

# Resolving the phylogenetic placement of *Porobeltraniella* and allied genera in the *Beltraniaceae*

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**Abstract** The circumscription of *Beltraniaceae* has thus far been based on the unique morphological characters of its genera, although these assumptions have never been tested phylogenetically because of a lack of DNA sequence data. The Indian Western Ghats is rich with diverse strains of *Beltrania* and similar genera, and therefore an attempt was made to re-collect as many species as possible, to study their taxonomy, and to resolve their phylogeny based on LSU and ITS nrDNA sequences. This study provides the first phylogenetic placement of genera *Beltraniella*, *Porobeltraniella*, *Pseudobeltrania* and *Hemibeltrania* in the *Beltraniaceae* based on fresh specimens, type studies and molecular

phylogenetic analyses. Furthermore, *Subramaniomyces* appears to be phylogenetically related to beltranioid genera, and is therefore included in *Beltraniaceae*. However, the phylogenies of the hyphomycete genus *Parapleurotheciopsis* and the perithecial genus *Pidoplitchkoviella* appear to change with data selection and phylogenetic analytical method used, and remain unresolved.

**Keywords** Asexual Ascomycota · *Beltrania* · *Beltraniella* · Phylogeny · *Pseudobeltrania* · Taxonomy · *Xylariales*

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## Introduction

The genus *Beltrania* was established with the type species *B. rhombica* found on *Citrus limonum* by Penzig (1882) in Italy. This genus was described with distinguishing characters such as setae with radially lobed basal cells, conidiophores with separating cells and biconic conidia with a hyaline transverse band and apical tubular appendage. Subsequently, Saccardo (1886) established the tribe *Beltranieae* within the didymosporous *Dematieae* Fr. (or *Dematiaceae* Fr. sensu Saccardo 1889) to accommodate *Beltrania* Penzig, represented by two species, *B. rhombica* Penzig and *B. querna* Harkness. Hennings (1902) shortly thereafter added *Pseudobeltrania* to this complex, emphasizing the absence of setae and separating cells and the presence of radially lobed basal cells and biconic conidia with a hyaline transverse band. While working on leaf litter of *Odina wodier* from India, Subramanian (1952) proposed the genus *Beltraniella*, typified by *B. odinae* Subram., with distinctive lageniform conidia with a truncate base, rostrate apex, and a hyaline transverse band at the equatorial zone. Later, Batista and Bezerra (1960) established the genus *Beltraniopsis* with type species *B. esenbeckiae* Bat. & J.L. Bezerra (on dead

leaves of *Esenbeckia macrocarpa* from Brazil), characterized by setiform conidiophores, radially lobed basal cells, separating cells and biconic conidia with an obtuse base, rostrate apex, and a hyaline transverse band at the equatorial zone. Manoharachary et al. (2003) erected a monotypic *Beltraniomyces* from India for *B. lignicola* Manohar., D.K. Agarwal & Rao, a fungus with unbranched conidiophores and scarred rather than denticulate conidiogenous loci. Gusmão (Fernando and Gusmão 2004) proposed the genus *Porobeltraniella*, typified by *P. porosa* (Piroz. & S.D. Patil) Gusmão, including two *Beltraniella* species known only from India, distinguished by the conspicuous pores around the equatorial part of the conidia and the setiform extension of conidiophores.

The illustrated monograph of Pirozynski (1963) on *Beltrania* and related genera introduced a broader concept for this group, emphasizing the aseptate, biconic conidia in all genera, and considered it a natural group. He unified the *Beltrania* complex, including *Beltrania*, *Beltraniella* Subram. (*Ellisiopsis* Batista & Nascimento), *Beltraniopsis* Batista & Bezerra, and *Pseudobeltrania* Hennings, and further added *Hemibeltrania*, typified by *H. cinnamomi* Deighton, and the synnematous genus *Rhombostilbella* Zimm., typified by *R. rosea* Zimm., under *Beltraniaceae*.

Kendrick (1980), as part of an overview of generic concepts in hyphomycetes, provided a definition of polythetic genus concepts that stabilized the *Beltrania* complex; he stated that genera in the *Beltrania* complex must have at least three major characters in common, such as dark setae, setae or conidiophores with radially lobed bases, swollen separating cells, biconic conidia and conidia with a hyaline equatorial band. Considering Kendrick's recommendations, *Hemibeltrania* and *Rhombostilbella* were excluded from the *Beltrania* complex. Seifert et al. (2011) provided the most recent key to the beltranioid complex including nine genera.

In this study, samples collected from different microhabitats of the Western Ghats were subjected to morphological and molecular comparison for authentication. This paper aims to re-evaluate the morphological characteristics and establish the phylogenetic placement of beltranioid genera in the family *Beltraniaceae*.

## Materials and methods

Conidia were directly isolated from infected leaves and leaf litter observed under a Nikon stereomicroscope (model SMZ1500 with Digital camera; Nikon, Tokyo, Japan). Single conidial cultures were established on 2 % potato dextrose agar (PDA) and 2 % malt extract agar (MEA; HiMedia, Mumbai, India) plates. For taxonomic studies and photomicrographs, a ZEISS Axio Imager 2 microscope (Carl Zeiss, Oberkochen, Germany) was used. Conidia and conidiophores were

mounted in lactic acid cotton blue and measured using AxioVision version 4.8 software, with 30 observations per structure. Culture characteristics were studied on MEA and PDA (Crous et al. 2009). Colony colours were determined using the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978). Herbarium specimens were deposited in the Ajrekar Mycological Herbarium (AMH), and the derived cultures were accessioned and preserved in the National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India.

## DNA extraction, amplification, and phylogenetic analyses

Colonies were grown on MEA plates, and genomic DNA was extracted following the rapid salt extraction method of Aljanabi and Martinez (1997). Fragments of the large subunit nuclear ribosomal DNA (LSU) gene and internally transcribed spacer region or ITS 1-5.8S nrDNA-ITS 2 (ITS) regions were amplified using primer pairs LROR (Rehner and Samuels 1994) and LR7 (Vilgalys and Hester 1990) for LSU and primer pairs ITS5 and ITS4 (White et al. 1990) for ITS. PCR conditions, sequence alignments, and phylogenetic analyses (Bayesian and parsimony analyses for LSU, parsimony only for ITS) followed the protocols of Crous et al. (2006). To create the LSU phylogeny, reference sequences of the *Sordariomycetes* were downloaded from GenBank from the studies of Jaklitsch et al. (2016) and Hernández-Restrepo et al. (2016). *Neofabraea kienholzii* (GenBank KR858873; *Dermateaceae*, *Helotiales*, *Leotiomyces*) was used as the outgroup sequence. The ITS alignment was created by adding sequences obtained through MegaBLAST searches of the NCBI GenBank nucleotide database of the cultures sequenced in the present study, and by downloading sequences from the NITE Biological Resource Centre, Japan, database. Many ITS sequences from GenBank are from the Thomas et al. (2016) study. The robustness of the parsimony trees was evaluated by 1000 bootstrap replicates (Hillis and Bull 1993), and statistics were calculated for tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC). Sequence data were deposited in GenBank. Details of isolates and accession numbers validated in the phylogenetic analyses of family *Beltraniaceae* are given in Table 1.

