as one species) and *Da. cf*. *adpressus* (a fully supported clade with two samples, estimated as either one or two species). The other main clade is well-supported (pp = 0.97) and includes four putative species, *i.e*. *De. concrescens* (fully supported clade with three specimens), *De. ellipsosporum* (fully supported clade with seven specimens), *De. ciprense* (fully supported clade with three specimens), *De. aff*. *ciprense* (one isolated specimen), and *De. oblongisporum* (fully supported clade with three specimens). The branches connecting these five putative species received full support except for the sister relationship between *De. aff*. *ciprense* and *De. oblongisporum*, which is unsupported (pp = 0.9).

Within each putative species (cluster) in the scheme of seven species, all included specimens had a high posterior probability (pp > 0.9) of belonging to the cluster they were assigned, except for the two specimens of *Da. cf*. *adpressus*. In this case, the probability that they belonged to the same species was pp = 0.54. The posterior probability that any specimen belonged to a different species to which it was assigned was very low (pp < 0.001).

Taxonomy

Dendrodacrys J.C. Zamora, A. Savchenko, Á. González-Cruz, Prieto-García, Olariaga & Ekman, ***gen. nov.*** MycoBank MB 842993.

Etymology: From the Greek δένδρον (*dendron*, branched like a tree) and δάκρυ (*dacry*, tear), so as to refer to a genus of *Dacrymycetaceae* with branched hyphidia.

Typus: *Dendrodacrys ellipsosporum* J.C. Zamora, A. Savchenko, Á. González-Cruz, Prieto-García, Olariaga & Ekman

Description: *Basidiocarps* firm- to soft-gelatinous when fresh, xerotolerant or not, ± sessile and with or without a rooting base, pulvinate to depressed, yellow-orange to brown. *Hymenium* amphigenous or ± confined to the upper part of the fruitbody, then with a distinct sterile cortex. *Clamp-connections* present except in one of the currently included taxa. Terminal cells of cortical/marginal hyphae ± cylindrical to narrowly clavate, thin- to thick-walled. Internal hyphae and subhymenial hyphae mostly thin-walled. *Basidia* 2-spored, often cylindrical to clavate, more rarely ± urniform; apex U- to W-shaped, rarely Y-shaped. *Hyphidia* present, distinct, simple to moderately branched, reaching or surpassing the level of the young basidia, but only sometimes forming a conspicuous layer on them. Recently

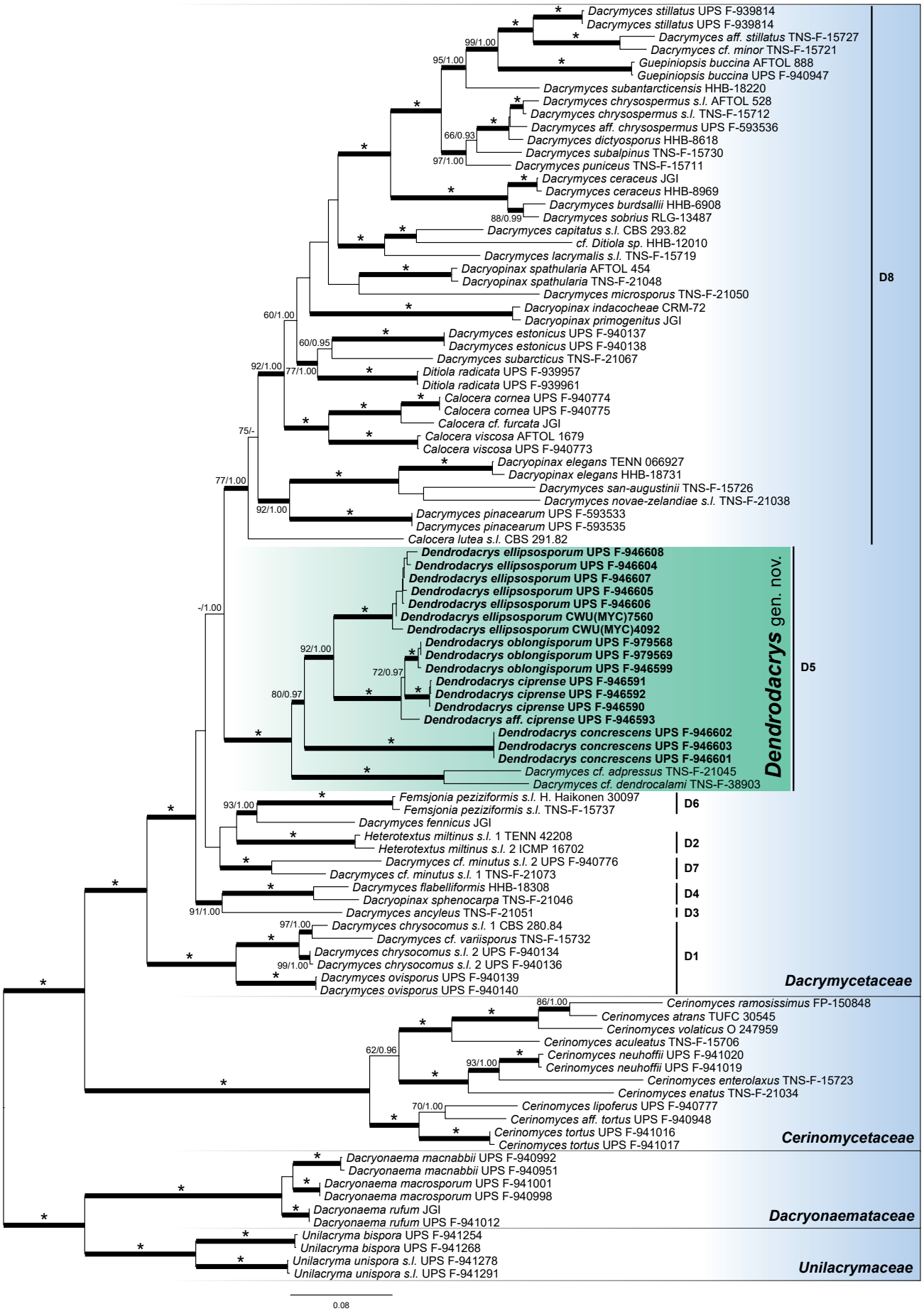

Fig. 1. Maximum likelihood phylogram of the class *Dacrymycetes*, with bootstrap (bs) and posterior probabilities (pp) values indicated at species level or above. Thickened branches are considered well-supported (bs ≥ 75 % and pp ≥ 0.95), asterisks (*) denote full support (bs = 100 %, pp = 1.00), and other values are included only when bs ≥ 60 % and pp ≥ 0.9. Notation D1–D8 in *Dacrymycetaceae* follows Zamora & Ekman (2020) for convenience, and the new genus *Dendrodacrys* is highlighted. Samples with newly generated data are indicated in bold.

