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Research Article

Mystery unveiled: *Diacanthodes* Singer – a lineage within the core polyporoid clade

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Diacanthodes (Polyporales, Basidiomycota) is a fungal genus with stipitate basidiomata and a combination of ornamented and dextrinoid basidiospores that is unique among the poroid fungi. Although some hypotheses based on morphological features speculated about the phylogenetic relationships of the taxon, they have never been tested based on molecular data. We performed molecular phylogenetic analyses including specimens of *Diacanthodes* from the Neotropics and Africa using the internal transcribed spacers (ITS1-5.8S-ITS2 = ITS) and the D1–D2 domains of the 28S gene of the nuclear rDNA regions, as well as the translation elongation factor 1-alpha (TEF-1α) protein-coding gene. Our study revealed *Diacanthodes* as a member of the 'core polyporoid' clade within the Polyporales. Two new species from South America: *Diacanthodes cerebriporoides* and *D. neotropicalis*, a new combination *D. coffeae* from Africa and notes on the other *Diacanthodes* species are presented. Basidiospore morphology in *Diacanthodes* and related genera is discussed in the phylogenetic context.

Key words: Dextrinoid basidiospore, neotropical polypores, Polyporales, scanning electron microscopy, systematics, wood-decay fungi

Introduction

Fungal taxonomy and systematics have been based mainly on morphology for a long time. The advance of molecular techniques represents a change of paradigm in the conception and weight of morphological characters. This has been evident in polypore fungi where in many cases molecular phylogenetic analyses have confirmed taxonomic hypotheses based on morphology and the homogeneity of morphological characters that sustained them. In this context, the advances of molecular techniques have strongly contributed to a better understanding of the phylogenetic relationships between fungal taxa. However, many phylogenetic relationships are still inferred only from morphological

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characters. This is the case of the polypore genus *Diacanthodes* Singer that is composed of species with stipitate basidiomata growing from soil, presenting a dimitic hyphal system, generative clamped hyphae, and a particular type of basidiospores ornamented with warts and with a dextrinoid reaction in Melzer's reagent (Fidalgo, 1962; Ryvarden & Johansen, 1980; Singer, 1945). The combination of ornamented and dextrinoid basidiospores has not been reported for any polypore and therefore the phylogenetic position of the genus remains elusive (Binder et al., 2013). However, based on single or combined morphological characters, different hypotheses about the phylogenetic relationships of this genus have been suggested.

On one hand it has been suggested that *Abortiporus* Murrill and *Diacanthodes* are closely related genera, sharing a similar macromorphological aspect and hyphal system, while the main difference lies in the basidiospore morphology (Fidalgo, 1962). Based on the basidiospore morphology, the dimitic hyphal system and the basidiomata habit (growing on the ground), a relationship with *Bondarzewia* Singer has also been suggested (Gilbertson & Ryvarden, 1986; Ryvarden & Johansen, 1980; Singer, 1945). However, *Bondarzewia* presents simple septate generative hyphae and amyloid basidiospores, and is now placed in the order Russulales (Hibbett & Binder, 2002; Moncalvo et al., 2002; Song et al., 2016).

In turn, through morphological phylogenetic analysis based on differently weighted characters a relationship with *Heterobasidion* and *Pachykytospora* (Quanten, 1997) has been suggested. *Heterobasidion* presents generative hyphae with simple septa and amyloid basidiospores and is currently recognized as a poroid member of Bondarzewiaceae, Russulales (Miller et al., 2006). *Pachykytospora* shares a similar hyphal system with *Diacanthodes*, but presents ornamented cyanophilous basidiospores without reaction in Melzer's reagent, producing resupinate basidiomata on wood (Zhou et al., 2019).

It has been suggested that the basidiospore ornamentation of *Diacanthodes* species seems to be included in a hyaline exospore membrane (Corner, 1989). This observation is supported by the published images of scanning electron microscopy, where the ornamentations can be observed as deep undulations and not as true spines as seen under optical microscope (Ryvarden & Johansen, 1980). The ornamented endosporium covered by a hyaline exosporium has been described for Ganodermataceae among Polyporales (Costa-Rezende et al., 2017, 2020), however, most of the species described in the Ganodermataceae present a coloured endospore without a dextrinoid reaction.

Amyloid reaction and simple septa are important morphological characters for Russulales (Miller et al., 2006). In turn, the dextrinoid reaction shows intrageneric variability, e.g., *Perenniporia*, *Microporellus*. Taxa that exhibit a dimitic hyphal system with clamp connections together with ornamented basidiospores or with dextrinoid reaction are, so far, related to the traditional core polyporoid clade (Binder et al., 2013). Although the combination of characters of the basidiospores of *Dicanthodes*, i.e., ornamented and dextrinoid, is not reported in any taxa belonging to the core polyporoid clade, we support the hypothesis that *Diacanthodes* is a member of the core polyporoid clade. Moreover, although the combination of morphological features of *Diacanthodes* suggests different affinities within that clade, we hypothesize that *Diacanthodes* is related to taxa presenting thick-walled and dextrinoid basidiospores.

In order to assess the phylogenetic relationships of *Diacanthodes* and test the above-mentioned hypotheses, we studied specimens from the tropical regions of South America and Africa using molecular phylogenies and morphological analyses.

Materials and methods

Specimens and morphological studies

The studied specimens are deposited in CORD, CTES, HUA, and TAAM, herbarium acronyms follow Thiers (2020) (continuously updated, http://sweetgum.nybg.org/science/ih/). Microscopic examinations and measurements were done using Melzer's reagent, Cotton Blue and/or 3–5% KOH as mounting media. For the hyphal system study, sections of the basidiomata were incubated in hot (40 °C) 3% NaOH solution, then dissected under a stereo microscope and finally examined at 3% NaOH solution at room temperature (Decock et al., 2013). Melzer's reagent was used to check dextrinoid and amyloid reactions. The lack of reaction was denoted as IKI−. Forty basidiospores were measured, with 5% of the measurements at each end of the range given in parentheses, when relevant.

For ultrastructural analysis, basidiospores were observed in two different treatments. In the first one, fragments of the tubes were placed on stubs, and then coated with gold and observed under SEM. In the second one, we treated the basidiospores according to Costa-Rezende et al. (2017). Fragments of the tubes were placed in chromic acid (H_2CrO_4) crystals, covered by enough water drops to dissolve the crystals, and kept for around 20 minutes. Then, this solution and the tube fragments were filtered (0.45 µm filter) by vacuum,

adding enough water to remove the acid. The filter was dried at room temperature and finally scraped with a blade onto a stub with a drop of 70% alcohol. After the alcohol had dried at room temperature, the sample was coated with gold and observed under SEM. Chlamydospores were observed in the context and pilear surface without treatment. The analyses were performed using a scanning electronic microscope (SEM) Zeiss LEO 1450VP at Laboratorio de Microscopía Electrónica y Microanálisis (LABMEM)/Universidad Nacional de San Luis, Argentina and JEOL JSM-6390LV.

DNA extraction and sequencing

DNA was extracted from dried basidiomata following the protocol of Doyle and Doyle (1987) modified by Góes-Neto et al. (2005). Primer pairs ITS8-F/ITS6-R (Dentinger et al., 2009) and LR0R/LR7 (Vilgalys & Hester, 1990) were used to amplify the internal transcribed spacers (ITS) and large subunit (28S) rDNA regions, respectively. Primer pairs EF1-983F/EF1- 2218 R (Rehner & Buckley, 2005) were used to amplify the translation elongation factor 1-alpha (TEF-1α) protein-coding gene. Sanger sequencing was performed with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) following manufacturer's procedures. The same oligos were used as forward and reverse sequencing primers for the ITS, 28S, and TEF-1α.

Phylogenetic analyses

Chromatograms were manually edited using Geneious v. 6.1.8 (http://www.geneious.com). The sequences generated in this study were combined with ITS, 28S, and TEF-1α sequences retrieved from GenBank (NCBI) to compose two different datasets. The first dataset (1) was constructed to infer the phylogenetic position of *Diacanthodes* within the Agaricomycetes, including the main lineages of the group. Based on the results obtained from this first analysis, a second dataset (2) was constructed focused on the 'core poliporoid' clade. The newly generated sequences and additional sequences downloaded from GenBank are listed in Table 1.

ITS, 28S, and TEF-1α matrices for both datasets were individually aligned using MAFFT v.7 (Katoh & Standley, 2013), under the G-INS–i criteria. Then, they were manually inspected and edited using MEGA v.6 (Tamura et al., 2013). Potentially ambiguously aligned segments of ITS1-5.8S-ITS2 of dataset 1 were detected by Gblocks 0.91 b (Castresana, 2000), using the following block parameters: minimum number of sequences for conserved or flanking positions was one half of the total sequences, the maximum number of contiguous non-conserved positions was 16 bp, the minimum length of a block was 2 bp and all gap positions were included (within an appropriate block).

We used PartitionFinder v.2 (Lanfear et al., 2017) to estimate the best-fit partitioning strategy and the best-fit model of nucleotide evolution for both datasets, using seven data blocks (28S, ITS1, 5.8S, ITS2, *TEF* 1st, 2nd, and 3rd codon positions) in dataset 1 and eight data blocks (28S, ITS1, 5.8S, ITS2, *TEF* 1st, 2nd, and 3rd codon positions and *TEF* introns) in dataset 2, with the following settings applied for both datasets: branch lengths = linked, models = mr bayes, model_ selection = AICc and search = greedy.