## Results

### Phylogenetic analyses

The overall LSU phylogeny consisted of 65 sequences, including the outgroup sequence. A total of 741 characters including alignment gaps were included; 230 characters were parsimony-informative, 75 were variable and parsimony-uninformative, and 436 characters were constant. A total of 102

**Table 1** Isolates and accession numbers validated in the phylogenetic analyses of family *Beltraniaceae*. Isolates in **bold** were isolated/sequenced in the present study

Species	Strain accession no.	Country/Locality	Substrate	GenBank no.		References
				ITS	LSU	
<i>Acrocordiella occulta</i>	RS9	Austria	<i>Ribes petraeum</i>	–	KT949893	Jaklitsch et al. 2016
<i>Amphibambusa bambusicola</i>	MFLUCC 11-0617	Chiang Rai, Thailand	On dead culm of <i>Bambusa</i>	–	KP744474	Liu et al. 2015
<i>Anthostomella eucalyptorum</i>	CBS 120036	Indonesia	<i>Eucalyptus</i> sp.	–	DQ890026	Crous et al. 2006
<i>Apiospora bambusae</i>	ICMP 6889	Coromandel, New Zealand	<i>Bambusa</i>	–	DQ368630	Tang et al. 2007
<i>Apiospora setosa</i>	ATCC 58184	New Zealand	<i>Bambusa</i> sp.	–	AY346259	Huhndorf et al. 2004
<i>Arecophila bambusae</i>	HKUCC 4794	Philippines	On dead culm of <i>Bambusa</i>	–	AF452038	Jeewon and Edward 2003
<i>Arthrinium phragmites</i>	CPC 18900	Bomarzo, Italy	Culms of <i>Phragmites australis</i>	–	KF144956	Crous and Groenewald 2013
<i>Arthrinium saccharicola</i>	CBS 463.83	Harderbos, Netherlands	Dead culms of <i>Phragmites australis</i>	–	KF144968	Crous and Groenewald 2013
<i>Balansia hemingsiana</i>	AEG96-27a	Georgia, USA	<i>Panicum</i> sp.	–	AY489715	Castlebury et al. 2004
<i>Beltrania pseudorhombica</i>	CPC 23656	Beijing, China	<i>Pinus tabulaeformis</i> needles	KJ869158	KJ869215	Crous et al. 2014
<i>Beltrania pseudorhombica</i>	JSP 01–10 A 1.2	Sao Paulo, Brazil	<i>Atta capiguara</i> nest	KR093912	–	Pereira et al. 2016
<i>Beltrania querna</i>	ICMP:15825	Auckland, New Zealand	<i>Quercus ilex</i> dead leaf	EF029240	–	Cooper 2006 unpubl.
<i>Beltrania querna</i>	NBRC 7543	Unknown	Unknown	00754301	00754301	–
<i>Beltrania querna</i>	NBRC 6884	Unknown	Unknown	00688401	00688401	–
<i>Beltrania querna</i>	NBRC 32637	Spain	<i>Quercus</i> dead leaf	03263701	03263701	–
<i>Beltrania querna</i>	BCRC 34620	Taiwan	Unknown	GU905994	–	Chen 2010 unpubl.
<i>Beltrania rhombica</i>	NBRC 8857	Unknown	Unknown	00885701	00885701	–
<i>Beltrania rhombica</i>	NBRC 100226	Chiba, Japan	Litter	10022601	10022601	–
<i>Beltrania rhombica</i>	NBRC 100558	Ishikawa, Japan	<i>Abies firma</i> needles	11055001	11055001	–
<i>Beltrania rhombica</i>	<b>CPC 27482</b>	Malaysia	<i>Acacia crassipes</i> leaves	KX519515	KX519521	This paper
<i>Beltrania rhombica</i>	DWM34	Yunnan, China	Unidentified plant	KM357317	–	Zhou 2014 unpubl.
<i>Beltrania</i> sp.	RV-2015 isolate 114.2.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Myrcia fallax</i> endophyte	KP133187	–	Thomas et al. 2016
<i>Beltrania</i> sp.	RV-2015 isolate 114.1.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Myrcia fallax</i> endophyte	KP133186	–	Thomas et al. 2016
<i>Beltrania</i> sp.	RV-2015 isolate 37.4.2	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133184	–	Thomas et al. 2016
<i>Beltraniella carolinensis</i>	111.3.3	Reserva Los Cedros, Imbabura, Ecuador	<i>Nectandra lineatifolia</i> endophyte	KP133179	–	Thomas et al. 2016
<i>Beltraniella carolinensis</i>	111.4.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Nectandra lineatifolia</i> endophyte	KP133173	–	Thomas et al. 2016
<i>Beltraniella carolinensis</i>	NBRC 9502	Unknown	<i>Persea borbonia</i> leaf	00950202	00950202	Unknown
<i>Beltraniella carolinensis</i>	111.1.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Nectandra lineatifolia</i> endophyte	KP133174	–	Thomas et al. 2016
<i>Beltraniella carolinensis</i>	Voucher 9502 (IFO)	Southeastern Forest Exp. Sta., USDA	<i>Persea borbonia</i> leaf	–	DQ810233	Bahl 2006 unpubl.
<i>Beltraniella endiandrae</i>	CPC 22193	New South Wales, Australia	<i>Endiandra introrsa</i> leaves	KJ869128	KJ869185	Crous et al. 2014