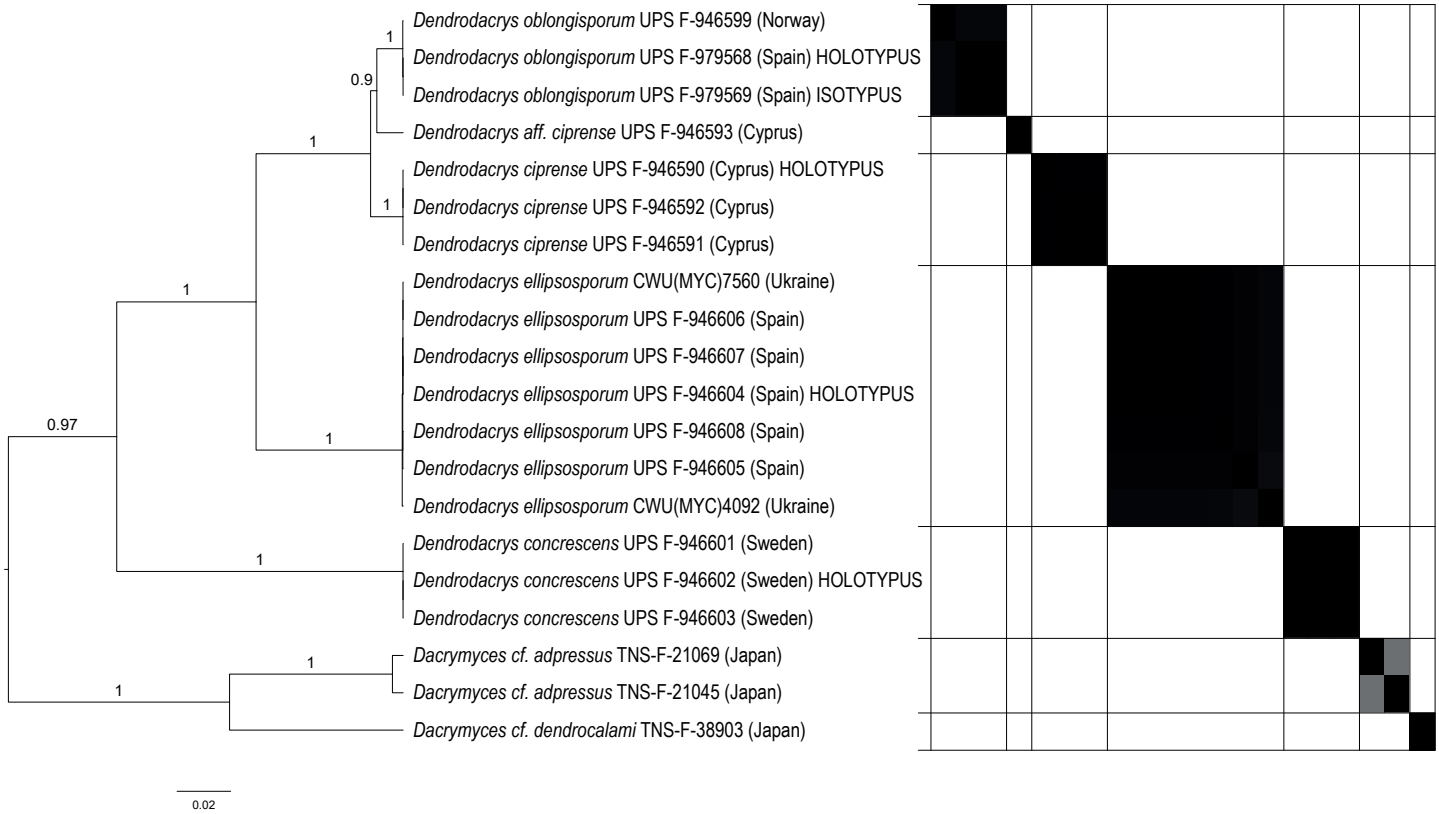

Fig. 2. STACEY species delimitation analysis. Chronogram with posterior probabilities at and above species level, and similarity matrix. Clusters separated by lines indicate the scheme of putative species with highest posterior probability.

discharged and still aseptate basidiospores uninucleate. Mature basidiospores 0–3-septate, thin- to thick-walled, hyaline, subglobose to cylindrical-allantoid. *Spore print* cream to orange, also visible from the spore pruinescence on the basidiocarps (*e.g*. Fig. 3C). *Microconidia* infrequent, ellipsoid to cylindrical. *Cell cytoplasm* with abundant lipid bodies, and with carotenoids ± visible under the light microscope, sometimes inconspicuous. Brownish diffuse parietal pigments sometimes visible in the cortical/marginal hyphae.

Included species: *Dendrodacrys ciprense*, *De. concrescens*, *De. ellipsosporum*, *De. oblongisporum*, *De. paraphysatum*. Two additional species provisionally identified as "*Da. cf. dendrocalami*" and "*Da. cf. adpressus*" are also included here.

Dendrodacrys ciprense J.C. Zamora ***sp. nov.*** MycoBank MB 842994. Fig. 3.

Etymology: The adjectival specific epithet refers to the country where the known specimens were found.

Typus: **Cyprus**, Lemesos, Mesa Potamos, picnic area, on *Pinus brutia* branches, 2 Dec. 2017, *J.C. Zamora* (**holotype** UPS F-946590).

Description: *Basidiocarps* gelatinous, (0.2–)0.4–1.5(–1.8) mm in diam, slightly erumpent and pustulate when very young, becoming pulvinate to applanate, often with an inconspicuous central root-like projection, gregarious and sometimes partially coalescing but retaining evidence of the individual origin; in hydrated state amber coloured to orangish when young, soon orangish brown to brown, ± dark brown when old; dark brown to blackish when dried. *Hymenium* ± confined to the upper part of the basidiocarps, irregularly spreading to the margins; sterile cortex often distinct, or at least with a sterile area in the lower part of the basidiocarps. Terminal cells of cortical/marginal hyphae narrowly clavate to slightly fusiform or almost cylindrical, 5.4–9.0 µm diam, ± thick-walled, with walls not clearly gelatinised but cells often embedded in a gelatinous matrix, sometimes with secondary simple septa, with a brownish, diffuse parietal pigmentation well-visible especially in the darkest basidiocarps. Internal hyphae 1.8–5.0 µm diam, thin- to slightly thick-walled, clamped, some with roughened walls. *Hyphidia* frequent, conspicuous, moderately to densely branched, rarely simple, 2.1–4.4(–5.7) µm diam (wider towards the base and becoming thinner in the upper half or third), often with 1–2 clamped septa throughout their length, reaching or surpassing the level of the young basidia but not forming a layer on them. Young basidia cylindrical to narrowly clavate; mature basidia 49.5–74.4 × 4.4–9.5 µm, with two subapical sterigmata, 16.5–34.0 × 3.9–6.0 µm; basidium apex often slightly protruding. *Basidiospores* thin-walled, (13.6–)16.4–20.1 × (5.5–)6.0–8.9 µm, 2.2 ≤ Q ≤ 3.2 (n = 20), cylindrical-allantoid to slightly arachiform, becoming 3-septate at maturity, not constricted at septa or only slightly constricted, uninucleate prior to septation; hilar appendix conspicuous, *ca*. 1 µm long. Basidiospore germination not seen. Carotenoid contents present in the cytoplasm of most cells but rather inconspicuous, not bright yellow-orange.

Ecology and distribution: Only known from *Pinus brutia* forests in Cyprus. For a more accurate knowledge of its ecological preferences, the species should be looked for in other areas where the host is present (northeastern Mediterranean basin). Probably at least partially xerotolerant.

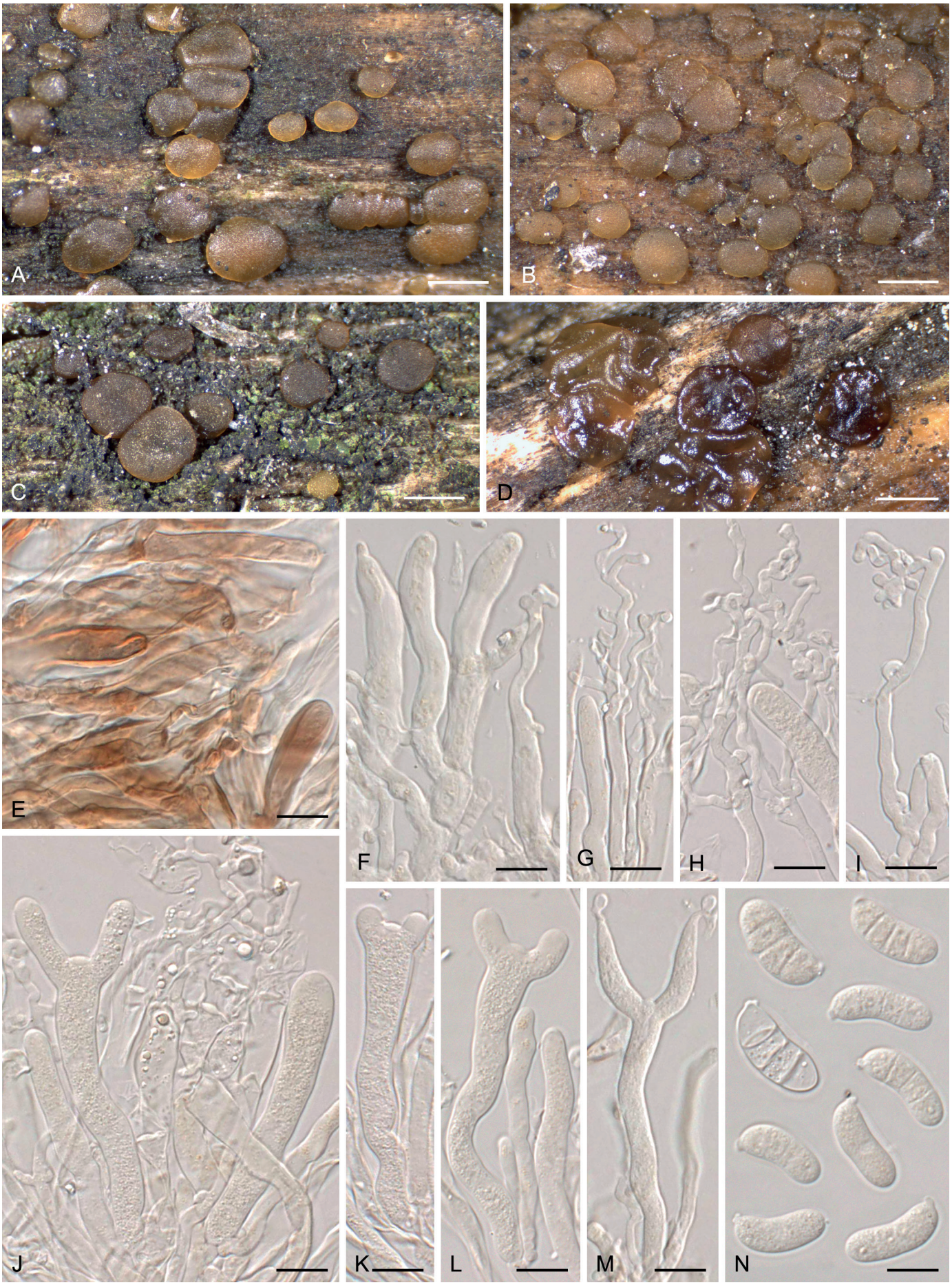

Fig. 3. *Dendrodacrys ciprense*. **A–D.** Macromorphology; basidiocarps in fresh conditions. **E–N.** Micromorphology. **E, F.** Terminal cells of cortical hyphae. **G–I.** Hyphidia. **J–M.** Basidia. **N.** Basidiospores. A–C, F–H, J–N from UPS F-946590 (holotype); D, E, I from UPS F-946592. Scale bars: A–D = 1 mm; E–N = 10 µm.