Bayesian inference (BI) and Maximum likelihood (ML) phylogenetic analyses were applied to the datasets. Bayesian inference was performed using the defined partitions and evolutionary models in MrBayes 3.2 (Ronquist et al., 2012) with two independent runs, each one beginning from random trees with four simultaneous independent chains, performing 5×10^7 replications, sampling one tree every 1×10^3 generation. The first 25% of the sampled trees were discarded as burn-in, while the remaining ones were used to reconstruct a 50% majority-rule consensus tree and to calculate Bayesian posterior probabilities (BPP) of the clades.

Maximum likelihood searches were conducted with RAxML-HPC v.8.2.3 (Stamatakis, 2014), available in the CIPRES science gateway (Miller et al., 2010; http://www.phylo.org/). The analysis first involved 100 ML searches, each one starting from one randomized stepwise-addition parsimony tree, under a GTRGAMMA model, with all other parameters estimated by the software. Only the best-scored likelihood tree from all the searches was kept to assess the reliability of the nodes. Multiparametric bootstrapping replicates under the same model were computed, allowing the program to halt bootstrapping automatically by the autoMRE option. An additional alignment partition file was used to force RAxML software to search for a separate evolutionary model for each defined partition.

A node was considered strongly supported if it showed a BPP $\geq$ 0.95 and/or BS $\geq$ 80% (Hyde et al., 2013). Hereafter, support values are presented as BPP/BS in the text. The final alignment and the retrieved topologies were deposited in TreeBASE (http://www.treebase.org/treebase/index.html), under accession ID: 24885.

Results

Phylogenetic inference

Dataset 1 included sequences from 62 fungal specimens, with 2718 characters, of which 1443 were constant and 923

Table 1. Taxon sampling, specimen-voucher information, and GenBank accession numbers of sequences used in this study. **T**= type specimen. New sequences generated in this study are highlighted in bold.

Species	***Voucher***	***ITS***	***28S***	***TEF-1α***
Abortiporus biennis (Bull.) Singer	EL65-03	JN649325	JN649325	JX109892
Abundisporus fuscopurpureus (Pers.) Ryvarden	Cui 10950	KC456254	KC456256	KF181154
Abundisporus roseoalbus (Jungh.) Ryvarden	Dai 12269	KC415908	KC415910	KF181131
Abundisporus violaceus (Wakef.) Ryvarden	Ryvarden 32807	KF018127	KF018135	KF181132
Amanita brunnescens G.F. Atk.	AFTOL-ID 673	AY789079	AY631902	AY881021
Amylocorticium subsulphureum (P. Karst.) Pouzar	CFMR:HHB-13817	GU187506	GU187562	GU187680
Antrodia serialis (Fr.) Donk	KHL 12010	NR_154676	JX109844	JX109898
Aseroe rubra Labill.	OSC122632	–	DQ218625	DQ219261
Armillaria mellea (Vahl) P. Kumm.	AFTOL-ID 449	AY789081	AY700194	AY881023
Athelia arachnoidea (Berk.) Jülich	CBS 418.72	–	GU187557	GU187672
Athelia epiphylla Pers.	CFMR: FP-100564	GU187501	GU187558	GU187676
Auricularia heimuer F. Wu, B.K. Cui & Y.C. Dai	Heishan	LT716073	KY418889	KY419082
Auricularia polytricha (Mont.) Sacc.	TUFC12920	AB871752	AB871733	–
Blumenavia rhacodes Möller	ICN 177266	MG817719	MG817730	MH061937
Boletinellus merulioides (Schwein.) Murrill	AFTOL-ID 575	DQ200922	DQ534581	DQ056287
Boletus edulis Bull.	Be3	–	KF030282	GU187682
Bondarzewia berkeleyi (Fr.) Bondartsev & Singer	Dai 12759	KJ583202	KJ583216	KX066138
Bondarzewia mesenterica (Schaeff.) Kreisel	DD 348/06	KM243328	KM243331	KX066147
Bresadolia uda (Jungh.) Audet	WD1878	AF518756	AB368108	–
Bresadolia uda	Cui11071	KX851642	KX851695	KX851794
Brevicellicium sp.	LISU:178590	HE963775	HE963776	–
Colospora andalasii Miettinen & Spirin	Miettinen X1442	KT361629	KT361629	–
Coriolopsis cf. byrsina (Mont.) Ryvarden	FP 105050-SP	JN165001	AY351954	JN164879
Crassisporus leucoporus B.K. Cui & Xing Ji	Cui 16801	MK116488	MK116497	MK122986
Crassisporus macroporus B.K. Cui & Xing Ji	Cui 14468	MK116486	MK116495	MK122984
Crassisporus microsporus B.K. Cui & Xing Ji	Cui 16221	MK116487	MK116496	MK122985
Cristataspora coffeata (Murrill) Robledo, Costa-Rezende & de Madrignac Bonzi	Robledo 3183	MN077526	MN077560	MN061695
Cristataspora flavipora (Berk.) Robledo, Costa-Rezende & de Madrignac Bonzi	Robledo 3288	MN077521	MN077555	MN061694
Cryptoporus volvatus (Peck) Shear	Cui 16468	MG847207	MG847216	MG867694
Cryptoporus volvatus	CBS 432.48	MH856424	MH867970	–
Dacrymyces sp.	AFTOL-ID 528	DQ205684	AY691892	DQ028587
Daedalea quercina (L.) Pers.	Miettinen 12662	JQ700296	JQ700296	JX109912
Daedaleopsis confragosa (Bolton) J. Schröt.	PRM92163	HG973502	–	HG973511
Daedaleopsis septentrionalis (P. Karst.) Niemelä	H6035	HG973499	–	HG973507
Daedaleopsis tricolor (Bull.) Bondartsev & Singer	Cui 8301	KU892426	KU892468	KX838423
Daedaleopsis tricolor	Dai 8349	KU892432	KU892470	KX838422
Datronia mollis (Sommerf.) Donk	RLG6304sp	JN165002	JN164791	JN164901
Datronia mollis	Dai11456	JX559253	JX559292	–
Datronia stereoides (Fr.) Ryvarden	Niemela3020	KC415178	KC415195	–
Datronia stereoides	Holonen	KC415179	KC415196	–
Diacanthodes cerebriporoides	**Robledo 1876**	**MK913639**	**MK913635**	–
Diacanthodes cerebriporoides	**Robledo 1891**	**MK913641**	**MK913637**	–
***Diacanthodes cerebriporoides* T**	**Robledo 3026**	**MK913642**	**MK913638**	**MK991767**

(*continued*)

Table 1. Continued.

Species	*Voucher*	*ITS*	*28S*	*TEF-1α*
***Diacanthodes neotropicalis* T**	**Palacio 105**	**MK913640**	**MK913636**	–
Diacanthodes coffeae	**TAAM 134226**	**MN366249**	–	–
Dichomitus squalens (P. Karst.) D.A. Reid	LE2588894	KM411455	KM411471	KM411486
Donkioporia expansa (Desm.) Kotl. & Pouzar	P185	AJ249501	AJ583428	–
Donkioporia expansa	P188	HM536087	HM536052	HM536103
Donkioporiella mellea L.W. Zhou	IFP LWZ 20140622-15	NR154014	NG059141	–
Donkioporiella mellea	LWZ 20140622-12	KX258957	KX258955	–
Earliella scabrosa (Pers.) Gilb. & Ryvarden	PR1209	JN165009	JN164793	JN164894
Echinochaete maximipora Sotome & T. Hatt.	WD2559	AB462314	AB462302	–
Echinochaete russiceps (Berk. & Broome) D.A. Reid	TFM F-24255	AB462318	AB462306	–
Flammeopellis bambusicola Y.C. Dai, B.K. Cui & C.L. Zhao	Dai 13443	KF698748	KF698759	KF725879
Fomes fomentarius (L.) Fr.	Isolate 7	FJ865440	JX470537	JX481269
Fomitella supina (Sw.) Murrill	JV0610	KF274645	KF274646	KJ410718
Fomitiporia mediterranea M. Fisch.	AFTOL-ID 688	AY854080	AY684157	AY885149
Foraminispora rugosa (Berk.) Costa-Rezende, Drechsler-Santos & Robledo	DHCR560	MF409963	MF409955	MF421241
Funalia gallica (Fr.) Bondartsev & Singer	FP91663T	JN165012	–	–
Funalia rigida (Berk. & Mont.) Peck	BJFC12680	KC867381	KC867454	–
Funalia sanguinaria (Klotzsch) Zmitr. & V. Malysheva	Cui 5444	KC867387	KC867463	–
Funalia trogii (Berk.) Bondartsev & Singer	RLG4286sp	JN164993	JN164808	JN164898
Ganoderma australe (Fr.) Pat.	DHCR 411	MF436675	MF436672	MF436677
Ganoderma lucidum (Curtis) P. Karst.	K 175217	KJ143911	–	KJ143929
Gautieria crispa E.L. Stewart & Trappe	OSC61308	–	DQ218484	DQ219244
Geastrum berkeleyi Massee	RGC06-168	–	KC581986	KC758620
Geastrum mirabile Mont.	TNS:KH-JPN10-711	JN845108	JN845226	–
Geastrum schweinitzii (Berk. & M.A. Curtis) Zeller	MA-Fungi 36141	KF988438	KF988568	–
Gelatoporia subvermispora (Pilát) Niemelä	H:Heikki Kotiranta 20823	FN907911	FN907911	–
Gomphus brunneus (Heinem.) Corner	BR034190-46	–	AY574680	–
Gloeophyllum trabeum (Pers.) Murrill	1320	HM536094	HM536067	HM536113
Grammothele sp.	BJFC004386	KX832048	KX832057	KX838433
Grammothele subargentea (Speg.) Rajchenb.	CBS 413.66	MH858842	MH870481	–
Grammothele fuligo (Berk. & Broome) Ryvarden	MUCL 45066	GQ355956	AJ406506	–
Grammothelopsis subtropica B.K. Cui & C.L. Zhao	Cui 9035	JQ845094	JQ845097	KF181124
Grammothelopsis subtropica	Cui 9041	JQ845096	JQ845099	KF181133
Guepiniopsis buccina (Pers.) L.L. Kenn	AFTOL-ID 888	DQ206986	AY745711	DQ028588
Haploporus cylindrosporus L.L. Shen, Y.C. Dai & B.K. Cui	Dai 15643	KU941853	KU941877	KU941940
Haploporus latisporus Juan Li & Y.C. Dai	Dai 11873	KU941847	KU941871	KU941934
Haploporus nepalensis (T. Hatt.) Piątek	Dai 12937	KU941855	KU941879	KU941942
Haploporus odorus (Sommerf.) Bondartsev & Singer	Dai 11296	KU941845	KU941869	KU941932
Haploporus septatus L.L. Shen, Y.C. Dai & B.K. Cui	Dai 13581	KU941843	KU941867	KU941930
Haploporus subpapyraceus L.L. Shen, Y.C. Dai & B.K. Cui	Dai 13580	KU941841	KU941865	KU941928