**Table 1** (continued)

Species	Strain accession no.	Country/Locality	Substrate	GenBank no.		References
				ITS	LSU	
<i>Beltraniella japonica</i>	NBRC 30443	Kagoshima, Japan	<i>Quercus phillyraeoides</i> litter	03044301	03044301	–
<i>Beltraniella odinae</i>	NBRC 6774	Unknown	Unknown	00677401	00677401	–
<i>Beltraniella portoricensis</i>	BCRC 34590	Taiwan	Unknown	GU905993	–	Chen 2010 unpubl.
<i>Beltraniella portoricensis</i>	NBRC 30666	Miyazaki, Japan	Oak tree litter	03066601	03066601	–
<i>Beltraniella portoricensis</i>	NBRC 9079	Unknown	Unknown	00907901	00907901	–
<i>Beltraniella portoricensis</i>	CX1	Canelones, Uruguay	<i>Eugenia uruguayensis</i> fresh fallen leaves	KU212349	–	García-Laviña et al. 2016
<i>Beltraniella portoricensis</i>	<b>NFCCI 3993</b>	Kerala, India	<i>Mangifera indica</i> fallen leaves	KX519516	KX519522	This paper
<i>Beltraniella</i> sp.	37.2.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133182	–	Thomas et al. 2016
<i>Beltraniella</i> sp.	37.1.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133183	–	Thomas et al. 2016
<i>Beltraniella</i> sp.	37.2.2	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133176	–	Thomas et al. 2016
<i>Beltraniella</i> sp.	37.4.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133180	–	Thomas et al. 2016
<i>Beltraniella</i> sp.	37.3.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133175	–	Thomas et al. 2016
<i>Beltraniella</i> sp.	37.3.2	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133178	–	Thomas et al. 2016
<i>Beltraniopsis neolitiseae</i>	CPC 22168	New South Wales, Australia	<i>Neolitsea australiensis</i> leaves	KJ869126	KJ869183	Crous et al. 2014
<i>Beltraniopsis</i> sp.	RV-2015 isolate 37.3.3	Reserva Los Cedros, Imbabura, Ecuador	<i>Persea pseudofasciculata</i> endophyte	KP133172	–	Thomas et al. 2016
<i>Beltraniopsis</i> sp.	RV-2015 isolate 27.3.1	Reserva Los Cedros, Imbabura, Ecuador	<i>Myrcia fallax</i> endophyte	KP133171	–	Thomas et al. 2016
<i>Cainia desmazieri</i>	CAI	Spain	<i>Telina monspessulana</i>	–	KT949896	Jaklitsch et al. 2016
<i>Cainia graminis</i>	CBS 136.62	Rocca Maima, France	<i>Sesleria albicans</i>	–	AF431949	Lumbsch et al. 2002
<i>Ciliochorella castaneae</i>	HHUF:28799	Hachimandai-shi, Japan	<i>Cercidiphyllum japonicum</i> leaves	–	AB433277	Endo et al. 2008
<i>Clypeosphaeria mamillana</i>	CLM	France	<i>Cornus alba</i>	–	KT949897	Jaklitsch et al. 2016
<i>Collodiscula japonica</i>	CJ	Jiu Xi, China	Dead culms of <i>Phyllostachys</i>	–	JF440974	Jaklitsch and Voglmayr 2012
<i>Cryptovalsa rabenhorstii</i>	CreI	–	<i>Hippocrepis emeris</i>	–	KC774567	Jaklitsch et al. 2014
<i>Diaporthe eres</i>	AR3538	Austria	<i>Acer campestre</i>	–	AF408350	Castlebury et al. 2002
<i>Diaporthe phaseolorum</i>	FAU458	Mississippi, USA	<i>Caperonia palustris</i>	–	AY346279	Huhndorf et al. 2004
<i>Diatrype disciformis</i>	AFTOL-ID 927	Netherlands	<i>Alnus</i> sp. decayed wood	–	DQ470964	Spatafora et al. 2006
<i>Diatrype palmicola</i>	MFLUCC 11-0020	Chiang Rai Province, Thailand	On dead branch of <i>Caryota urens</i>	–	KP744482	Liu et al. 2015

**Table 1** (continued)

Species	Strain accession no.	Country/Locality	Substrate	GenBank no.		References
				ITS	LSU	
<i>Gelasinospora tetrasperma</i>	CBS 178.33	Hudson Bay, Manitoba	Dung of <i>Lagopus</i> sp.	–	DQ470980	Spatafora et al. 2006
<i>Harknessia viterboensis</i>	CPC 10843	Italy	<i>Eucalyptus</i> leaves	–	JQ706242	Crous et al. 2012
<i>Harknessia weresubiae</i>	CPC 17670	Victoria, Australia	<i>Eucalyptus</i> leaf litter	–	JQ706244	Crous et al. 2012
<i>Hemibeltrania cinnamomi</i>	<b>NFCCI 3997</b>	Kerala, India	<i>Cinnamomum malabattrum</i> leaves	KX519517	KX519523	This paper
<i>Hemibeltrania cinnamomi</i>	<b>NFCCI 3695</b>	Kerala, India	<i>Cinnamomum malabattrum</i> leaves	KT119564	KT119565	This paper
<i>Hemibeltrania cinnamomi</i>	CL12WA	Malaysia	<i>Cinnamomum iners</i>	JQ621881	–	Refaei and Santhanam unpubl.
<i>Idriella lunata</i>	CBS 112028 = INIFAT CO2/71-1	Corcovado, Brazil	Decaying leaf	–	KP858984	Hernandez-Restrepo et al. 2016
<i>Leiosphaerella lycopodina</i>	PL	Oberösterreich, St. Willibald, Grosser Salletwald, Austria	Stalks of <i>Lycopodium annotinum</i>	–	JF440975	Jaklitsch and Voglmayr 2012
<i>Leiosphaerella praeclara</i>	LP	Oberösterreich, St. Willibald, Grosser Salletwald, Austria	Twigs of <i>Vaccinium myrtillus</i>	–	JF440976	Jaklitsch and Voglmayr 2012
<i>Lepteutypa fückelii</i>	LEF	Belgium	<i>Tilia cordata</i>	KT949902	–	Jaklitsch et al. 2016
<i>Lepteutypa sambuci</i>	RS	UK	<i>Sambucus nigra</i>	–	KT949904	Jaklitsch et al. 2016
<i>Lepteutypa uniseptata</i>	Voucher 6349 (HKUCC)	Shing Mun Reservoir, N.T., Hong Kong	Unknown wood	–	DQ810219	Bahl 2006 unpubl.
<i>Microdochium lycopodium</i>	CBS 109398	Germany	On <i>Phragmites australis</i>	–	KP858941	Hernandez-Restrepo et al. 2016
<i>Microdochium phragmitis</i>	CBS 285.71	Poland	On <i>Phragmites australis</i>	–	KP858949	Hernandez-Restrepo et al. 2016
<i>Nectria cinnabarina</i>	CBS 255.47	Netherlands	<i>Ulmus</i> stem	–	GQ505997	Hirooka et al. 2010
<i>Neoclaviceps monostipa</i>	INBio 6-141	Limón Province, Costa Rica	Unknown panicoid grass	–	AF245293	Sullivan et al. 2001
<i>Neofabraea kienholzii</i>	CBS 126461	USA	<i>Malus domestica</i>	–	KR858873	Chen et al. 2015
<i>Neopestalotiopsis protearum</i>	CPC 1765	Zimbabwe	Living leaves of <i>Leucospermum cuneiforme</i>	–	JN712564	Crous et al. 2011
<i>Parapleurotheciopsis inaequiseptata</i>	MUCL 41089	Brazil	Rotten leaf	–	EU040235	Crous et al. 2007
<i>Pestalotiopsis knightiae</i>	CBS 114138	New Zealand	<i>Knightia</i> sp.	–	KM116227	Maharachchikumbura et al. 2014
<i>Pestalotiopsis malayana</i>	CBS 102220	Malaysia	Stem of <i>Macaranga triloba</i> colonized by ants	–	KM116238	Maharachchikumbura et al. 2014
<i>Phlogicylindrium eucalyptorum</i>	CBS 111689	Australia	<i>Eucalyptus nitens</i>	–	KF251708	Quaedvlieg et al. 2013
<i>Phlogicylindrium uniformae</i>	CBS 131312	New South Wales, Australia	<i>Eucalyptus cypellocarpa</i> leaves	–	JQ044445	Crous et al. 2011
<i>Pidoplitchkoviella terricola</i>	CBS 180.77	Kiev region, Ukraine	<i>Quercus rubra</i> rhizosphere	–	AF096197	Suh and Blackwell 1999
<i>Porobeltraniella porosa</i>	<b>NFCCI 3994</b>	Mulshi, Maharashtra, India	<i>Gnetum ula</i> leaf litter	KX519518	KX519524	This paper
<i>Porobeltraniella porosa</i>	<b>NFCCI 3995</b>	Mulshi, Maharashtra, India	<i>Gnetum ula</i> leaf litter	KX519519	KX519525	This paper
<i>Porobeltraniella porosa</i>	<b>NFCCI 3996</b>	India	<i>Gnetum ula</i> leaf litter	KX519520	KX519526	This paper
<i>Pseudapiospora corni</i>	PCO	Austria	<i>Cornus alba</i>	–	KT949907	Jaklitsch et al. 2016