Additional specimens examined: **Cyprus**, Lemesos, Mesa Potamos, on *Pinus brutia* branches, 2 Dec. 2017, *J.C. Zamora*, UPS F-946591; Platres, on *Pinus brutia* branch, 3 Dec. 2017, *J.C. Zamora*, UPS F-946592.

Notes: This species can be easily distinguished by the combination of dark basidiocarps, often with distinct brownish parietal pigments but not very conspicuous carotenoids, distinctly branched hyphidia, and cylindrical-allantoid, thin-walled, 3-septate mature basidiospores. There are very few *Dacrymyces s.l.* described with branched hyphidia, 3-septate ± allantoid basidiospores, and non-yellow/orange basidiocarps. We studied part of the type material of *Dacrymyces paraphysatus* (the holotype, NY00738304, and one isotype, K[M] 8355) and *Da. enatus* var. *macrosporus* (the holotype, BPI725717, and four isotypes, NY03684200, LSU00135945, TAAM192134, and K[M] 95953, the last one annotated as "*Dacrymyces dendrohyphidia* P. Roberts", *nom. herb.*). These taxa clearly differ from *De. ciprense* by having distinctly thick-walled and differently sized basidiospores: in *Da. paraphysatus* 12.8–16.0 × 5.2–6.0 µm measured in cotton blue from the holotype, (13.4–)13.9–17.6(–22.1) × 5.7–7.4(–7.9) µm measured in KOH from the isotype; 13.5–17.5(–21) × 5–7 µm from McNabb (1973); in *Da. enatus* var. *macrosporus* 13.1–15.1(–15.4) × (5.3–)5.4–6.6(–6.8) µm measured in cotton blue from BPI725717, NY03684200, LSU00135945, and TAAM192134, (10.1–)12.1–16.6 × 5.4–7.2 µm measured in KOH from K(M) 95953; 11–15.5 × 4.5–5.5(–6.5) µm from McNabb (1973). In addition, in these taxa some basidiospores are constricted at septa and pigmented, the hyphidia are 1–2(–2.5) µm wide (rather constant through their length), heavily branched, forming a conspicuous layer on the hymenium, sometimes pigmented, and the basidiocarps are either individually larger or may form masses of some cm in extent (McNabb 1973, and own observations). Furthermore, *Da. paraphysatus* and *Da. enatus* var. *macrosporus* seem to be restricted to tropical areas, occurring on angiosperm wood (McNabb 1973). These two taxa clearly belong to the new genus *Dendrodacrys*, and we have combined *Da. paraphysatus* for being the one validly published at species level.

We have found one specimen (UPS F-946593), inhabiting a *Cistus* branch, that is morphologically and phylogenetically close to *De. ciprense*, but differs by having paler coloured basidiocarps, with almost indistinct brownish parietal pigments, barely inflated and ± thin-walled terminal cells of cortical hyphae, slightly narrower basidia (4.2–6.8 µm wide), slightly smaller basidiospores (14.5–17.2[–19.1] × 5.7–7.3 µm), and simple to sparingly branched hyphidia. Also, all sequenced DNA regions place it as close but substantially different from *De. ciprense*, and the STACEY analysis considered it as another putative species (Fig. 2). These results suggest that this sample probably represents a different species, but we cannot properly evaluate its intraspecific variation based on a single specimen and refrain from describing it here.

Dendrodacrys concrescens J.C. Zamora & Ekman, ***sp. nov.*** MycoBank MB 842995. Fig. 4.

Etymology: The specific epithet is an adjectival form based on the participle of the Latin verb *concresco* ("grow together"), and it refers to the habit of the basidiocarps, growing closely aggregated.

Typus: **Sweden**, Öland, Böda par., Lindreservatet, on a fallen *Pinus sylvestris* trunk, 3 Oct. 2017, *J.C. Zamora*, (**holotype** UPS F-946602; **isotypes** in G and H).

Description: *Basidiocarps* gelatinous to soft-gelatinous, 0.2–1 mm diam, erumpent when very young and later spreading on the substrate, pustulate to pulvinate, growing in densely aggregated groups and coalescing to form masses of several cm^2, partially retaining evidence of pustular origin at least when fresh; orange to yellowish orange or ochraceous orange in hydrated state, becoming orangish brown when dried and being reduced to a varnish-like layer on the substrate. *Hymenium* ± amphigenous, irregularly spreading to the margins; sterile areas around the margin often visible in young basidiocarps, becoming inconspicuous when basidiocarps coalesce. Terminal cells of marginal hyphae ± cylindrical, 3.3–6.3 µm diam, thin- to ± thick-walled, apex sometimes pointed, with hyaline walls and some cytoplasmic, yellow-orange carotenoids. Internal hyphae (1.5–)2.0–4.0 µm diam, mostly thin-walled, clamped. *Hyphidia* unevenly distributed, conspicuous only in some areas, moderately to densely branched, transitioning to simple towards the margin, 2.8–3.4 µm diam (wider towards the base and becoming thinner in the upper third); often with 1–2 clamped septa throughout their length, reaching ± the same level of young basidia, or some surpassing them. Young basidia cylindrical to narrowly clavate; mature basidia (27.3–)29.3–49.3(–52.1) × 4.5–6.2 µm, with two apical or subapical sterigmata, 16.3–36.5 × 2.7–3.9 µm, apex of the mature basidium rarely protruding. *Basidiospores* thin-walled, 12.0–16.2(–18.1) × 4.8–6.3 µm, 2.0 ≤ Q ≤ 3.3 (n = 40), cylindrical-allantoid to slightly arachiform, becoming 3-septate at maturity, not visibly constricted at septa, uninucleate prior to septation; hilar appendix conspicuous, *ca*. 1 µm long. Germinating basidiospores producing cylindrical microconidia, *ca*. 5.0–7.0 × 2.0–3.0 µm (few germinating basidiospores seen). Carotenoid contents very conspicuous in the majority of the cells of the basidiocarps, bright yellow-orange.

Ecology and distribution: All studied specimens come from the hemiboreal zone in Sweden, but one GenBank accession (LC492199, released after our datasets were compiled) corresponds to a nrLSU sequence identical to ours of *De. concrescens*, having been generated from a Japanese specimen (HNo1210, Shirouzu *et al.* 2020). Even if the species grows on a relatively common substrate, *i.e*. ± old, decorticated logs of *Pinus sylvestris*, it seems to be rare, since it was only encountered three times during intense sampling between 2017 and 2020. We have not found any additional specimens in GB, H, O, S, or UPS herbaria. It does not seem to tolerate desiccation well, as the cells of the collected specimens quickly died when the samples were dried.

Additional specimens studied: **Sweden**, Öland, Böda par., Trollskogens NR, on a fallen *Pinus sylvestris* trunk, 5 Oct. 2017, *J.C. Zamora*, UPS F-946601; Uppland, Uppsala, Norra Lunsen NR, on an old, fallen *Pinus sylvestris* log, 19 Nov. 2017, *J.C. Zamora*, UPS F-946603.

Notes: *Dendrodacrys concrescens* is easy to recognise in the field by its conspicuous and dense masses of fused small basidiocarps, on decorticated *Pinus* logs. When dried, it resembles a thin layer of varnish and the individual basidiocarps become indistinguishable. Two species share some morphological similarities with *De. concrescens* according to the literature, *viz*.