(*continued*)

Table 1. Continued.

Species	*Voucher*	*ITS*	*28S*	*TEF-1α*
Haploporus subtrameteus (Pilát) Y.C. Dai & Niemelä	Dai 4222	KU941849	KU941873	KU941936
Haploporus thindii (Natarajan & Koland.) Y.C. Dai	Cui 9373	KU941851	KU941875	KU941938
Haploporus tuberculosus (Fr.) *Niemelä & Y.C. Dai*	KA11	JX124705	–	JX109907
Haploporus tuberculosus	15559	KU941857	KU941881	–
Heterobasidion annosum (Fr.) Bref.	DAOM73191	–	AF287866	DQ028583
Hornodermoporus latissimus (Bres.) B.K. Cui & Y.C. Dai	Cui 6652/6625	HQ876604	JF706340	KF181134
Hornodermoporus martius (Berk.) (Berk.) Teixeira	Cui 7992	HQ876603	HQ654114	KF181135
Hydnum repandum L.	BB 07.341	–	KF294643	JX192980
Hysterangium aggregatum J.W. Cribb	H4262	–	DQ218489	DQ219146
Inonotus linteus (Berk. & M.A. Curtis) Teixeira	MUCL 47139	GU461973	GU462002	GU461936
Jaapia argillacea Bres.	CBS252.74	GU187524	GU187581	GU187711
Kavinia alboviridis (Morgan) Gilb. & Budington	O102140	–	AY574692	DQ219250
Lentinus badius (Berk.) Berk.	DED07668	KP283480	KP283518	–
Lentinus crinitus (L.) Fr.	DSH9243C	KP283495	KP283523	–
Lentinus squarrosulus Mont.	CUI6513	KP283482	KP283516	–
Lenzitopsis sp.	Yuan 2952 LZ 2011	JN169798	JN169794	–
Lepidostroma caatingae Sulzbacher & Lücking	Sulzbacher 1479	KC170320	KC170318	–
Lepidostroma calocerum (G.W. Martin) Oberw.	R05	–	FJ171737	–
Leptosporomyces raunkiaeri (M.P. Christ.) Jülich	CFMR: HHB-7628	GU187528	GU187588	GU187719
Lignosus hainanensis B.K. Cui	Dai 10670	NR154112	NG060261	–
Lignosus rhinocerotis (Cooke) Ryvarden	Benjamin143	–	KX900694	KX900833
Lignosus rhinocerotis	Pen94	JQ409359	AB368074	–
Lopharia cinerascens (Schwein.) G. Cunn.	FP105043sp	JN165019	JN164813	JN164900
Megasporia ellipsoidea (B.K. Cui & P. Du) B.K. Cui & Hai J. Li	Cui 13854	MK116483	MK116492	MK122981
Megasporia guangdongensis B.K. Cui & Hai J. Li	Cui 9130	NR120301	NG042651	MG867698
Megasporia hengduanensis B.K. Cui & Hai J. Li	Cui 8076	NR120104	NG042652	KF286337
Megasporoporia bannaensis B.K. Cui & Hai J. Li	Dai 12306	NR120300	NG042607	KF494979
Melanoderma microcarpum B.K. Cui & Y.C. Dai	Dai 8116	KF495002	KF495012	–
Melanoderma microcarpum	Dai 11521	HQ678174	HQ678176	KF482758
Microporellus violaceocinerascens (Petch) A. David & Rajchenb.	Cui8459	HQ654113	HQ876606	KF181136
Microporellus violaceocinerascens	MUCL 45229	FJ411106	FJ393874	–
Microporus affinis (Blume & T. Nees) Kuntze	Cui 8818	KX880614	KX880654	KX880874
Microporus subaffinis (Lloyd) Imazeki	Dai 11712	KX880616	KX880656	KX880876
Microporus vernicipes (Berk.) Kuntze	Dai 9283	KX880618	KX880658	KX880926
Neodictyopus sp.	CulTENN11501	AF516561	AJ487945	–
Neolentinus adhaerens (Alb. & Schwein.) Redhead & Ginns	DAOM 214911	–	HM536071	–
Neolentinus lepideus (Fr.) Redhead & Ginns	DAOM:208668	–	HM536077	HM536122
Pachykytospora wasseri Zmitr., Malysheva & Spirin	LE814872	KM411456	KM411472	KM411487

(*continued*)

Table 1. Continued.

Species	*Voucher*	*ITS*	*28S*	*TEF-1α*
Perenniporia hainaniana B.K. Cui & C.L. Zhao	Cui 6365	JQ861744	JQ861760	KF181139
Perenniporia gomezii Rajchenb. & J.E. Wright	Dai 13719	KX900674	KX900724	KX900851
Perenniporia medulla-panis (Jacq.) Donk	Cui 3274	JN112792	JN112793	KF181137
Perenniporia subacida (Peck) Donk	Cui 10053	KF495006	KF495017	KF286327
Perenniporia substraminea B.K. Cui & C.L. Zhao	Dai 10781	KF495007	KF495018	KF494983
Perenniporiella chaquenia Robledo & Decock	MUCL 47648	FJ411084	FJ393856	HM467610
Perenniporiella micropora (Ryvarden) Decock & Ryvarden	MUCL 43581	FJ411086	FJ393858	HM467608
Perenniporiella neofulva (Lloyd) Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852	HM467599
Perenniporiella pendula Decock & Ryvarden	MUCL 47129	FJ411082	FJ393854	HM467600
Perenniporiopsis minutissima (Yasuda) C.L. Zhao	Cui 10979	KF495003	KF495013	KF286310
Perenniporiopsis minutissima	Dai 11643	HQ876602	KF495015	KF286309
Phallus costatus (Penz.) Lloyd	MB02040	–	DQ218513	–
Phanerochaete chrysosporium Burds.	AFTOL-ID 776	AY854086	–	AY885155
Phlebia unica (H.S. Jacks. & Dearden) Ginns	KHL 11786	EU118657	EU118657	JX109889
Picipes badius (Pers.) Zmitr. & Kovalenko	Cui 10853	KU189780	KU189811	KU189929
Podoscypha venustula (Speg.) D.A. Reid	LR40821c	JX109851	JX109851	JX109910
Podoserpula sp.	ZJL2015015	KU324484	KU324487	–
Polyporellus arcularius (Batsch) Fr.	Cui 11398	KU189766	KU189797	KU189911
Polyporellus brumalis (Pers.) P. Karst.	Cui 10750	KU189765	KU189796	KU189910
Polyporellus ciliatus (Fr.) P. Karst.	Wei1582	KU189767	KU189798	KU189912
Polyporus grammocephalus Berk.	WD2351	AB587627	AB368090	–
Polyporus guianensis Mont.	CulTENN11288	AF516564	AJ487947	–
Polyporus leprieurii Mont.	CulTENN10489	AF516567	AJ487949	–
Polyporus squamosus (Huds.) Fr.	AFTOL 704	DQ267123	AY629320	DQ028601
Polyporus subvarius C.J. Yu & Y.C. Dai	Yu2	AB587632	AB587621	KU189924
Polyporus tubaeformis (P. Karst.) Ryvarden & Gilb.	WD1839	AB587634	AB368101	–
Polyporus tuberaster (Jacq. ex Pers.) Fr.	Dai 12462	KU507580	KU507582	KU507590
Polyporus umbellatus (Pers.) Fr.	Pen13513	KU189772	KU189803	KU189917
Polyporus varius (Pers.) Fr.	Dai 13874	KU189777	KU189808	KU189923
Polyporus virgatus Berk. & M.A. Curtis	CulTENN11406	AF516582	AJ488123	–
Porogramme albocincta (Cooke & Massee) Gibertoni	PR1478T	KY948725	KY948838 -	–
Porogramme albocincta	TL 9894/03	JX109854	–	–
Punctularia strigosozonata (Schwein.) P.H.B. Talbot	LR40885	AY463456	AY586702	–
Pyrofomes demidoffii (Lév.) Kotl. & Pouzar	MUCL 41034	FJ411105	FJ393873	–
Pyrofomes demidoffii	PRM869999	KY940249	KY940260	–
Pyrofomes juniperinus (H. Schrenk) Vlasák & Spirin	221923	KY940257	KY940265	–
Pyrofomes juniperinus	222395	KY940258	KY940264	–
Sclerogaster columellatus (Zeller) Fogel	Trappe8098	–	FJ435972	–
Skeletocutis yuchengii Miettinen & A. Korhonen	FBCC 1132	KY953056	KY953056	KY953109
Sparsitubus nelumbiformis L.W. Hsu & J.D. Zhao	Cui 8497	KX880631	KX880670	KX880887
Thelephora ganbajun M. Zang	ZRL20151295	LT716082	KY418908	KY419093
Thelephora vialis Schwein.	Thv1	–	AJ406478	–
Theleporus membranaceus Y.C. Dai & L.W. Zhou	Dai 12075	JN411120	KX880673	KX880889