**Table 1** (continued)

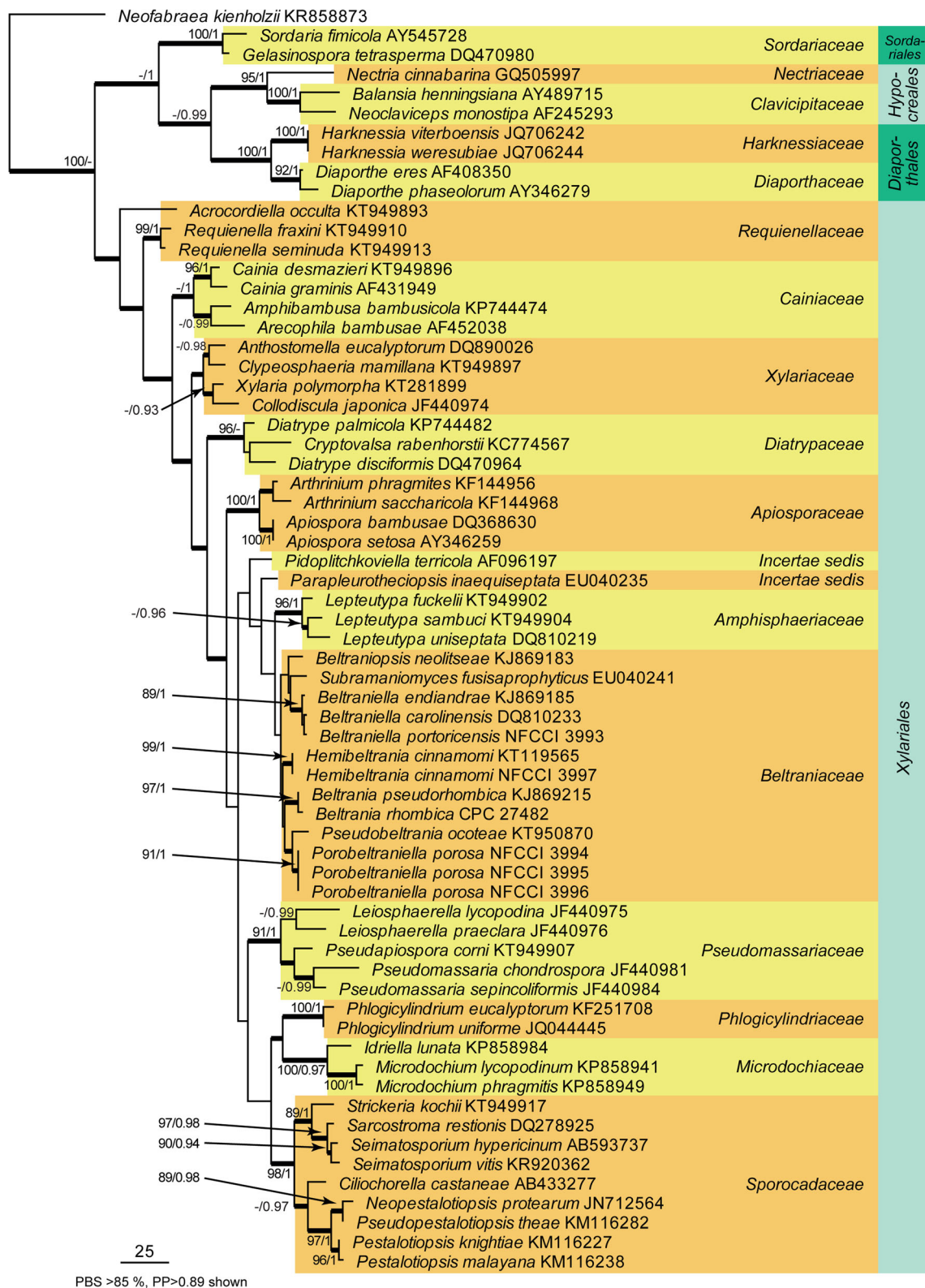
Species	Strain accession no.	Country/Locality	Substrate	GenBank no.		References
				ITS	LSU	
<i>Pseudobeltrania ocoteae</i>	<b>CPC 26219</b>	La Reunion, France	<i>Ocotea obtusata</i> leaves	KT950856	KT950870	Crous et al. 2015
<i>Pseudomassaria chondrospora</i>	PC	Botanical Garden of University of Vienna, Austria	Corticated branch of <i>Tilia</i> sp.	–	JF440981	Jaklitsch and Voglmayr 2012
<i>Pseudomassaria sepincoliformis</i>	PS	Niederösterreich, Giesshübl, Kleiner Sattelberg, Austria	<i>Rosa</i> sp.	–	JF440984	Jaklitsch and Voglmayr 2012
<i>Pseudopestalotiopsis theae</i>	MFLUCC 12-0055	Thailand	<i>Camellia sinensis</i>	–	KM116282	Maharachchikumbura et al. 2014
<i>Requienella fraxini</i>	RS3	Norway	<i>Fraxinus excelsior</i>	–	KT949910	Jaklitsch et al. 2016
<i>Requienella seminuda</i>	RS13	Greece	<i>Olea europaea</i>	–	KT949913	Jaklitsch et al. 2016
<i>Sarcostroma restionis</i>	CBS 118153	Jonkershoek, South Africa	<i>Ischyrolepis</i> cf. <i>sieberi</i> dead culm	–	DQ278925	Lee et al. 2006
<i>Seimatosporium hypericinum</i>	NBRC 32647	Switzerland	<i>Hypericum</i> sp.	–	AB593737	Tanaka et al. 2011
<i>Seimatosporium vitis</i>	MFLUCC:14-0051	Italy	Dead stem of <i>Vitis vinifera</i>	–	KR920362	Senanayake et al. 2015
<i>Sordaria fimicola</i>	Carolina Biological Supply Company 15-6291	–	–	–	AY545728	unpubl.
<i>Strickeria kochii</i>	C138	Austria	<i>Robinia pseudoacacia</i>	–	KT949917	Jaklitsch et al. 2016
<i>Subramaniomyces fusisaprophyticus</i>	CBS 418.95	Cuba	Leaf litter	–	EU040241	Crous et al. 2007
<i>Xylaria polymorpha</i>	MUCL 49884	France	Decaying wood <i>Quercus</i> sp.	–	KT281899	Senanayake et al. 2015