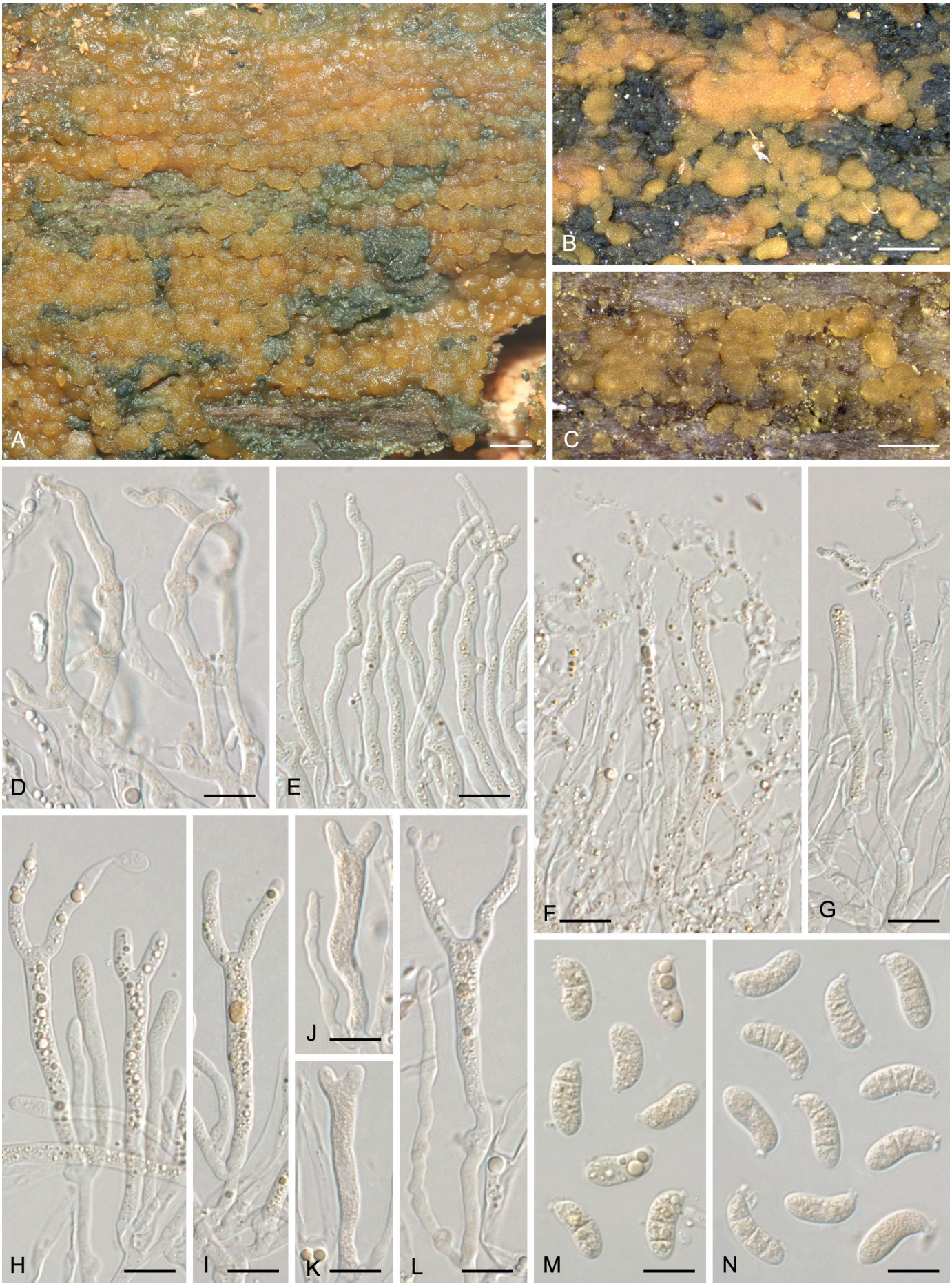

Fig. 4. *Dendrodacrys concrescens*. **A–C.** Macromorphology; basidiocarps in fresh conditions. **D–N.** Micromorphology. **D, E.** Terminal cells of marginal (D) and submarginal (E) hyphae. **F, G.** Hyphidia. **H–L.** Basidia. **M, N.** Basidiospores. A, B, E–I, M from UPS F-946602 (holotype); C, D, J–L, N from UPS F-946603. Scale bars: A–C = 1 mm; D–N = 10 µm.

Dacrymyces adpressus and *Da. fennicus*. We have studied type material of both. *Dacrymyces adpressus* has simple or indistinct hyphidia and larger individual basidiocarps, not so conspicuously fusing as they grow, and the lectotype was collected on angiosperm wood (Grognot 1863, McNabb 1973). *Dacrymyces fennicus*, considered as a synonym of *Da. adpressus* by McNabb (1973), shares the habitat with *De. concrescens*, and we have even found both species growing on a single log. However, *Da. fennicus* produces larger, well-separated basidiocarps that are normally not applanate and only sometimes coalesce. In addition, the hyphidia are often indistinct and always simple. Specimens identified as *Da. adpressus* from Japan (that likely do not represent *Da. adpressus s.str*.), and a specimen of *Da. fennicus* with a sequenced genome are well-distinguished from *De. concrescens* based on the available molecular data (Fig. 1). In particular, the ITS1 sequences of *De. concrescens* are highly deviant from those of any other species in the *Dacrymycetes*. In Shirouzu *et al.* (2020), HNo1210 (see "Ecology and distribution" above) was considered to be an unidentified clade (Clade O).

Dendrodacrys ellipsosporum J.C. Zamora, A. Savchenko, Á. González-Cruz, Prieto-García, Olariaga & Ekman, ***sp. nov.*** MycoBank MB 842996. Fig. 5.

Etymology: The specific epithet is a compound adjective referring to the shape of the basidiospores, based on the ancient Greek ελλειψοειδή (ellipsoid) and σπορά (spora).

Typus: **Spain**, Madrid, Becerril de la Sierra, on *Juniperus thurifera* exposed branches, 30 Dec. 2017, *J.C. Zamora et al.* (**holotype** UPS F-946604; **isotypes** in G and H); *idem*, on *Juniperus oxycedrus* wood, UPS F-946610 (**isotype**).

Description: *Basidiocarps* gelatinous to firm-gelatinous, (0.3–) 0.5–2.0 mm diam, at first erumpent, pustulate or pulvinate, but soon becoming applanate and slightly pezizoid when dried, often with a central root-like projection, gregarious but sometimes partially coalescing; in hydrated state orangish yellow when young, soon amber coloured to dull orange or brownish orange, chestnut brown when old; orangish brown to blackish when dried. *Hymenium* confined to the upper part of the basidiocarps or sometimes spreading to the margins, sterile cortex more or less distinct, or at least always with a sterile area in the lower part of the basidiocarps. Terminal cells of cortical/marginal hyphae ± cylindrical to irregularly dilated, (3.3–)4.1–7.6(–9.0) µm diam, thin- to more or less thick-walled, often with secondary simple septa, with a brownish, diffuse parietal pigmentation especially in the darkest basidiocarps. Internal hyphae 2.0–6.0 µm diam, thin- to slightly thick-walled, clamped, some with a roughened surface. *Hyphidia* rather common, distinct, most of them sparingly branched but varying from simple to rather densely branched, 2.1–3.6 µm diam (rather constant throughout their length or somewhat wider towards the base), often with 1–2 clamped septa throughout their length, reaching or surpassing the level of young basidia but not forming a conspicuous layer on them. Young basidia cylindrical to narrowly clavate or narrowly obpyriform; mature basidia (33.5–)40.0–73.0(–82.0) × (5.3–)6.3–12.8 µm, with two subapical sterigmata, 18.0–44.0 × 4.7–6.8 µm, apex of the mature basidium often slightly protruding. Basidium wall sometimes thickened. *Basidiospores* thin-walled, 13.9–25.7(–26.8) × (7.0–)9.7–14.2(–15.5) µm, 1.2 ≤ Q ≤ 2.2 (n = 50), commonly ellipsoid to narrowly ovoid, but rather variable from almost subglobose to lacrymiform/pyriform, 0–1(–3)-septate at maturity, not to sometimes slightly constricted at septa, uninucleate prior to septation; hilar appendix conspicuous, *ca*. 1.0–1.5 µm long. Basidiospore germination by the formation of hyphae or, more frequently, producing ellipsoid to narrowly ellipsoid conidia, *ca*. 5.0–6.0 × 2.0–2.5 µm (few germinating basidiospores observed). Carotenoid contents present in the cytoplasm of most cells, but particularly visible at basidia and basidiospores, sometimes inconspicuous and often of a dull orangish cream to moderately orange.

Ecology and distribution: Rather common in the Mediterranean forests, woodlands, and scrub biome of the Iberian Peninsula and Balearic Islands, always associated with *Juniperus spp*. Also found in the southern coast of Crimea. The species is highly xerotolerant and prefers exposed branches, undergoing repeated cycles of dryness and hydration.