(*continued*)

Table 1. Continued.

Species	*Voucher*	*ITS*	*28S*	*TEF-1α*
Trametes betulina (L.) Pilát	HHB9942sp	JN164983	JN164794	JN164895
Trametes gibbosa (Pers.) Fr.	L11664sp	JN164943	JN164800	JN164896
Trametes membranacea (Sw.) Kreisel	PRSC82	JN164945	JN164805	JN164893
Trechispora sp.	PBM418	DQ411534	AY647217	DQ059051
Truncospora detrita (Berk.) Ryvarden	MUCL 42649	FJ411099	FJ393866	–
Truncospora ochroleuca (Berk.)	MUCL 39563	FJ411097	FJ393864	–
Truncospora ornata Spirin & Bukharova	Cui 5714	HQ654103	HQ654116	KF181150
Tylopilus plumbeoviolaceus (Snell & E.A. Dick) Snell & E.A. Dick	MB06-056	–	KF030350	KF030439
Tyromyces chioneus (Fr.) P. Karst.	FD-4	KP135311	KP135291	–
Vanderbylia fraxinea (Bull.) D.A. Reid	Cui 8885	HQ876611	JF706344	KF286295
Vanderbylia robiniophila (Murrill) B.K. Cui & Y.C. Dai	Cui 5644	HQ876609	JF706342	KF181145
*Vanderbylia vicina (*Lloyd) D.A. Reid	MUCL 44779	FJ411095	FJ393862	–
Vanderbylia sp.	Dai 6891	JQ861738	KF495019	KF286293
Vuilleminia comedens (Nees) Maire	AFTOL-ID 1247	DQ398959	AF518666	–
Yuchengia narymica (Pilát) B.K. Cui, C.L. Zhao & K.T. Steffen	Dai 6998	JN048775	JN048794	KF181149

parsimony informative. Dataset 2 included sequences from 126 fungal specimens, with 3264 characters, of which 1611 were constant and 1101 parsimony informative. The evolutionary models selected for dataset 1 were SYM + G (ITS1), SYM + I + G (5.8S), GTR + G (ITS2), GTR + I + G (28S), GTR + I + G (TEF-1α 1st codon pos.), GTR + I + G (2nd codon pos.) and GTR + G (3rd codon pos.). For dataset 2 the selected models were GTR + I + G (ITS1), K80 + I + G (5.8S), GTR + G (ITS2), GTR + I + G (28S), GTR + I + G (TEF-1α introns), F81 + I + G (TEF-1α 1st codon pos.), GTR + I + G (2nd codon pos.) and GTR + G (3rd codon pos.).

The topology recovered in our phylogenetic analysis of the Agaricomycetes showed that *Diacanthodes* is positioned in the 'core polyporoid' clade within Polyporales (Fig. 1). The phylogenetic analysis of the extended set of taxa of the 'core polyporoid' clade (Fig. 2a–b) is overall consistent with previous results (Binder et al., 2013; Justo et al., 2017) and *Diacanthodes* species were recovered as monophyletic within the 'ganoderma' clade with maximum support (1/100). However, its affinities within 'ganoderma' clade remained uncertain. *Diacanthodes* grouped with *Yuchengia* B.K. Cui & Steffen and *Perenniporia subacida* (Peck) Donk with weak support (0.87/52). The morphological analyses of the sequenced specimens of *Diacanthodes*, in addition to the phylogenetic evidence, showed that they represented two new morphospecies, and will hence be described below in the Taxonomy section. In the light of these results, taxonomic implications on the broad concept of *D. novo-guineensis* and its heterotypic synonyms will also be discussed.

Taxonomy

Diacanthodes Sing. Lloydia 8:141, 1945.

Description. Basidiomata annual, solitary, stipitate, pileus circular, centrally depressed to flat, flexible when fresh, brittle when dry, pilear surface tomentose to hispid or strigose at the centre, whitish, brownish yellow to ochraceous brown, darkening when dry, context heterogeneous with an upper layer lax and darker than the lighter and dense lower layer; pores circular, angular to labyrinthic. Hyphal system dimitic, generative hyphae clamped, skeletal hyphae thick walled to solid, hyaline; cystidia present in some species, clavate, thick-walled; basidiospores subglobose, ellipsoid to drop-shaped or lacrymoid, hyaline to yellowish, slightly thick-walled, ornamented with warts, conical, columnar or rounded and blunt, often elongated or fused and forming short crests, dextrinoid; chlamydospores present in some species, in the pilear surface and/or context subglobose, thick walled, yellowish brown to brown. Growing on the ground.

Type species. *Diacanthodes philippinensis* (Pat.) Singer, Lloydia 8(3): 141 (1945).

Remarks. The genus is characterized by the unique morphological combination of ornamented dextrinoid basidiospores and solitary stipitate basidiomata growing out of soil. The genus is phylogenetically placed in the 'core polyporoid' clade, however its affinities with other genera are uncertain.

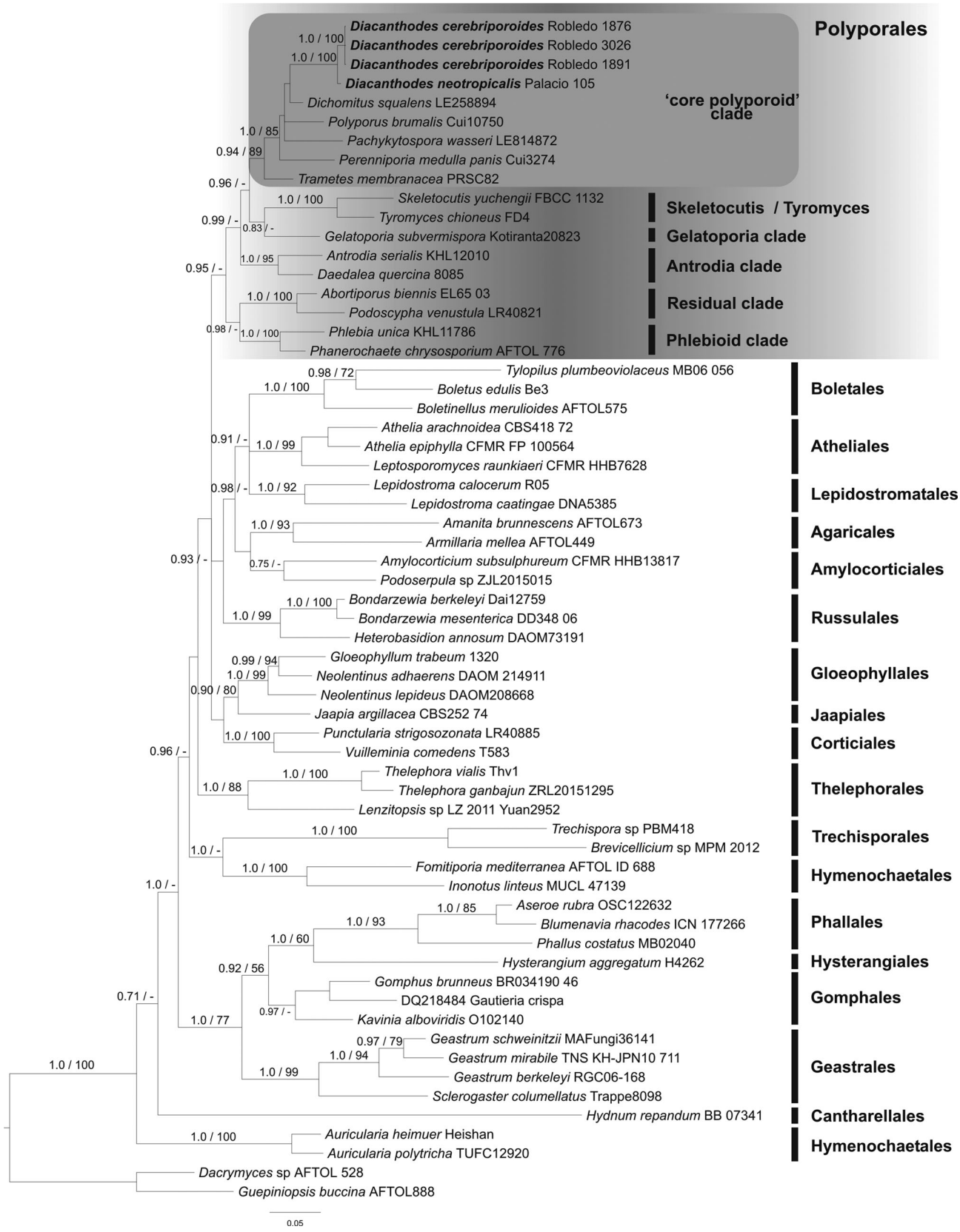


Fig. 1. Bayesian tree (BI) positioning *Diacanthodes* among Agaricomycetes based on concatenated ITS, 28S, and TEF-1α sequence data. Bayesian posterior probability above 0.7 and bootstrap values above 50% are shown.