equally most parsimonious trees were obtained, the first of which is shown in Fig. 1 (TL = 1 193, CI = 0.381, RI = 0.688, and RC = 0.262). In the Bayesian analysis, the LSU partition had 287 unique site patterns, and the analysis ran for 1,545,000 generations, at which point the average standard deviation of split frequencies fell below 0.01, resulting in 30,902 trees, of which 23,178 trees were used to calculate the posterior probabilities that are mapped onto Fig. 1. The partition was analysed using dirichlet (1,1,1) state frequency distribution and inverse gamma-shaped rate variation across sites (GTR+I+G).

In the LSU phylogeny, the family *Beltraniaceae* Nann. (Nannizzi 1934) remained united, but neither the parsimony nor the Bayesian analysis provided statistical support. However, all genera represented by more than one sequence were monophyletic and well-supported, with the exception of *Beltrania*, which was less well-supported in the parsimony analysis. The genera *Beltraniopsis*, *Subramaniomyces* and *Beltrania* clustered together without support, as did *Pseudobeltrania* and *Porobeltraniella*. Although the same general terminal clades were obtained between the Bayesian and parsimony phylogenies, the backbone order of the families was incongruent (data not shown, see TreeBASE). In particular, the phylogeny

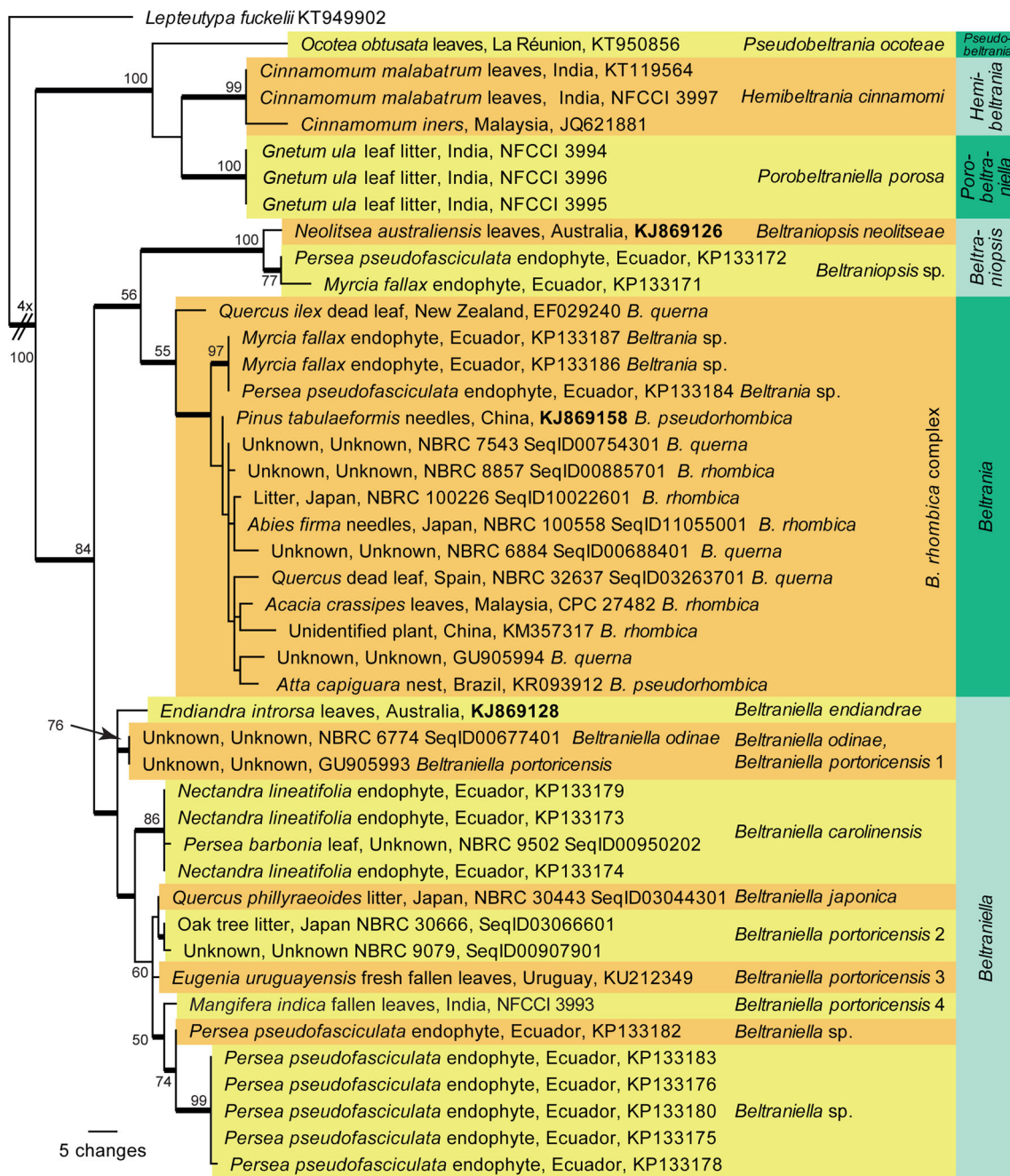
obtained with MrBayes resulted in a tree that was not congruent with other studies (for example, the *Amphisphaeriaceae* represented by *Lepteutypa* clustered inside the *Beltraniaceae*, and the *Microdochiaceae* moved to the most basal position in the tree, even outside the other included orders). It is quite possible that this was caused by homoplasy in the dataset (HI = 0.619), because phylogenies based on smaller and larger numbers of included LSU sequences resulted in the same phenomena. Interestingly, the phylogenetic position of *Pidoplitchkoviella terricola* and *Parapleurotheciopsis inaequiseptata* seems to vary with the number and diversity of included sequences and the kind of phylogenetic method used. Depending on the analysis, these species in some cases were associated with the *Beltraniaceae* and in others were not; *Subramaniomyces fusisaprophyticus* was always associated with this family.