Additional specimens examined: **Spain**, Balearic Islands, Ibiza, Alla dins, Pollença, on *Juniperus phoenicea* wood, 7 Dec. 2018, *I. Olariaga*, UPS F-946606; Castilla-La Mancha, Guadalajara, Tamajón, near ermita de la Virgen de los Enebrales, on *Juniperus thurifera* branches, 28 Dec. 2019, *J.C. Zamora, J. Señoret, B. Zamora, P.L. Aznar & S. Pardillo*, UPS F-979748; Guadalajara, Turmiel, entre Anquela del Ducado y Turmiel, junto a la carretera CM-2107, fallen *Juniperus thurifera* log, 24 Jan. 2016, *I. Olariaga*, UPS F-946613; Madrid, Becerril de la Sierra, on unidentified wood, 16 Jan. 2010, *J.C. Zamora, J.C. Campos, Á. González, F. Prieto & G. Sánchez*, UPS F-946609; Madrid, Colmenarejo, colada de Cabeza Aguda, on *Juniperus oxycedrus* branches, 28 Dec. 2012, *J.C. Zamora, F. Prieto & Á. González*, UPS F-946608; Madrid, Colmenarejo, Cercados del Huerto, on *Juniperus oxycedrus* dead branches, 24 Dec. 2019, *J.C. Zamora, I. Olariaga, Á. González, F. Prieto & B. Zamora*, UPS F-979765; Madrid, Colmenarejo, Presa Vieja, on *Juniperus oxycedrus* branches, 24 Dec. 2019, *J.C. Zamora, I. Olariaga & B. Zamora*, UPS F-979756; Madrid, Hoyo de Manzanares, Finca La Ladera, on *Juniperus oxycedrus* exposed branches, 11 Jan. 2018, *I. Olariaga, J.C. Zamora, F. Pancorbo & L.A. Parra*, UPS F-946605; *ibid.*, on *Juniperus oxycedrus* branch, still attached to the tree, 4 Jan. 2018, *I. Olariaga*, UPS F-946611; Madrid, Hoyo de Manzanares, Finca Las Viñas, on *Juniperus oxycedrus* branch, still attached to the tree, 19 Dec. 2017, *M. Prieto & I. Olariaga*, UPS F-946612; Madrid, Lozoya, on *Juniperus thurifera* wood, 13 Dec. 2009, *Á. González, F. Prieto, B. Zamora & J.C. Zamora*, UPS F-946607. **Ukraine**, Crimea, Greater Yalta, Mys Martyan Nature Reserve, on *Juniperus excelsa* twig, 1 Jul. 2004, *A. Bereznitskyi*, CWU(MYC)4092, LE262836; *ibid.*, 30 Jun. 2004, *S. Klimova*, CWU(MYC)4093, LE262830; *ibid.*, Mys Martyan Nature Reserve, cape Nikitin, unidentified wood, 2 Jun. 2004, *S. Klimova, A. Bereznitskyi*, CWU(MYC)7560.

Notes: This species is easily distinguished by its ovoid to cylindrical-ellipsoid, thin-walled basidiospores with 0–3 transverse septa at maturity that never become muriform, a morphology that is unique in *Dacrymyces s.l.* Besides, the combination of relatively large and dull-coloured basidiocarps, large basidia, conspicuous hyphidia, and xeric habitat on exposed *Juniperus* wood further distinguishes it from any other known species. There are, however, two other accepted species in the *Dacrymycetes* with typically ovoid to ellipsoid basidiospores. The first is *Dacrymyces ovisporus*, which has shorter, subglobose to broadly ovoid basidiospores, becoming muriform at maturity due to the formation of transverse, longitudinal and oblique septa (Brefeld 1888, McNabb 1973), simple hyphidia, and larger basidiocarps that are bright orangish

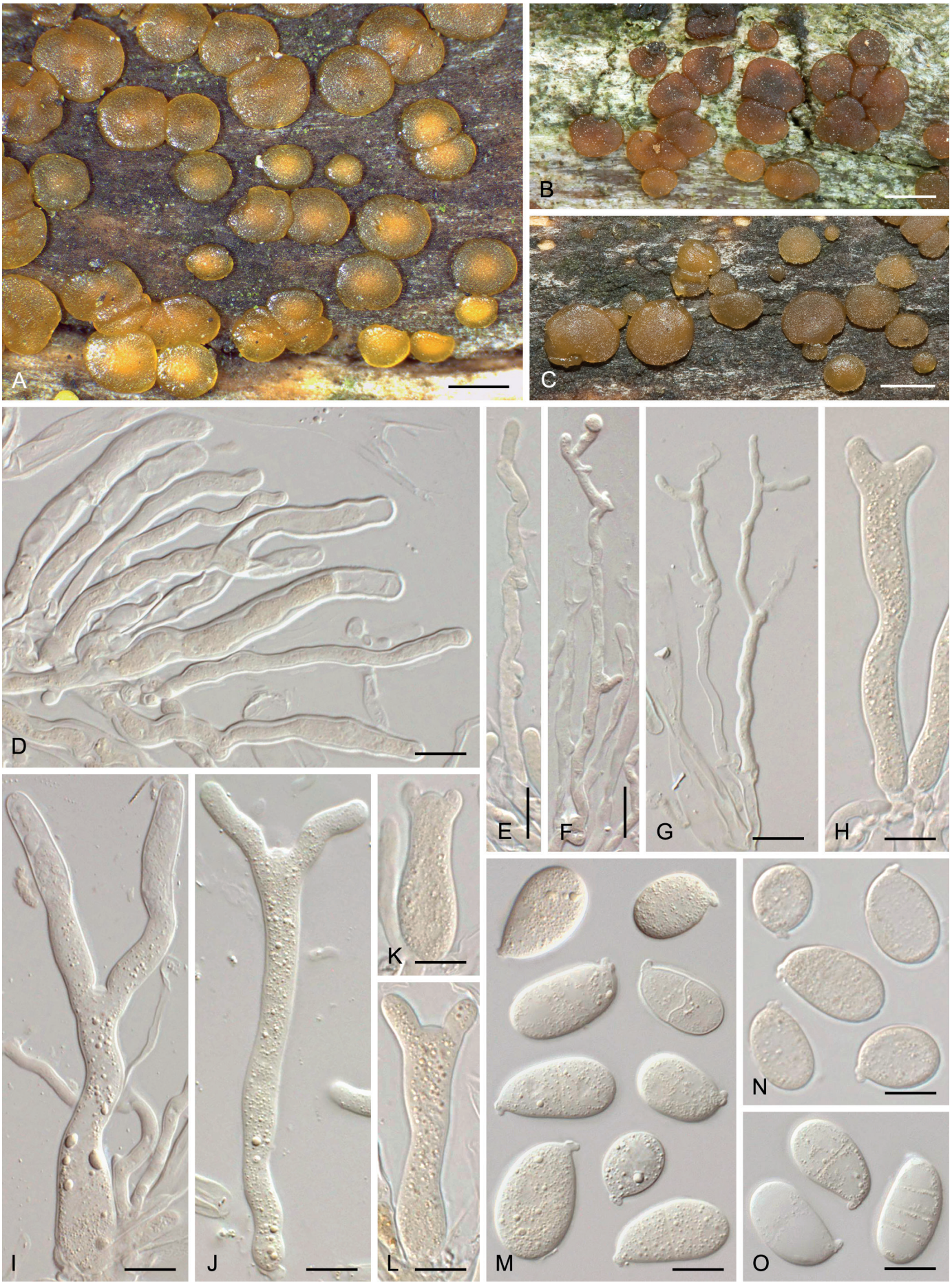

Fig. 5. *Dendrodacrys ellipsosporum*. **A–C.** Macromorphology of fresh basidiocarps. **D–O.** Micromorphology. **D.** Terminal cells of cortical hyphae. **E–G.** Hyphidia. **H–L.** Basidia. **M–O.** Basidiospores. A, B, D, F, G, I, J, M from UPS F-946604 (holotype); C, O from UPS F-946607; E, K, L, N from UPS F-946610 (isotype); H from UPS F-946605. Scale bars: A–C = 1 mm; D–O = 10 µm.

yellow. This species is typically found in *Pinus* wood and stains the substrate yellow (Torkelsen 1997). In addition, the specimens of *Da. ovisporus* included in the phylogenetic analyses show no close relationship with our samples of *De. ellipsosporum* (Fig. 1). Two of the Ukrainian studied specimens (CWU[MYC]4092 and CWU[MYC]4093) were indeed cited as *Da. ovisporus* in Malysheva & Akulov (2011). The second species with similarly shaped basidiospores is *Unilacryma bispora*. The dull colours and the presence of branched hyphidia are reminiscent of *De. ellipsosporum*, but the basidiocarps of the latter are larger, some carotenoid pigment is usually visible in the cytoplasm contents, the basidiospores never become muriform as in *U. bispora*, and septa in internal hyphae are always clamped. This species is also not closely related to *De. ellipsosporum*, belonging to a different family (Fig. 1).