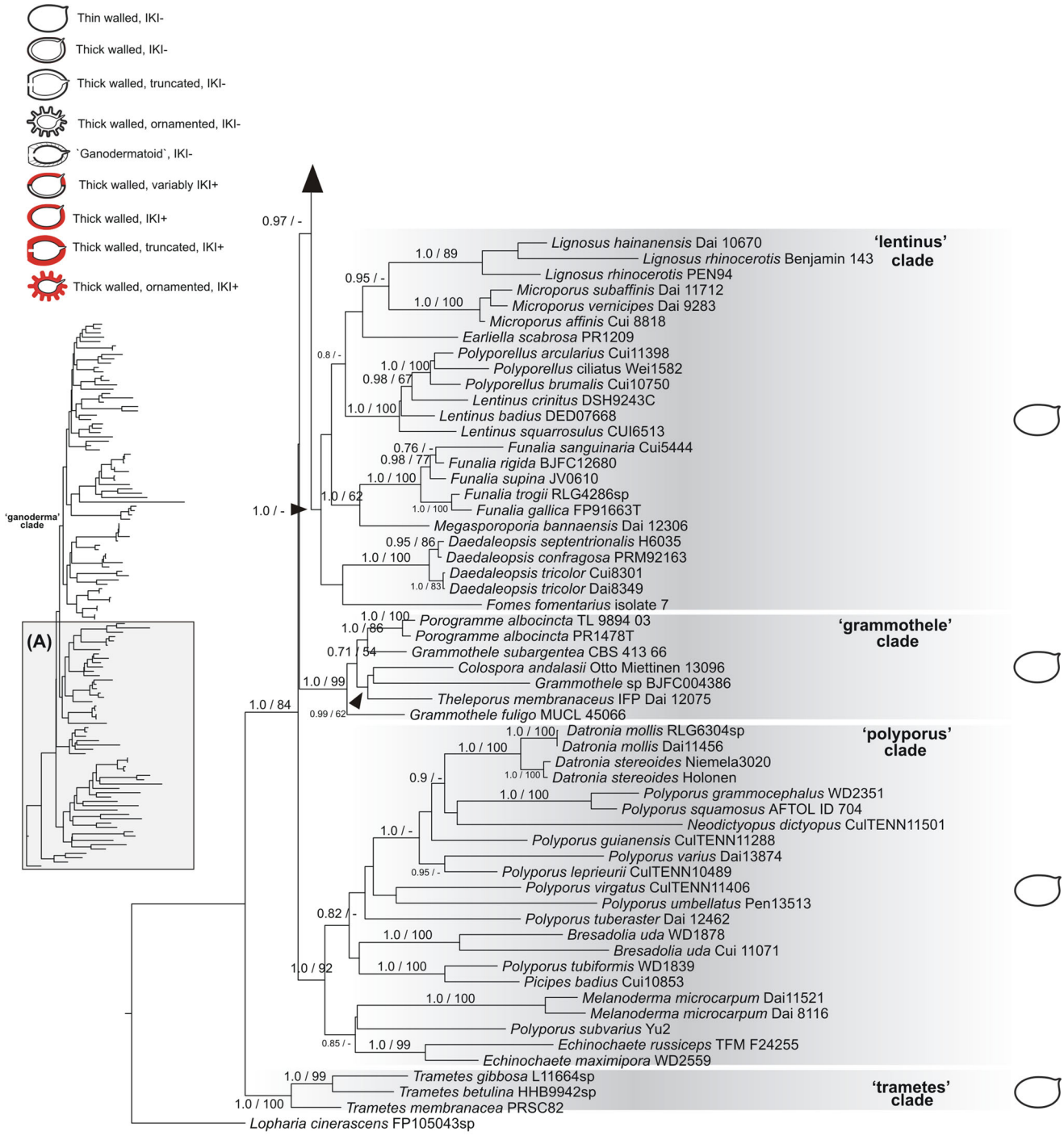


Fig. 2. Maximum likelihood (ML) tree positioning *Diacanthodes* among the 'core polyporoid' clade based on concatenated ITS, 28S, and TEF-1α sequence data. Bayesian posterior probability above 0.7 and bootstrap values above 50% are shown. Basidiospore morphology is schematized for each clade, red colour indicates dextrinoid reaction (IKI + in the graphic legend). * *Perenniporia gomezii* (thick-walled, dextrinoid with a germ pore) grouped inside *Megasporia* (thin-walled).

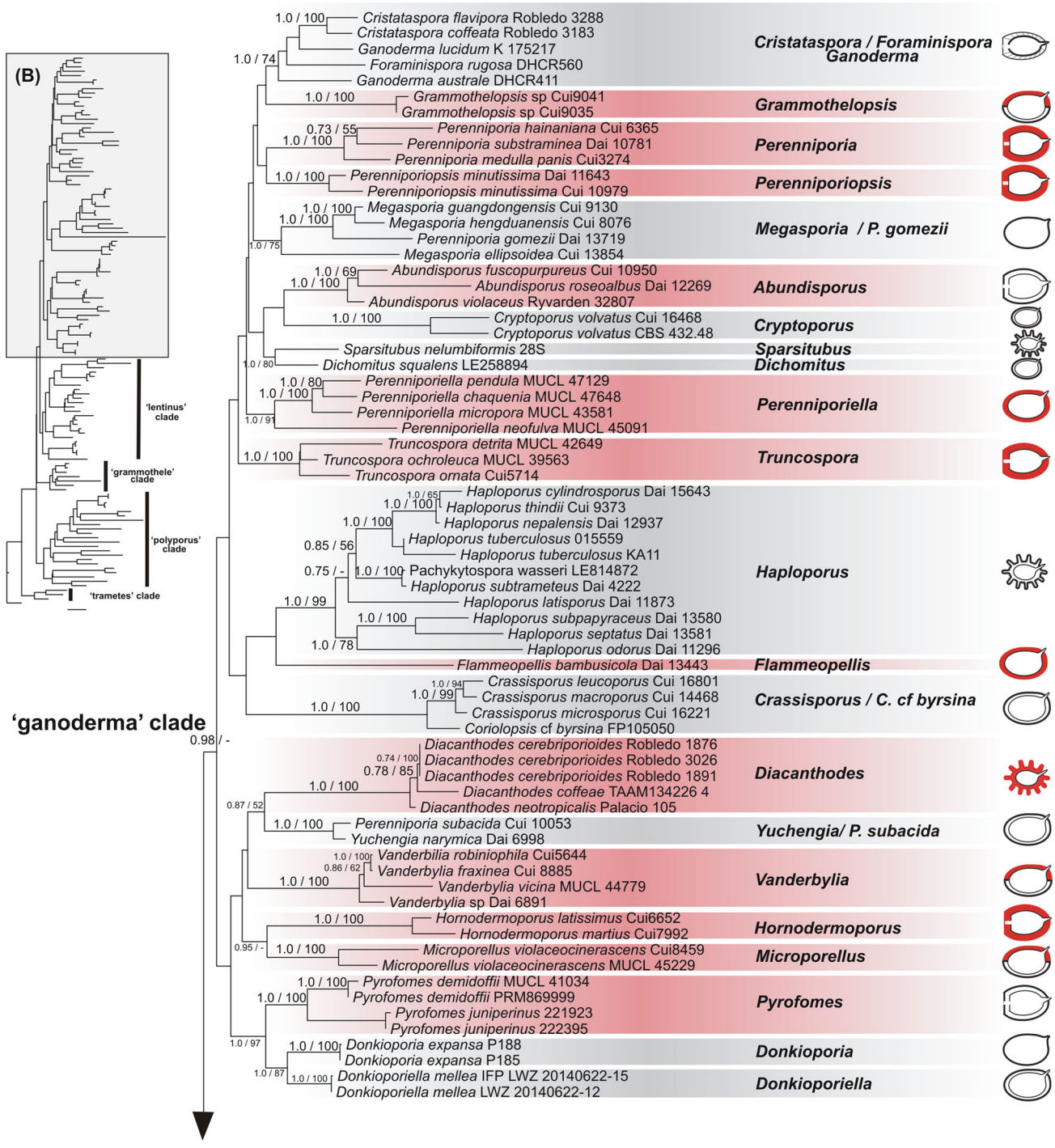
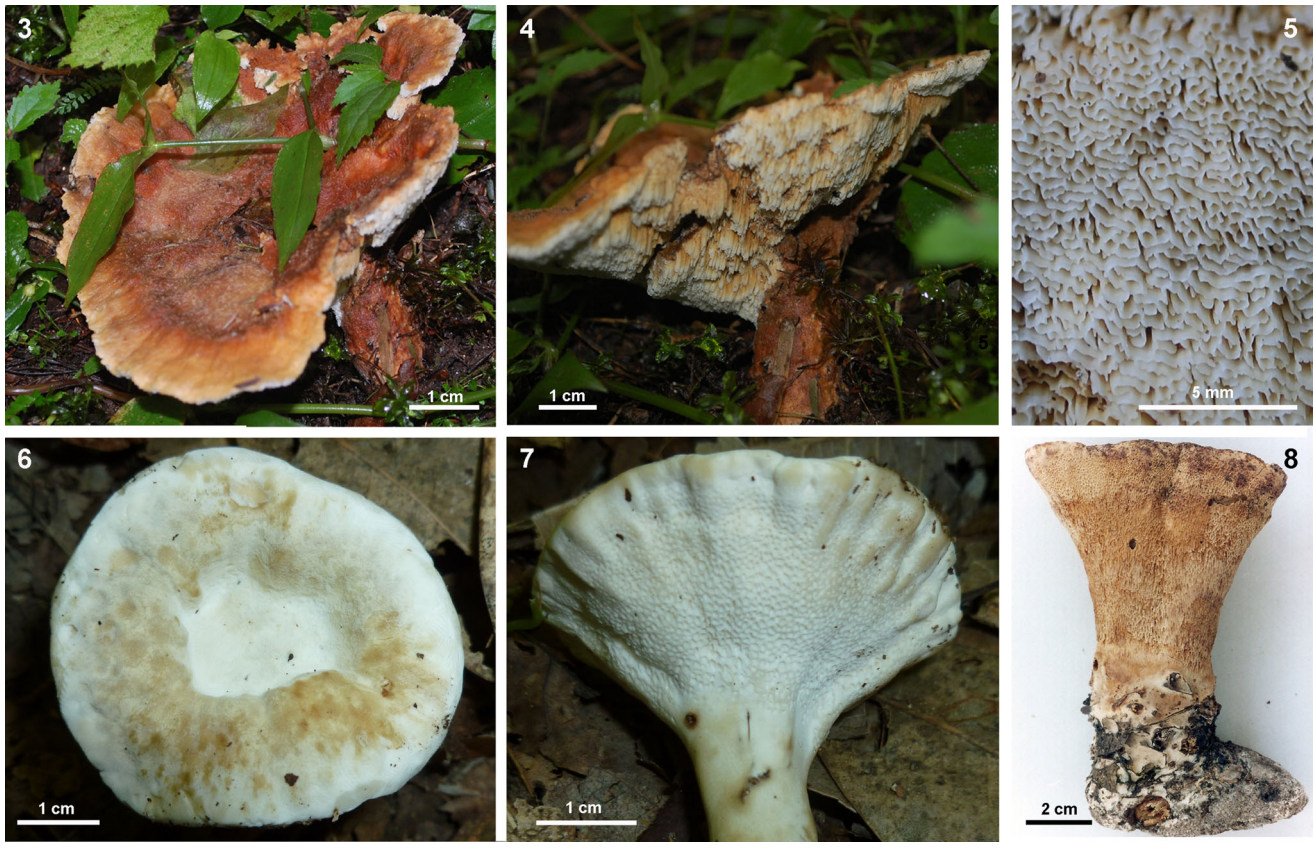