The ITS phylogeny consisted of 44 sequences, including the outgroup sequence. A total of 497 characters including alignment gaps were included in the phylogenetic analyses; 89 characters were parsimony-informative, 57 were variable and parsimony-uninformative, and 361 characters were constant. The maximum of 1000 equally most parsimonious trees were obtained, the first of which is shown in Fig. 2 (TL = 258,



**Fig. 1** The first of 102 equally most parsimonious trees obtained from a heuristic search with 25 random taxon additions of the LSU sequence alignment of selected families in the Xylariales. The scale bar represents the number of changes; bootstrap support values of the parsimony analysis (MP-BS; >85 %) and Bayesian posterior probability values (BPP; >0.89) are shown at the nodes (MP-BS/BPP). Genera and

families are indicated to the right of the tree in blocks of different colours. Thickened lines represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Neofabraea kienholzii* (GenBank KR858873; *Dermateaceae*, *Helotiales*, *Leotiomycetes*)



**Fig. 2** The first of the maximum of 1000 equally most parsimonious trees obtained from a heuristic search with 25 random taxon additions of the ITS sequence alignment of genera in the *Beltraniaceae*. The *scale bar* represents the number of changes, and bootstrap support values of the parsimony analysis are shown at the nodes. Species and genera are indicated to the right of the tree in blocks of different colours, and known ex-type cultures are printed in **bold**. Strains are indicated with

isolation source, country of isolation, culture and/or sequence accession number and, in some cases, the species name under which the sequence was downloaded. *Thickened lines* represent those branches present in the parsimony strict consensus tree. The tree was rooted to *Lepteutypa fuckelii* (GenBank KT949902; *Amphisphaeriaceae*, *Xylariales*, *Sordariomycetes*)

CI = 0.674, RI = 0.870, and RC = 0.587). Although all beltranioid genera recognised here reflect the LSU topology depicted in Fig. 1, several clades comprising those genera were weakly supported at their basal nodes, e.g. *Beltrania* and *Beltraniella*. In the ITS phylogeny, *Pseudobeltrania* and *Porobeltraniella* clustered together with *Hemibeltrania* in a

well-supported clade. Isolates of *Beltrania* clustered in a poorly supported clade, and based on the ITS data, it was not possible to distinguish species in this genus, with isolates labeled as *Beltrania querna* and *Beltrania rhombica* being intermixed. *Beltraniella* appears to represent a species-rich genus, although not all internal nodes were well-supported.



*Beltraniella portoricensis* clustered at several positions in the phylogenetic tree, and sequences from type or authentic material are needed to fix its phylogenetic position.

## Taxonomy

Generic concepts in the *Beltraniaceae* are traditionally based on key morphological characters such as the presence of dark setae, setiform conidiophores or conidiophores arising from the substrate, radially lobed conidiophores or seta base, swollen separating cells, conidial shapes, size, colour,

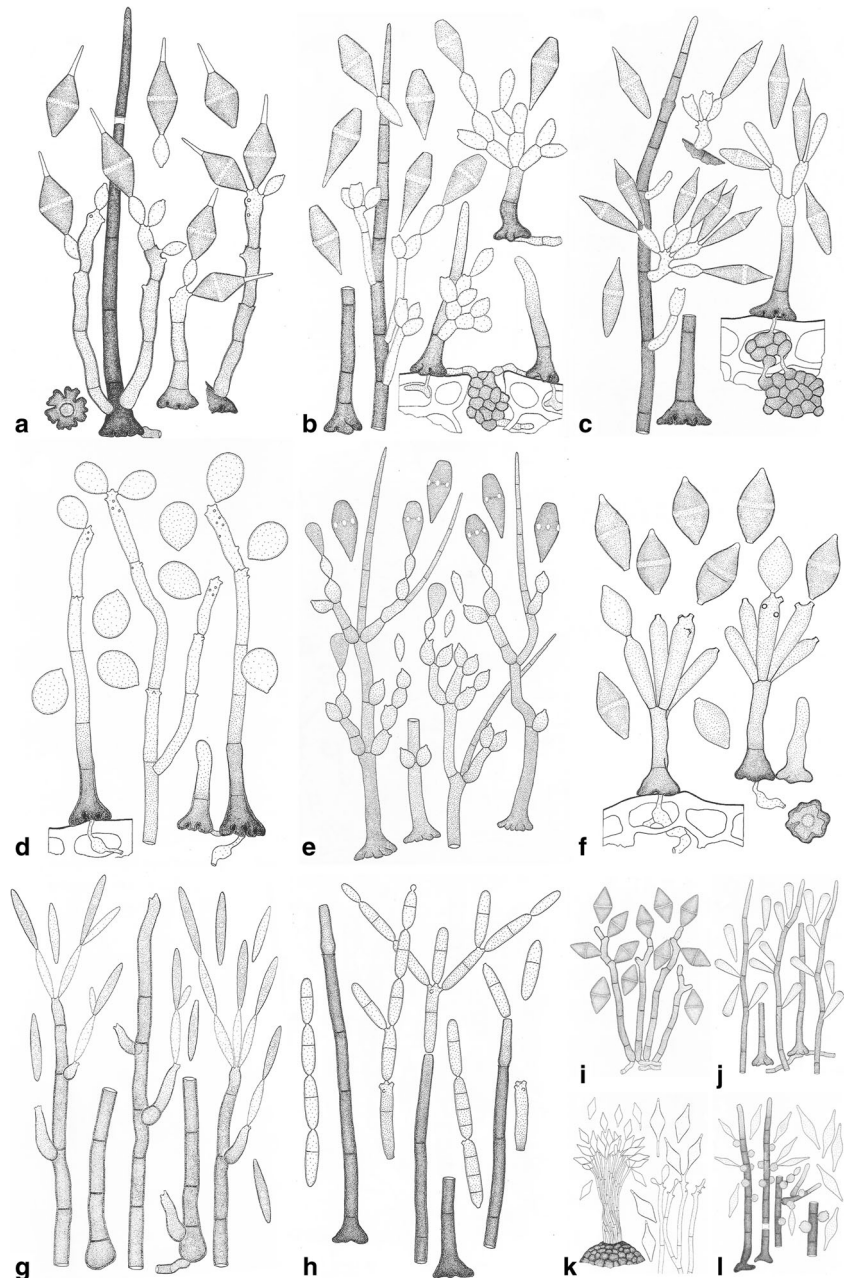
ornamentation, beaked or apiculate tip, apical appendage, and presence of equatorial hyaline band or hyaline pores. An illustrated comparison of the distinguishing features of the different genera is provided in Fig. 3.