Among other species names described in the literature for taxa that could be closely related to the new species, we found the old name *Da. castaneus* (Rabenhorst 1844). The data from the protologue are vague, but there are three characteristics that may agree with *De. ellipsosporum*. The first and most important one is the spore shape, which is defined as ovoid. In addition, the sporocarps are said to be brownish (hence, the epithet), and were found on a dry, dead branch. Unfortunately, no further data on the substrate or the ecology were indicated, and no iconography or specimens are cited. On the other hand, there are some characters that do not match well with *De. ellipsosporum*, and even raise doubts about the belonging of *Da. castaneus* to *Dacrymycetaceae*. The spores are said to have a dark central part and a bright edge, as if the cytoplasm was dark and the wall hyaline, something remarkably unusual for a species in *Dacrymycetaceae*. The hyphae are also said to have brown areas. The sporocarps are described as rounded, but the dimensions indicate they can be up to twice longer than wide when fresh, almost disappearing when dried, while in *De. ellipsosporum* they are almost circular and remain very conspicuous when dried, being easily visible in the field. It should be taken into account that Rabenhorst included in his concept of *Dacrymyces* (as "*Dacryomyces*") species that nowadays we know belong to other groups, *e.g*. *Da. violaceus* and *Da. fragiformis*, which are most likely members of the *Tremellomycetes*. Therefore, the name *Da. castaneus* could refer to a non-*Dacrymycetes* jelly fungus. The dark interior of the spores already caused Fries (1874) to express doubts about its classification. The name *Da. castaneus* has not been in use during the last century and was interpreted differently by other mycologists in the past. For example, Saccardo (1888) suggested that the spores mentioned in the protologue could actually be conidia, and also indicated that the species was found on lemon tree branches in Portugal and Germany, a substrate on which we would never expect *De. ellipsosporum* to occur. Neuhoff (1936) suggested that it may represent *Exidia badio-umbrina*, while Kennedy (1958) listed it as a possible, yet dubious, synonym of *Dacrymyces enatus* var. *enatus*. McNabb (1973) agreed with Kennedy (1958) while noting that original material could not be traced. Donk (1966) considered it as a *nomen dubium*, and judging by the information indicated above, we agree with this decision and do not see any advantage to rescuing this name. A possible neotypification of *Da. castaneus*, the only currently possible choice to fix the application of the name, would be difficult and subjective, since the lack of original material and the insufficient data contained in the diagnosis do not allow to make an informed and objective decision. For all these reasons, we prefer to describe *De. ellipsosporum* as a well-defined new species, and to reject *Da. castaneus* as a *nomen dubium* and *ambiguum* for the time being, at least until any original material could be found.

Dendrodacrys oblongisporum J.C. Zamora & Ekman, ***sp. nov***. MycoBank MB 842997. Fig. 6.

Etymology: The specific epithet is a Latin compound adjective of *oblongus* and *spora* (with a Greek origin but treated as Latin), and refers to the shape of the basidiospores.

Typus: **Spain**, Madrid, Bustarviejo, close to Puerto de Canencia, on *Juniperus communis subsp. alpina* dead branches, 28 Dec. 2019, *J.C. Zamora, P.L. Aznar, S. Pardillo, J. Señoret & B. Zamora* (**holotype** UPS F-979568); *idem*, UPS F-979569 (**isotype**). Note – the holotype and isotype are two different individuals, collected in well-separated bushes but treated as duplicates following Art. 8.2.

Description: *Basidiocarps* gelatinous to firm-gelatinous, (0.2–) 0.4–1.2 mm diam, barely erumpent when young, pulvinate to applanate, some becoming ± pezizoid when drying up, gregarious or in small groups, rarely coalescing; yellowish orange, ochraceous orange to amber coloured in hydrated state, becoming orange to orangish brown when dried. *Hymenium* ± confined to the upper part of the basidiocarps, irregularly spreading to the margins; sterile cortex often distinct, or at least with a sterile area in the lower part of the basidiocarps. Terminal cells of marginal hyphae narrowly clavate to cylindrical, 3.9–6.9 µm diam, ± thick-walled, with hyaline walls and some cytoplasmic, often not very conspicuous, yellow-orange carotenoids. Internal hyphae 1.5–4.0 µm diam, mostly thin-walled, clamped. *Hyphidia* present, often moderately branched but varying from simple to ± densely branched, 2.0–3.8 µm diam (width rather constant throughout their length or somewhat wider towards the base; bumps sometimes present), often with 1–2 clamped septa throughout their length, reaching the level of basidia or some surpassing them. Young basidia cylindrical to narrowly clavate; mature basidia 42.5–70.0(–92.0) × 5.0–7.8 µm, with two subapical sterigmata, 17.0–34.5 × 3.8–6.2 µm, apex of the mature basidium slightly protruding or not. *Basidiospores* thin-walled or with walls slightly thickened when old, 13.5–18.5(–19.0) × 6.3–9.4 µm, 1.6 ≤ Q ≤ 2.4 (n = 41), often oblong but varying from ellipsoid, narrowly ovoid, to ± cylindrical-allantoid, becoming 3-septate at maturity, not constricted at septa or only slightly, uninucleate prior to septation; hilar appendix conspicuous, *ca*. 1.0 µm long. Basidiospore germination not seen. Carotenoid contents visible in the majority of the cells but especially in the basidia, cream-orange to yellow-orange.

Ecology and distribution: Insufficiently known. This species has been found in only two distant localities, one in the Mediterranean forests, woodlands and scrub biome (central Iberian Peninsula, submediterranean climate due to the high elevation), and the other in the taiga biome (southwestern Scandinavia, hyperhumid southern boreal to hemiboreal coniferous forests). In both places, *De. oblongisporum* inhabited thin branches of coniferous trees and bushes, still attached to the living plants. It may be a widespread but uncommon species, or overlooked due to its small size and macroscopic similarity with many other *Dacrymyces s.l*. species. At least partly xerotolerant.

Additional specimen studied: **Norway**, Sogn og Fjordane, Førde, Viafjellet, on *Pinus sylvestris* branches, 5 Jul. 2018, *S. Ekman*, UPS F-946599.

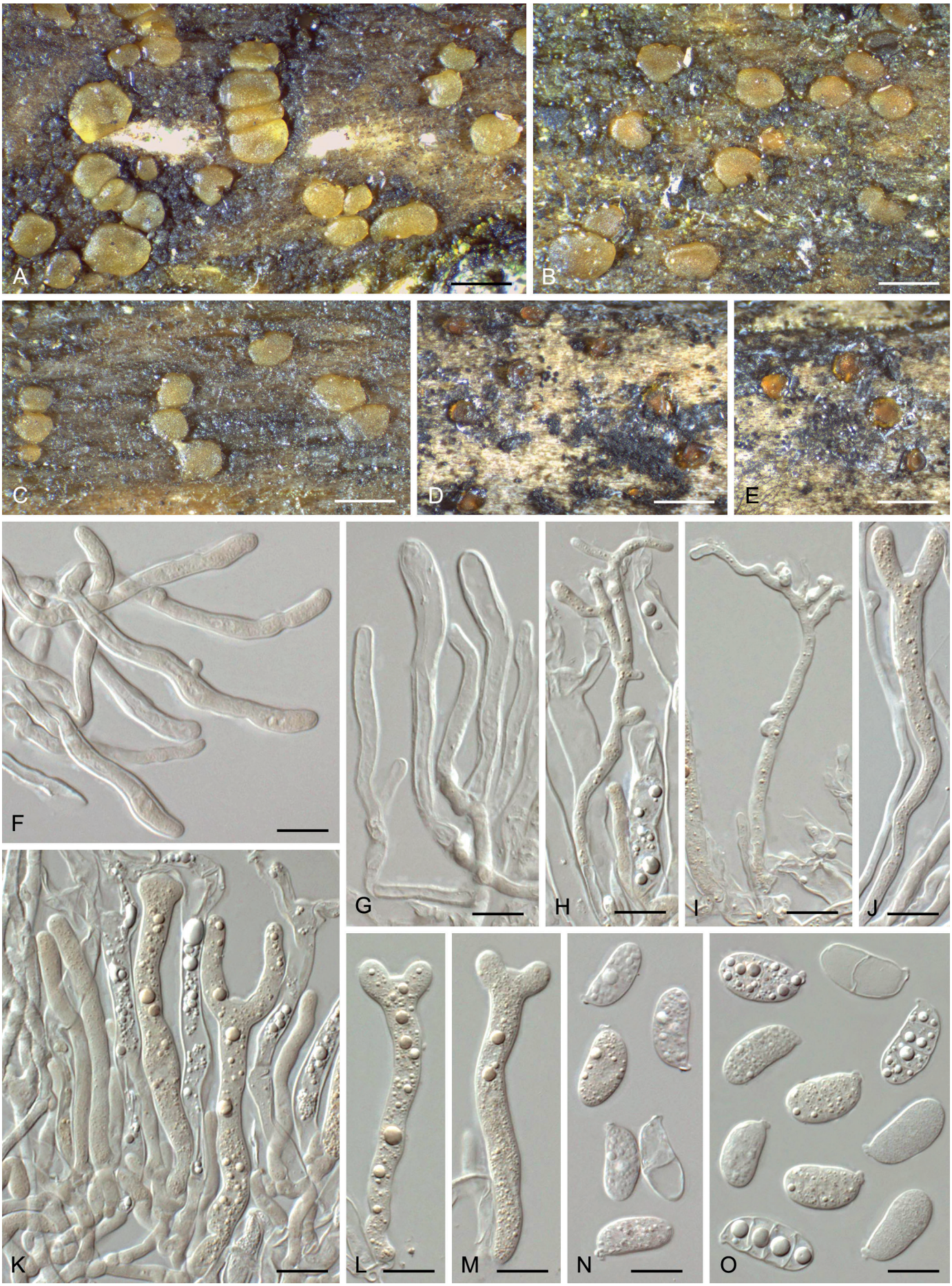

Fig. 6. *Dendrodacrys oblongisporum*. **A–E.** Macromorphology; hydrated (A–C) and dried (D, E) basidiocarps. **F–O.** Micromorphology. **F, G.** Terminal cells of cortical hyphae. **H, I.** Hyphidia. **J–M.** Basidia. **N, O.** Basidiospores. A–C, G–I, K–M, O from UPS F-979568 (holotype); D–F, J, N from UPS F-946599. Scale bars: A–E = 1 mm; F–O = 10 µm.