Fig. 2. Continued.

Diacanthodes cerebriporoides Robledo & Urcelay sp. nov.
Figs 3–5

MycoBank accession. MB830988.

Typification. ARGENTINA. Catamarca: La Merced, on the ground in a montane forest with Myrtaceae, 18 Feb 2016, *Robledo 3026* (holotype, CORD) 28°06′06″S, 65°36′58″O, 1000 m asl on the ground, GenBank accession numbers: ITS = MK913642, 28S = MK913638, TEF-1α = MK991767.



Figs 3–8. Macroscopic features of *Diacanthodes* species. 3–5, *Diachantodes cerebriporoides* (Holotype, *Robledo 3026*). 3–4, general view *in situ*. 5, detail of the pore surface showing the labyrinthic shape. 6–8, *Diachantodes neotropicalis*. 6–7, general view *in situ* (Holotype, *Palacio 105*). 8, general view (*O. Popoff 178*).

Etymology. Referring to the brain-like irregular configuration of the hymenophore.

Description. Macroscopic characters: basidiomata annual, solitary, central to laterally stipitate, sometimes shortly stipitate to sessile, single; pileus circular, flat to depressed in the centre, 12 cm in diameter and 0.8 cm thick, often reniform to flabelliform, flexible when fresh, then the tissue agglutinate becoming dense to resinous, and brittle when dried; pileus surface glabrous to finely tomentose, light brown azonate when fresh to light brown to ochraceous brown and glabrous when drying, base rugulose; margin acute, decurved when dry; pore surface cream, yellowish to whitish toward the margin when fresh, and brownish ochraceous to blackish when drying; pores angular, labyrinthic, 4–5 per mm, pores collapsing and shrinking when drying; dissepiments entire; tubes agglutinated, resinous and friable when drying, up to 0.5 cm long, concolorous with pore surface; context heterogeneous, zonate, with a lower layer dense and cream-coloured, and an upper layer lax and darker, often with a black resinous middle layer up to 0.3 cm thick. Stipe cylindrical, mostly circular in section, central to eccentric, short and robust, straight, up to 2.5 cm long and 1 cm wide, with a wider nodular partially buried base, occasionally with a very short stipe and the pileus arising from the nodular base. Stipe surface with presence of decurrent pores in the upper part, smooth, whitish to pale brown when fresh, concolorous with the pilear surface, darkening when dry. Context zonate, whitish in the centre, light brown towards the surface, corky, slightly more fibrous and cottony to the centre. Microscopic characters: hyphal system dimitic, IKI-, generative hyphae hyaline with clamps, mostly thin-walled in the trama to slightly thick-walled to the context, clamp-connections double, and swollen up to 15 µm diam. Skeletal hyphae hyaline, thick-walled, 4–6 µm diameter, straight to sinuous, abundant on the trama and context, and dominating in the stipe. Cystidia absent. Basidia not observed. Basidiospores ellipsoid $5-6\times4-5$ µm, finally ornamented with rounded and blunt warts to elongated and forming short crests, hyaline to yellowish, thin to slightly thick-walled, dextrinoid, mature spores deposited in the pilear surface present a stronger dextrinoid reaction than those present in the tubes. Chlamydospores absent.

Ecology and distribution. Forming basidiomata on soil from a nodular base; known from Yungas Mountain rain forests, north-western Argentina, from where it was previously reported as *D. novo-guineensis* (Robledo & Rajchenberg, 2007; Rajchenberg & Robledo, 2013).

Specimen studied. ARGENTINA, Salta: Parque Nacional El Rey, Los Lobitos, 9 Mar 2005, 24°41′42″S, 64°36′43″O, 895 m asl, from soil, *Robledo 612* (CORD). Ibid., 25 Mar 2007, 24°41′40″S, 64°36′43.7″O, 876 m asl, from soil, *Robledo 1066* (CORD). Jujuy: Parque Nacional Calilegua, Sendero Pedemontano, 2 Apr 2008, 23°45′18″S, 64°51′13″O, 720 m asl, from soil, *Robledo 1891, 1876* (CORD). Ibid., Sendero Guaraní, 24 Mar 2011, 23°45′66.1″S, 64°51′15.0″W, 627 m asl, from soil, *Niveiro 2252, 2253* (CTES).

Diacanthodes coffeae (Wakef.) Robledo, K. Põldmaa & Ryvarden comb. nov.
[MB#833321]

Basionym. *Polyporus coffeae* Wakef., Bulletin of Miscellaneous Information (Royal Botanical Gardens, Kew) 1917: 308, 1917 [MB#185071].

Remarks. *Polyporus coffeae* was described from Uganda growing on the roots of coffee trees, hence its name. Due to the economic importance of the diseases it causes, the species has frequently been reported, as *D. novo-guineensis*, *Diacanthodes* sp. or as *Polyporus coffeae*. Our morphological analyses showed that the African specimen examined has basidiospores of similar size to those observed in the holotypes of *Polyporus coffeae* and *D. novo-guineensis* (Table 2), although chlamydospores and cystidia were not observed. The phylogenetic analyses showed that *D. coffeae* presents an intermediate position between the Neotropical species, grouped with *D. cerebriporoides* that also lacks chlamydospores.

Specimens examined: MALAWI. Mulanje Mountains Forest Reserve, Lichenja plateau, 1800–2000 m asl, 15°58′S, 35°30′E, 10 Mar 1973, *Ryvarden 11293* (TAAM 134226).

Diacanthodes neotropicalis Palacio & Robledo **sp. nov.**
Figs 6–14

MycoBank accession. MB 830968.

Typification. COLOMBIA. CESAR: Valledupar, Santuario de Vida Silvestre Los Besotes, Tropical Dry Forest, 10°34′30.9″N, 73°16′ 61.6″W, 692 m asl, 15 Sep 2012, *Palacio 105* (holotype, HUA185578), GenBank accession numbers: ITS = MK913640, 28S = MK913636.

Etymology. Referring to the neotropical region from where it is currently known.