## *Beltraniella*

*Beltraniella* includes species with setiform conidiophores and polyblastic, sympodial, denticulate conidiogenous cells (Subramanian 1952). In addition to the type species, four species, namely *B. humicola* P. Rama Rao, *B. nilgirica* Piroz. & S.D. Patil, *B. spiralis* Piroz. & S.D. Patil, and *B. vateriae* K.

**Fig. 3** Members of the family *Beltraniaceae* and similar genera.

**a** *Beltrania rhombica*. **b** *Beltraniella odinae*. **c** *Beltraniopsis esenbeckiae*. **d** *Hemibeltrania cinnamomi*. **e** *Porobeltraniella porosa*. **f** *Pseudobeltrania cedrelae*. **g** *Subramaniomyces indicus*. **h** *Parapleurotheciopsis inaequiseptata*. **i** *Beltraniomyces lignicola*. **j** *Parabeltrania persianiae*. **k** *Rhombostilbella rosea*. **l** *Kiliophora fusispora*. (Reproduced with permission from Seifert et al., *Genera of Hyphomycetes*, 2011)



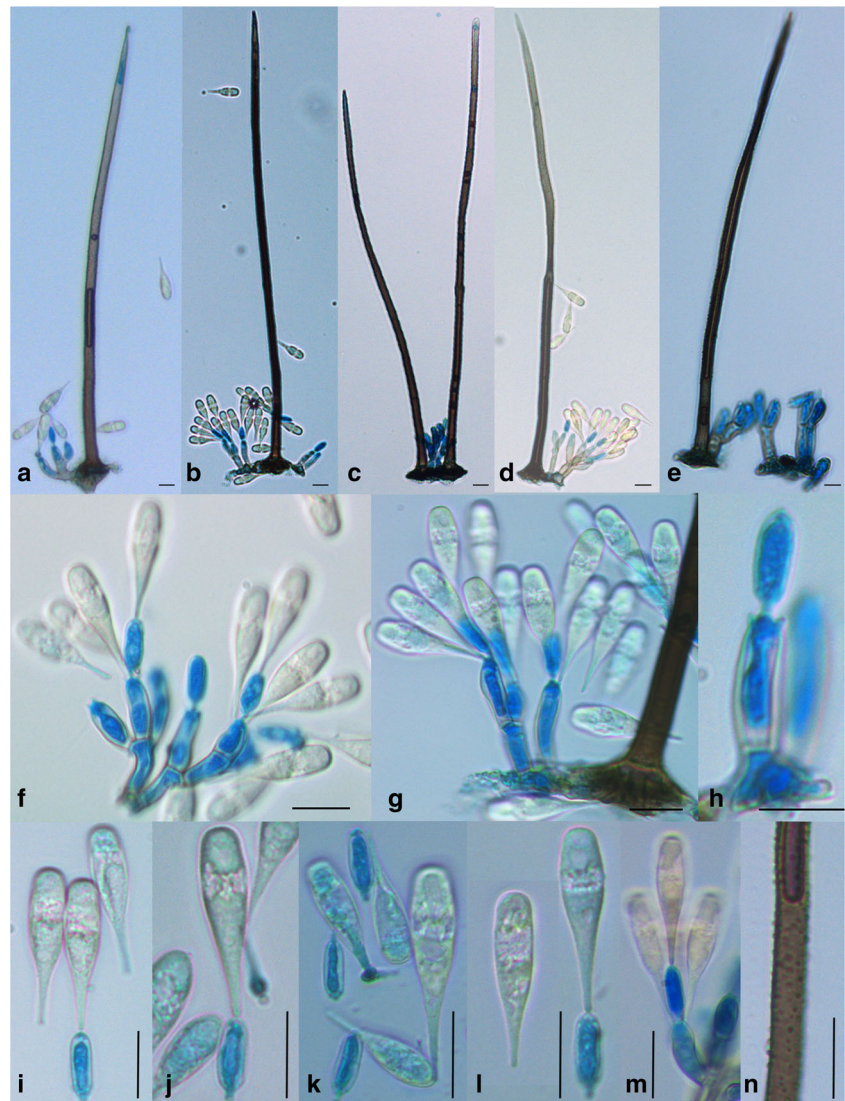
Swapna, Nagaveni, Kunwar & Manohar., were added to this genus from India. *Beltraniella porosa* and *B. patilii* were later transferred to *Porobeltraniella*. Presently, 20 accepted species are recorded in the genus *Beltraniella* (MycoBank (Robert et al. 2005) accessed 23 July 2016). Stevens (1917) originally collected and identified *Ellisiella portoricensis* F. Stevens on a dead leaf of *Clusia rosea* from Arecibo, Desecheo Island, Puerto Rico, which was later recombined as *Beltraniella portoricensis* (F. Stevens) Piroz. & Patil (Pirozynski and Patil 1970). A recent collection of *B. portoricensis* was made from southern Western Ghats, and minor variations in conidial size were noted in this study.