Notes: *Dendrodacrys oblongisporum* resembles *Dacrymyces adpressus* and *Da. fennicus* based on literature. However, both species lack branched hyphidia, *Da. adpressus* occurs on angiosperm wood (Grognot 1863), and *Da. fennicus* typically grows on thick branches or logs of *Pinus*, not on thin branches or twigs as *De. oblongisporum*. The basidiospores in both *Da. adpressus* and *Da. fennicus* are also more distinctly allantoid, and the terminal cells of the cortical/marginal hyphae thin-walled. Finally, molecular data clearly separate *Da. fennicus* and *De. oblongisporum*, and the available DNA sequences from specimens identified as *Da. adpressus* from Japan (that probably do not represent *Da. adpressus s.str.*) also distinguish these species into well-separated clades (Fig. 1). From other species of *Dendrodacrys*, the combination of the basidiospore shape, presence of clamp-connections, and isolated, small yellow-orange basidiocarps is diagnostic. Specifically, *De. ciprense* produces darker, more brownish basidiocarps, and cylindrical-allantoid basidiospores ($2.2 \leq Q \leq 3.2$). Basidiocarps of *De. concrescens* are smaller and coalesce to form extense masses, the basidiospores are smaller and especially narrower (4.8–6.3 µm wide), and the ecology is also different, growing on fallen pine logs. *Dendrodacrys ellipsosporum* has larger and especially broader basidiospores [(7.0–)9.7–14.2(–15.5) µm wide], slightly broader basidia, and often darker basidiocarps. Finally, *Da. cf. dendrocalami* lacks clamp-connections and the basidiospores are thick-walled.

DISCUSSION

Is a new genus needed in *Dacrymycetaceae*?

Zamora & Ekman (2020) and Savchenko *et al.* (2021) showed that branched hyphidia seemed to be a common feature in *Cerinomycetaceae*, *Dacryonaemataceae* and *Unilacrymaceae*, and probably plesiomorphic in the last two families. By contrast, branched hyphidia in *Dacrymycetaceae* were only found in certain groups and seemed to be a secondary acquisition of this character state or a reversion to the plesiomorphic state. Specifically, until now, branched hyphidia have been found in only two small species groups of *Dacrymycetaceae*. One is the clade containing *Dacryopinax elegans* (generic type) and *Dacrymyces san-augustinii*, which is nested in the large group of mostly clampless species (clade D8). The other clade is D5, where most species have clamp-connections. The group including *Dacryopinax elegans* and *Da. san-augustinii* is morphologically very heterogeneous and difficult to diagnose, since *Dacryopinax elegans* has brownish, long-stalked, cochleariform to auricularioid basidiocarps with unilateral hymenium, and thick-walled, 3-septate basidiospores, while *Da. san-agustinii* (and also *Da. novae-zelandiae*, which lacks conspicuously branched hyphidia) has ± yellow-orange, sessile, cushion-shaped basidiocarps with a poorly defined sterile cortex, and thin-walled, multiseptate basidiospores.

Clade D5, on the other hand, is considerably more homogeneous and easier to diagnose, comprising species with sessile, cushion-shaped to flattened basidiocarps, branched hyphidia, and mature basidiospores with up to three septa. All known species in this group have clamp-connections, with the exception of *Da. cf. dendrocalami*, which seems to have lost them. From a phylogenetic point of view, clade D5 and the group containing *Dacryopinax elegans* are not closely related, so it is not possible to delimit a single, monophyletic genus that would include all *Dacrymycetaceae* species with branched hyphidia. In addition, it would be difficult to justify the inclusion of species in clade D5 even in a very broad and extended genus *Dacrymyces*, since that would imply merging well-known genera such as *Calocera s.str*. with *Dacrymyces*. Such an assemblage would hardly be diagnosable in terms of the most used characters in *Dacrymycetes* taxonomy, like the presence or absence of clamp-connections, basidiospore morphology (including shape, wall thickness, and septation), presence or absence of distinct hyphidia, development of a sterile cortex and terminal cells of its hyphae, or the morphology of the basidiocarps. Therefore, the recognition of clade D5 as a distinct genus does not imply oversplitting *Dacrymyces*, but on the contrary, it increases the diagnosability of the genera in *Dacrymycetaceae* and partially alleviates the rampant polyphyly of *Dacrymyces s.l*. Further generic rearrangements are expected in the future, but only after phylogenies with better resolution (especially in clade D8) are obtained and monographic studies of the different clades have been performed. To emphasize the character of branched hyphidia in the species currently included in clade D5, we chose the name *Dendrodacrys*. None of the included species contain the type of any validly published generic or infrageneric names in *Dacrymycetaceae*, most of which were already treated by Zamora & Ekman (2020) and Savchenko *et al.* (2021), so there is no other nomenclatural choice than proposing a new generic name.

Species delimitation in *Dendrodacrys*

STACEY results showed a rather clear assignment of most specimens to a single cluster (putative species), except for the uncertainty whether the two specimens of *Da. cf. adpressus* should be considered as one or two putative species. The amount of data for TNS-21069 is much smaller than for the other samples in the dataset, since only nrLSU data was available and only five substitutions separate the two *Da. cf. adpressus* specimens. This is clearly insufficient to get a reliable estimate of the population structure and possible speciation events within this species or species complex. By comparison, there are seven substitutions and one indel separating the two most distant nrLSU sequences of *De. ellipsosporum* [obtained from UPS F-946606 and CWU(MYC)4092], but thanks to the information contained in the remaining DNA regions, STACEY did not have problems to suggest that both samples belong to a same cluster, with very high posterior probability. As reported elsewhere, coalescence-based species delimitation is prone to oversplitting (*e.g*. Jackson *et al.* 2017, Sukumaran & Knowles 2017, Chambers & Hillis 2020, Leaché *et al.* 2019). Putative species suggested by such methods should be critically evaluated using all available data and not directly translated to nominal species. This is especially true when the amount of data is small, *e.g.* few specimens or populations per putative species, and/or few unlinked DNA regions with enough variation. Nevertheless, these two samples were mostly assigned to the same putative species in our analyses, which agrees with a conservative approach.

From a phenotypical point of view, the basidiospore morphology is demonstrated here to be particularly useful to characterise species in *Dendrodacrys*, being almost like a "fingerprint" for species recognition. In fact, the delimitation of the new species found during our study does not really appear to represent a challenge for the morphology- and coalescence-based species delimitation analyses. Among the proposed

new species, *De. concrescens* and *De. ellipsosporum* have a particularly striking morphology and very distinct DNA sequence data, making them unlikely to be confused with any other species. *Dendrodacrys ciprense* and *De. oblongisporum* are rather closely related according to our phylogenetic reconstructions, and both species produce non-coalescing, cushion-shaped basidiocarps and somewhat curved, thin-walled basidiospores. However, they are still well-defined and readily distinguishable on account of the colour of the basidiocarps, size and shape of the basidiospores, cell pigments, ecology, and molecular data (see Fig. 2 and observations under the mentioned taxa).

Notes on extra-European taxa

Dacrymyces dendrocalami is easily distinguished from the related taxa by clampless septa, wide basidia, sterigmata exceeding basidia in length, and spore shape. If the Japanese specimens are confirmed to belong to this taxon, then the species would be known from Japan and Taiwan, occurring on angiosperm wood. The characteristic dendroid hyphidia allow identification as *Dendrodacrys* even on a purely morphological basis, but we prefer to await the revision of the type material before proposing the required combination.

The Japanese *Dacrymyces cf. adpressus* is most likely an undescribed species. This angiosperm wood-dwelling species differs from the lectotype of *Da. adpressus* by the presence of abundant dendroid hyphidia. Yet another specimen (**Japan**, Wakayama, Mt. Shirami, on dead unidentified branches, 12 Oct. 2006, *T. Shirouzu* HNo. 554, TNS-F-21069) presumably belongs to this taxon, but we did not include it in the analyses due to the lack of data.

Dacrymyces paraphysatus and *Da. enatus* var. *macrosporus* are two morphologically close species that clearly belong to *Dendrodacrys* (see observations under *De. ciprense*). *Dacrymyces enatus* var. *macrosporus* is thus not closely related to *Da. enatus* var. *enatus* (syn. *Cerinomyces enatus*; see Savchenko *et al.* 2021), but its delimitation against *Da. paraphysatus* needs to be re-evaluated with additional specimens. Therefore, we do not make the combination in *Dendrodacrys* for the time being. Concerning *Da. paraphysatus*, we accept it as an independent species after studying the type material, and since it is already published at the species level, the combination can be safely made without risking the publication of a superfluous name:

Dendrodacrys paraphysatum (L.S. Olive) J.C. Zamora & A. Savchenko, ***comb. nov***. MycoBank MB 842998.
Basionym: *Dacrymyces paraphysatus* L.S. Olive, *Bull. Torrey Bot. Club* **85**: 106. 1958.