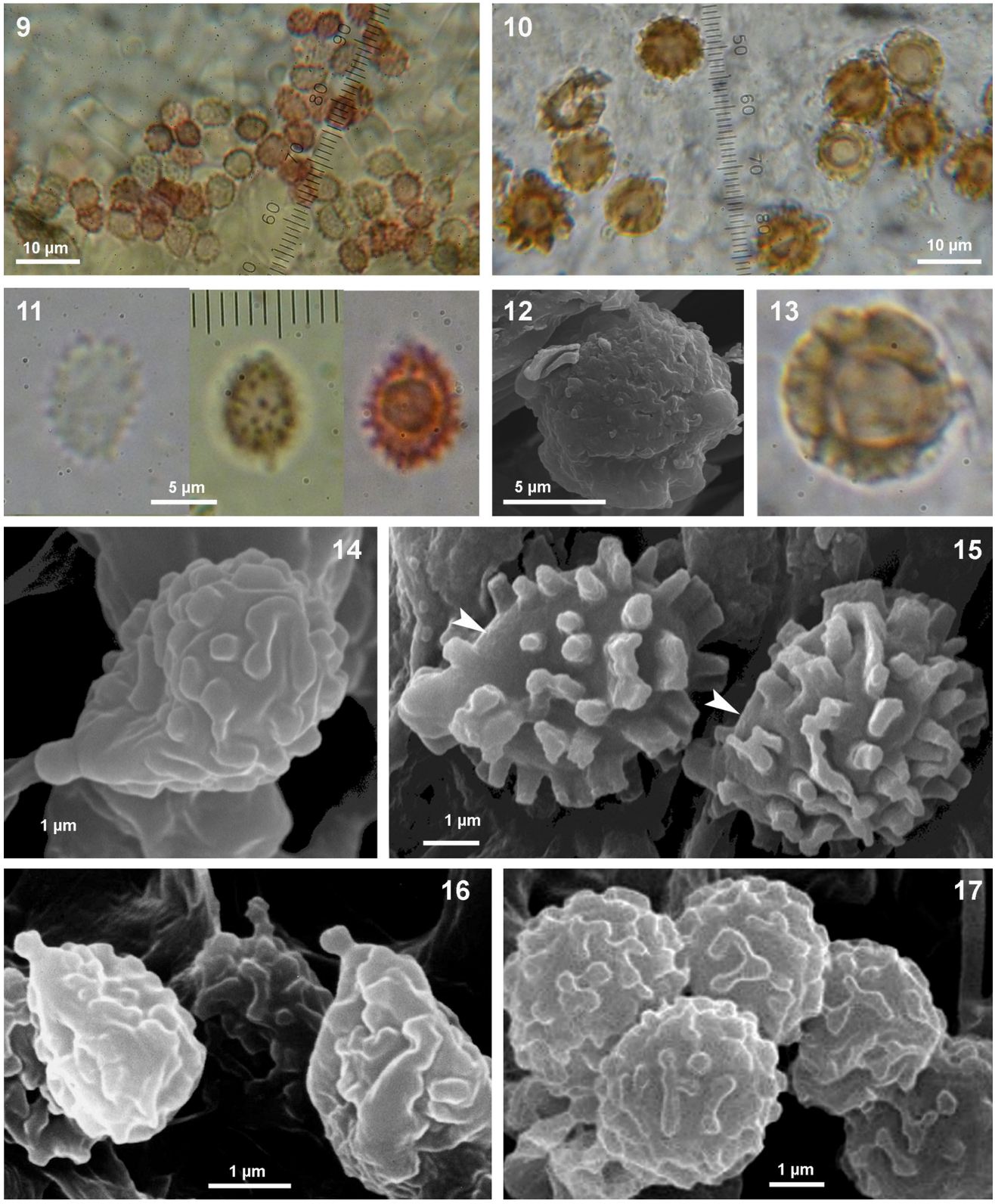
Description. Macroscopic characters: basidiomata centrally stipitate, solitary, fleshy when fresh, brittle when dry; pileus up to 7 cm diameter, circular to slightly infundibuliform, glabrous to velvety, white to pale brownish yellow; pore surface white to pale brownish yellow, pores circular to angular, 2–3 per mm, slightly elongated and irregular towards the stipe, dissepiments entire; margin rounded, sterile; stipe up to 3 cm long, circular in section up to 1 cm diameter, slightly swollen at the base, glabrous to velvety, yellowish to pale chestnut; context 0.4 cm thick, duplex, upper part yellowish cream, lower part white; tubes concolorous with the context. Microscopic characters: Hyphal system dimitic, IKI-; generative hyphae $3-6(-9)$ µm diameter, with clamps, hyaline, thin to thick-walled; skeletal hyphae $4-5(-8)$ µm diam, hyaline to yellowish, straight to tortuous, thick-walled to almost solid. Cystidia clavate, $20-36\times6-8(-10)$ µm, hyaline, slightly thick-walled, IKI-. Basidia clavate, $25-35\times6.5-9.5$ µm, 4-spored, sterigma up to 7 µm long. Basidiospores ellipsoid, broadly ellipsoid, to drop-shaped or lacrymoid $(6.0-)6.5-7.5\times(4.5-)5.0-6.5$ µm, hyaline, slightly thick-walled, under the light microscope ornamented with columns up to 1 µm, ornamentation absent in the suprahilar depression area, dextrinoid, the reaction is stronger as the basidiospores mature. Under the SEM the ornamentation is observed as short and blunt warts, after the treatment with chromic acid, discrete columns, occasionally merging to form short crests, are seen indicating that there is a hyaline layer, i.e., 'exosporium', covering the ornamentations, see Discussion. Chlamydospores subglobose $(7-)11-12\times(6.5-)10-11$ µm, thick walled, brown to yellowish brown, with irregular ornamentations, abundant in the pilear surface but also present in context.

Additional specimens examined. ARGENTINA. Corrientes: Riachuelo, 28 Jun 1987, *O. Popoff 178* (BAFC). Ibid., Concepción, Tabay, 28 Mar 1975, *M. Arbo 1063* (BAFC). PARAGUAY. Central: Asunción, 9 Jun 1973, *A. Schinini 12168* (BAFC, CTES).

Remarks. The study of specimens previously reported as *D. novo-guineensis* from NE Argentina and Paraguay (Atlantic rain forest and gallery forest) (Popoff & Wright, 1998, Popoff, 2000) showed the same macromorphological characters as observed in the Colombian specimen. Microscopically they share the presence of cystidia, morphology of basidiospores and the presence of chlamydospores. Chlamydospores of the Colombian specimens are larger ($11-12\times10-11$ µm) than those in the specimens from NE Argentina and Paraguay

Table 2. Morphological comparison of *Diacanthodes* species. **T**= type, n/d = no data.

Species Type locality	Reference (Voucher)	Basidiospores (size μm, shape)	Chlamydospores	Cystidia	Pores (size /mm, shape)
D. cerebriporoides Argentina	This study (**T**)	5 – 6 × 4 – 5 μm, ellipsoid	Absent	Absent	4 – 5 /mm, labyrinthic
D. coffeae Uganda	Fidalgo (1962) (**T**)	5.5 – 7.5 × 4 – 5.5 μm, ellipsoid	Absent	14 – 18 × 4 – 7 μm, thick-walled	n/d
	This study (TAAM 134226)	6 – 6.5 × 4 – 4.5 μm, ellipsoid	Absent	Not seen	2 – 4/mm, circular to angular to irregular shaped
D. fluminensis Brazil	Corner (1989) (**T**)	6.5 – 8 x 5.5 – 7 μm, broadly ellipsoid to subglobose,	Absent	Absent	150 – 250 μm wide, subcircular, more or less entire, dissepiments 40 – 110 μm thick, cream white.
D. griseus Malaysia	Corner (1989) (**T**)	5 – 6.5 × 4 – 4.5 μm, ellipsoid subglobose	Absent	Absent	medium size, subangular
D. neotropicalis Colombia	This study (**T**)	6.5 – 7.5 × 5.0 – 6.5 μm, ellipsoid to drop shaped or lacrimoid	(7–)11 – 12 × (7–)10 – 11 μm, globose, thick walled, with irregular ornamentations, brownish	26.4 – 36 × 6.4 – 8 μm, slightly thick-walled	2 – 3/mm, pores circular to irregular
D. novo-guineensis Papua New Guinea	Fidalgo (1962) (**T**)	6 – 7 × 4 – 5.5 μm, ellipsoid	5 – 7 × 3.5 – 5.5 μm, elliptic to subglobose	15 – 18 × 5.5 – 8.5 μm, thick-walled	n/d
	Quanten (1997) (**T**)	Not observed	5.6 – 6.3 × 4.2 – 5.4 μm, broadly ellipsoid to subglobose	n/d, thin walled except for the thickened apex	n/d
D. philippinensis Philippines	Patouilllard (1915) (**T**)	7 – 9 × 5 – 6 μm, ovoid to subglobose	n/d	Absent	n/d, angular, acute, lacerate, dentate, decurrent.
	Fidalgo (1962) (**T**)	5.5 – 7 × 4.5 – 6 μm	n/d	Absent	n/d



Figs 9–17. Microscopic features of *Diacanthodes neotropicalis* species. 9–15, Holotype, *Palacio 105*. 9, 11. Basidiospores under light microscopy mounted on Melzer's reagent. 10, 12–13, Chlamydospores. 14–15, Basidiospores under SEM, 14, general view showing ornamentation and apiculus, 15, after treatment with chromic acid, white arrows indicate suprahilar depression. 16–17, Basidiospores under SEM (*O. Popoff 178*).

(7 – 7.5 × 6.5 – 7 µm. Sequences could not be obtained for NE Argentina and Paraguay specimens.

Notes on *Diacanthodes* species:

Diachanthodes fluminensis Corner
Beihefte zur Nova Hedwigia 96: 24 (1989) [MB#136482]

Type specimens. Holotype: BRAZIL, Rio de Janeiro, Niteroi, Saco de São Francisco, 7 Mar. 1948, leg. E. J. H. Corner (E).

Remarks. The species is known only from the type specimen. The description provided by Corner (1989) regarding colour, size, shape and texture of basidiomata are reminiscent of *D. cerebriporioides*. However, the pores are described as subcircular and not as irregular as in *D. cerebriporoides*. Microscopically, *D. fluminensis* is characterized by particular generative hyphae described by Corner (1989) as 'producing lobes, obtuse processes or swellings, or inflating locally -15 mm wide, the walls thickening 1 – 2 µm in the old tissue in the lower part of the stem, the swellings and lobings forming bizarre structure …' (p. 24), characteristics that have not been observed in the other species. Basidiospores are broadly ellipsoid to sublglobose, 6.5 – 8 × 5.5 – 7 µm. Corner (1989) suggested it could be conspecific with *Polyporus asterosporus* Torrend, also described from Brazil. However, *Polyporus asterosporus* has smaller basidiospores (Fidalgo, 1962).

Diachantodes griseus Corner
Beih. Nova Hedwig. 96: 25, 1989 [MB#136483]

Type specimens. Holotype: MALAYSIA, Pahang, Tembeling, 10 Nov. 1930, leg. E. J. H. Corner (E).

Remarks. The species is known only from the type. It was described with densely and coarsely hispid-strigose pilear surface with erect fascicles of hyphae up to 3 mm long in the centre of the pileus, and the pilear surface and context with a particular livid grey colour. Corner (1989) described it as 'becoming very brittle in dried material, crumbling and impossible to tease apart, the walls swelling and becoming very hyaline in potash and Melzer's iodine …' (p. 25). Corner (1989) also described a variety [*D. griseus var. subglaber* Corner Beihefte zur Nova Hedwigia 96: 26, 1989, MB#136596; Holotype: MALAYSIA, Pahang, Tembeling, 10 Nov 1930] distinguished based on the lateral and attenuated upwards stipe, matt pilear surface, and the dried tissue not becoming brittle.

It has been suggested that *D. griseus* is a greyish form of *D. novo-guineensis* (Hattori, 2001). However, the particular macromorphology described above and the absence of cystidia (see Discussion) suggest that *D. griseus* could be considered an independent species. The relationship with *D. philippinensis*, that also lacks cystidia and is present in the same area, should be investigated through molecular analyses.

Diacanthodes novo-guineensis (Henn.) O. Fidalgo
Rickia 1: 149, 1962 [MB#536411]

Remarks. The species was described originally from Papua New Guinea as *Polyporus novoguineensis* Henn. It was transferred to *Diacanthodes* and a broad concept of the species was established including several synonyms (Fidalgo, 1962). Cystidia are reported as clavate and thin-walled (Quanten, 1997) or thick-walled (Fidalgo, 1962).

Diacanthodes philippinensis (Pat.) Singer
Lloydia 8: 141, 1945 [MB#286062]

Remarks. In the original description basidiospores were reported as 7 – 9 × 5 – 6, notably different from measurements later provided by Fidalgo (1962) (Table 2). The type specimen does not have cystidia, and chlamydospores were not reported. Molecular data are needed for specimens from Oceania in order to study their possible conspecificity with *D. novo-guineensis*.

Discussion

Basidiospore morphology and phylogenetic affinities of *Diacanthodes*

This study clarified the species delimitation and phylogenetic affinities of *Diacanthodes*. Our results revealed that *Diacanthodes* belongs to the 'core polyporoid' clade of Polyporales (Fig. 1); hence, affinities with the Russulales such *Bondarzewia* or *Heterobasidion* (Russulales) and *Abortiporus* ('residual clade', Polyporales) are ruled out. Within the 'core polyporoid' clade *Diacanthodes* was placed in the 'ganoderma' clade, where most of the affinities among genera remained uncertain. Therefore, the previously suggested affinities of *Diacanthodes* with *Pachykytospora* (Fidalgo, 1962; Gilbertson & Ryvarden, 1986; Ryvarden & Johansen, 1980; Singer, 1945; Quanten, 1997) should be further investigated.

The use of scanning electron microscopy (SEM) has been a useful tool in the study of Agaricomycetes with complex basidiospores such as Boletales and Russulales (Lee et al., 2017; Van de Putte et al., 2012; Wu et al., 2014), and taxa with double-walled basidiospores, such as Ganodermataceae species (Costa-Rezende et al., 2017, 2020 and references therein). By combining the

use of traditional optical microscopy and SEM, we suggest that *Diacanthodes* species have double-walled basidiospores consisting of an inner thick and ornamented endosporium, and a thin exosporium (*D. neotropicalis*, Figs 14–15). Corner (1989) suggested that basidiospores of *D. griseus* have a hyaline exosporium, which is in agreement with our observations. From a phylogenetic perspective, this could suggest that *Diacanthodes* could be related to the species traditionally classified in Ganodermataceae. However, as previously mentioned, the affinities among genera are unresolved. Transmission electron microscopy (TEM) analysis of the *Diacanthodes* basidiospore is needed to obtain a better understanding of its structure, as showed by Furtado (1962) for ganodermatoid polypores.

Basidiospore morphology has been traditionally used as an important character for taxonomy and to speculate about phylogenetic affinities among polypore species and genera (Ryvarden, 1991). Basidiospore features, i.e. wall thickness, ornamentation, dextrinoid reaction and presence of a germ pore, have a particular distribution and correlation with lineages of the 'core polyporoid' clade (schematized basidiospores, Fig. 2). Taxa presenting basidiospores with a dextrinoid reaction and/or a complex structure, i.e. thickened simple or a double wall, germ pore and/or ornamentation, are grouped in the 'ganoderma' clade where *Diacanthodes* is placed. This pattern of grouping/relationships of taxa with thin-walled hyaline basidiospores in a basal position and those with thick-walled basidiospores in a more derived group has been previously reported for Agarics (Garnica et al., 2007). Our results support the fact that the basidiospore morphology is a useful phylogenetic marker and hence has taxonomic value at genus level, as have been proposed for agarics (Garnica et al., 2007). In this scenario, we suggest that polypore taxa that present complex basidiospores (thick-walled and/or double walled, ornamented, presence of a germ pore, and the combination of these characters) would be related to the 'ganoderma' clade. This could be the case for *Phaeotrametes decipiens* (Berk.) J.E. Wright, a monospecific genus that has thick-walled brownish basidiospores, truncated at the apex with a germ pore (Robledo & Urcelay, 2009). The relationships of genera in the 'ganoderma' clade need further investigation, including analyses of additional protein-coding genes and studying additional ultrastructural characters, such as ornamentations and the ontogeny of the germ pores, by SEM and TEM.

Species diversity within Diacanthodes

The original description of *Diacanthodes* was based on the Australasian species *D. philippinensis* from the Philippines (Patouillard, 1915; Singer, 1945). Later, *D. novo-guineensis*, originally described from Papua New Guinea was included in the genus, and several taxa, including the type species, were then considered under synonymy of *D. novo-guineensis* in a broad morphological concept (Fidalgo, 1962). Following that broad morphological concept, several records of *D. novo-guineensis* have been reported from different parts of America, from southern USA (Gilbertson & Ryvarden, 1986), northern Mexico (Esqueda-Valle et al., 1999), Colombia (Setliff & Ryvarden, 1983; Vasco-Palacios & Franco-Molano, 2013), Brazil (Ryvarden & Meijer, 2002), Paraguay (Popoff & Wright, 1998), and northern Argentina (Robledo & Rajchenberg, 2007; Rajchenberg & Robledo, 2013). It was also reported from Africa (Ryvarden & Johansen, 1980).

The studied specimens from South America and Africa, initially identified as *D. novo-guineensis*, and descriptions of the type specimens of *Diacanthodes*, were shown to be morphologically heterogeneous, mainly in the basidiospore size, and in the presence/absence of cystida and chlamydospores (Table 2). Moreover, three lineages were recognized within *Diacanthodes* in the phylogenetic analysis – two from South America (*D. cerebriporoides* and *D. neotropicalis*) and one (*D. coffeae*) from Africa. All these three species differ in their morphology, that also distinguishes them from *D. novo-guineensis* based on the morphology of the type specimen (Table 2). Other species, still considered as synonyms of *D. novo-guineensis* should be further studied, as *Abortiporus subabortivus* Murrill (south-eastern USA) or *Polyporus asterosporus* Torrend (northern Brazil) to verify their status as distinct taxa.

To date, known *Diacanthodes* species are restricted to a tropical-subtropical distribution (Australasia, Africa, and America). As sequences available from only three species in two regions are available (*D. cerebroides* and *D. neotropicalis* from South America and *D. coffeae* from Africa), the phylogenetic affinities between species from distant biogeographic regions warrant further research.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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