Calocera arborea (Shirouzu *et al.* 2013b), which was considered part of clade D5 in Zamora & Ekman (2020), was not included in the present study pending a morphological revision of the available material, and the generation of additional DNA data. With only ITS and nrLSU sequences currently available, the phylogenetic position of this species varied between studies. For instance, in Shirouzu *et al.* (2013b, 2016, 2017) it did not form a clade with *Da. cf. adpressus* and *Da. cf. dendrocalami*. This species shares with *Dendrodacrys* the cushion-shaped fertile heads of the basidiocarps and the 3-septate mature basidiospores. However, it has strikingly long and branched stalks, which could be seen as an extraordinary development of the parts that are often rooting into the substrate in several other species of *Dacrymycetes*. Most importantly, branched hyphidia were not indicated in the protologue, but these structures are not always easy to find. Their absence should be confirmed before taxonomic conclusions are drawn.

Further details on the taxonomy of the cited additional non-European species, as well as an identification key for the genus *Dendrodacrys*, will be included in a forthcoming study.

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REFERENCES

Bouckaert RR, Drummond AJ (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evolutionary Biology* **17**: 42.

Bouckaert RR, Heled J, Kühnert D, *et al.* (2014). BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.

Brefeld O (1888). *Untersuchungen aus dem Gesammtgebiete der Mykologie* 7. Felix, Germany.

Chambers EA, Hillis DM (2020). The multispecies coalescent over-splits species in the case of geographically widespread taxa. *Systematic Biology* **69**: 184–193.

Donk MA (1966). Check list of European hymenomycetous *Heterobasidiae*. *Persoonia* **4**: 145–335.

Drummond AJ, Ho SYW, Phillips MJ, *et al.* (2006). Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4**: e88.

Ferencova Z, Rico VJ, Hawksworth DL (2017). Extraction of DNA from lichen-forming and lichenicolous fungi: A low-cost fast protocol using Chelex. *The Lichenologist* **49**: 521–525.

Fries EM (1874). *Hymenomycetes europaei*. Typis descripsip Ed. Berling, Uppsaliae, Sweden.

Gardes M, Bruns TD (1993). ITS primers with enhanced specificity for basidiomycetes. Application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Gargas A, Taylor JW (1992). Polymerase chain reaction. PCR primers for amplifying and sequencing nuclear 18S rDNA from lichenized fungi. *Mycologia* **84**: 589–592.

Grigoriev IV, Nikitin R, Haridas S, *et al.* (2014). MycoCosm portal: gearing up for 1 000 fungal genomes. *Nucleic Acids Research* **42**: D699–704.

Grognot A (1863). *Plantes Cryptogames - Cellulaires du department de Saone-et-Loire avec des tableaux synoptiques*. Michel Dejussieu, France.

Henriot A, Cheype J-L (2016). Piximètre. http://piximetre.fr/ [accessed 15 August 2021].

Huelsenbeck JP, Larget B, Alfaro MA (2004). Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. *Molecular Biology and Evolution* **21**: 1123–1133.

Jackson ND, Carstens BC, Morales AE, *et al.* (2017). Species delimitation with gene flow. *Systematic Biology* **66**: 799–812.

Jones G (2017). Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *Journal of Mathematical Biology* **74**: 447–467.

Jones G, Aydin Z, Oxelman B (2015). DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. *Bioinformatics* **31**: 991–998.

Kalyaanamoorthy S, Minh BQ, Wong TKF, *et al.* (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Kennedy LL (1958). The genus *Dacrymyces*. *Mycologia* **50**: 896–915.

Leaché AD, Zhu T, Rannala B, *et al.* (2019). The spectre of too many species. *Systematic Biology* **68**: 168–181.

Malysheva VF, Akulov AYu (2011). New records of *Dacrymyces ovisporus* and *Tremella diaporthicola* from the Ukraine. *Czech Mycology* **63**: 189–194.

Mason-Gamer RJ, Kellogg EA (1996). Testing for phylogenetic conflict among molecular data sets in the tribe *Triticeae*. *Gramineae*. *Systematic Biology* **45**: 524–545.

McNabb RFR (1965). Taxonomic studies in the *Dacrymycetaceae* V. *Heterotextus* Lloyd. *New Zealand Journal of Botany* **3**: 215–222.

McNabb RFR (1973). Taxonomic studies in the *Dacrymycetaceae* VIII. *Dacrymyces* Nees ex Fries. *New Zealand Journal of Botany* **11**: 461–524.

Nguyen L-T, Schmidt HA, Von Haeseler A, *et al.* (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.

Neuhoff W (1936). Die Gallertpilze Schwedens: *Tremellaceae*, *Dacrymycetaceae*, *Tulasnellaceae*, *Auriculariaceae*. *Arkiv för Botanik* **28A**: 1–57.

Oberwinkler F, Tschen J (1989). A new *Dacrymyces* species from Taiwan. *Transactions of the Mycological Society of Japan* **30**: 349–356.

R Core Team (2021). *R*: *A language and environment for statistical computing. R Foundation for Statistical Computing*. Vienna, Austria. https://www.R-project.org/ [accessed 15 Aug. 2021].

Rabenhorst L (1844). *Deutschlands Kryptogamenflora* 1. Kummer, Leipzig, Germany.

Rambaut A (2016). *FigTree v1.4*. Computer program and documentation distributed by the author. http://tree.bio.ed.ac.uk/software/figtree/.

Rambaut A, Drummond AJ, Xie D, *et al.* (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**: 901–904.

Rehner SA, Buckley E (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**: 84–98.

Reid DA (1974). A monograph of the British *Dacrymycetales*. *Transactions of the British Mycological Society* **62**: 433–494.

Ronquist F, Teslenko M, Mark P, *et al.* (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.

Saccardo PA (1888). *Sylloge Fungorum omnium hucusque cognitorum* 6(2). R. Friedländer & Sohn, Berlin, Germany.

Savchenko A, Zamora JC, Shirouzu T, *et al.* (2021). Revision of *Cerinomyces* (*Dacrymycetes*, *Basidiomycota*) with notes on morphologically and historically related taxa. *Studies in Mycology* **99**: 100117.

Shirouzu T, Hirose D, Tokumasu S (2007). Sequence analyses of 28S rRNA gene D1/D2 region suggests *Dacrymyces* (*Heterobasidiomycetes*, *Dacrymycetales*) is polyphyletic. *Mycoscience* **48**: 388–394.

Shirouzu T, Hirose D, Tokumasu S (2009). Taxonomic study of the Japanese *Dacrymycetes*. *Persoonia* **23**: 16–34.

Shirouzu T, Hirose D, Oberwinkler F, *et al.* (2013a). Combined molecular and morphological data for improving phylogenetic hypotheses in *Dacrymycetes*. *Mycologia* **105**: 1110–1125.

Shirouzu T, Ishikawa NK, Hirose D, *et al.* (2013b). A new Amazonian species of *Calocera* with dendroid and multi-headed basidiocarp. *Mycoscience* **54**: 252–256.

Shirouzu T, Uno K, Hosaka K, *et al.* (2016). Early-diverging wood-decaying fungi detected using three complementary sampling methods. *Molecular Phylogenetics and Evolution* **98**: 11–20.

Shirouzu T, Hosaka K, Nam K-O, *et al.* (2017). Phylogenetic relationships of eight new *Dacrymycetes* collected from New Zealand. *Persoonia* **38**: 156–169.

Shirouzu T, Matsuoka S, Doi H, *et al.* (2020). Complementary molecular methods reveal comprehensive phylogenetic diversity integrating inconspicuous lineages of early-diverged wood-decaying mushrooms. *Scientific Reports* **10**: 3057.

Stielow JB, Lévesque CA, Seifert KA, *et al.* (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* **35**: 242–263.

Suchard MA, Redelings BD (2006). BAli-Phy: simultaneous Bayesian inference of alignment and phylogeny. *Bioinformatics* **22**: 2047–2048.

Sukumaran J, Knowles LL (2017). Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 1607–1612.

Thiers B (2021). *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 01 Dec. 2021].

Torkelsen A-E (1997). *Dacryomycetales* Lindau. In: *Nordic Macromycetes, volume 3*: *Heterobasidioid, aphyllophoroid and gastromycetoid Basidiomycetes* (Hansen L, Knudsen H, eds). Nordsvamp, Denmark: 90–96.

Van de Put K (2014). Basidiemorfologie bij *Dacrymyces*. *Sterbeeckia* **33**: 41–44.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

White TJ, Bruns T, Lee S, *et al.* (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols*: *A guide to methods and applications* (Innis M, Gelfand J, Sninsky J, *et al.*, eds). Academic Press, USA: 315–322.

Zamora JC, Ekman S (2020). Phylogeny and character evolution in the *Dacrymycetes*, and systematics of *Unilacrymaceae* and *Dacryonaemataceae fam. nov*. *Persoonia* **44**: 161–205.