***Beltraniella portoricensis*** (F. Stevens) Piroz. & S.D. Patil, Can. J. Bot. 48 (3): 575 (1970) Fig. 4  
 ≡ *Ellisiella portoricensis* F. Stevens, Trans. Ill. St. Acad. Sci. 10: 203 (1917)

Colonies saprophytic, dark brown (6F5), forming irregular patches, velutinous. Setae simple, erect, straight, thick-walled, coarsely verrucose except at the base and apex, brown to reddish brown (6C5), 1–2 septate, arising from broad radially lobed basal cells tapering to an acute apex,  $100\text{--}200 \times 6\text{--}8 \mu\text{m}$ . Conidiophores simple or branched, pale brown or olivaceous, denticulate; separating cells hyaline, thin-walled, oval, fusiform or obovate, with a single denticle,  $9.5\text{--}10.5 \times 3\text{--}5 \mu\text{m}$ . Conidia smooth, lageniform to navicular, distal end truncate, proximal end rostrate, subhyaline to olivaceous with a hyaline transverse band,  $20\text{--}25\text{--}(31) \times 5.5\text{--}7 \mu\text{m}$ .

Specimen examined: INDIA. KERALA: Kasaragod, Karadka, on fallen leaves of *Mangifera indica* (Anacardiaceae), 9 Nov. 2015, K.C. Rajeshkumar (AMH 9789; culture NFCCI 3993).

**Fig. 4** *Beltraniella portoricensis* (AMH 9789). **a–e** Setae. **f–h** Conidiophore with separating cells and conidial attachment. **i–m** Conidial variations. **n** Verruculose setae ornamentation. Scale bars 10  $\mu\text{m}$



Note: In the ITS phylogeny, three downloaded and one self-generated sequence of *Beltraniella portoricensis* occur at four different positions within *Beltraniella*, each representing a different country and host, suggesting that this is probably a species complex. Sequences from type or authentic material (on *Odina wodier* from India) are needed to correctly designate the application of the name.

### *Hemibeltrania*

Pirozynski (1963) introduced *Hemibeltrania* as the causal fungus of cinnamon leaf spot in Sierra Leone, characterised by conidia described as ellipsoidal to obovate,  $12\text{--}16 \times 10\text{--}12 \mu\text{m}$ . There are 12 accepted species of *Hemibeltrania* documented in MycoBank (accessed 23 July 2016).

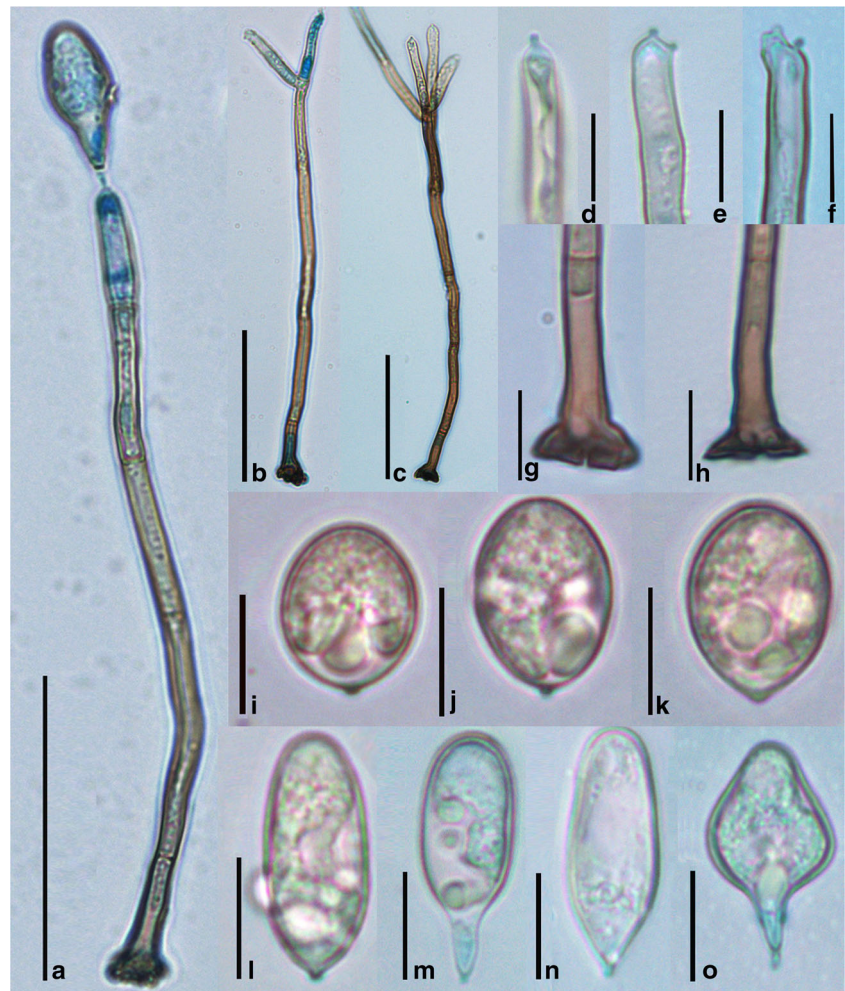
*Hemibeltrania cinnamomi* (Deighton) Piroz., Mycol. Pap. 90: 32 (1963) Fig. 5

≡ *Hansfordia cinnamomi* Deighton, Mycol. Pap. 78: 14 (1960)

Symptoms on leaves: necrotic, reddish brown to dark brown (7E6), amphigenous, irregular. On the natural substrate: conidiophores distinct, single or slightly branched towards the apex, erect, straight or flexuous, cylindrical, slightly sinuate, 4–8-septate, smooth, dark brown at the base, pale brown toward the apex, thin-walled,  $130\text{--}250 \times 3.5\text{--}6 \mu\text{m}$ , arising from a radially lobed basal cell,  $10\text{--}11.5 \mu\text{m}$  diam. Conidiogenous cells mono- and polyblastic, integrated or discrete, terminal, determinate or sympodially extended, denticulate; denticles cylindrical. Conidial secession schizolytic. Conidia solitary, limoniform, broadly ellipsoidal or obovate to irregular, rounded or obtuse at the apex, sometimes attenuate, subulate or rounded at the base with a small frill at the base, acrogenous becoming acropleurogenous after several sympodial extensions, unicellular, smooth,  $12\text{--}25 \times 8\text{--}13 \mu\text{m}$ , hyaline, subhyaline to pale olivaceous.

Specimen examined: INDIA. KERALA: Kaladi,  $10^{\circ}16'N$   $76^{\circ}43'E$ , on leaves of *Cinnamomum malabatum* (*Lauraceae*), 6 Dec. 2014, K.C. Rajeshkumar & K. Madhusudhanan (AMH 9667; culture NFCCI 3695 & NFCCI 3997).

**Fig. 5** *Hemibeltrania cinnamomi* (AMH 9667). **a–c** Conidiophore branching. **d–f** Denticulate conidiogenous cells. **g, h** Conidiophore bases. **i–o** Conidia. Scale bars (**a–c**)  $50 \mu\text{m}$ ; (**d–o**)  $10 \mu\text{m}$

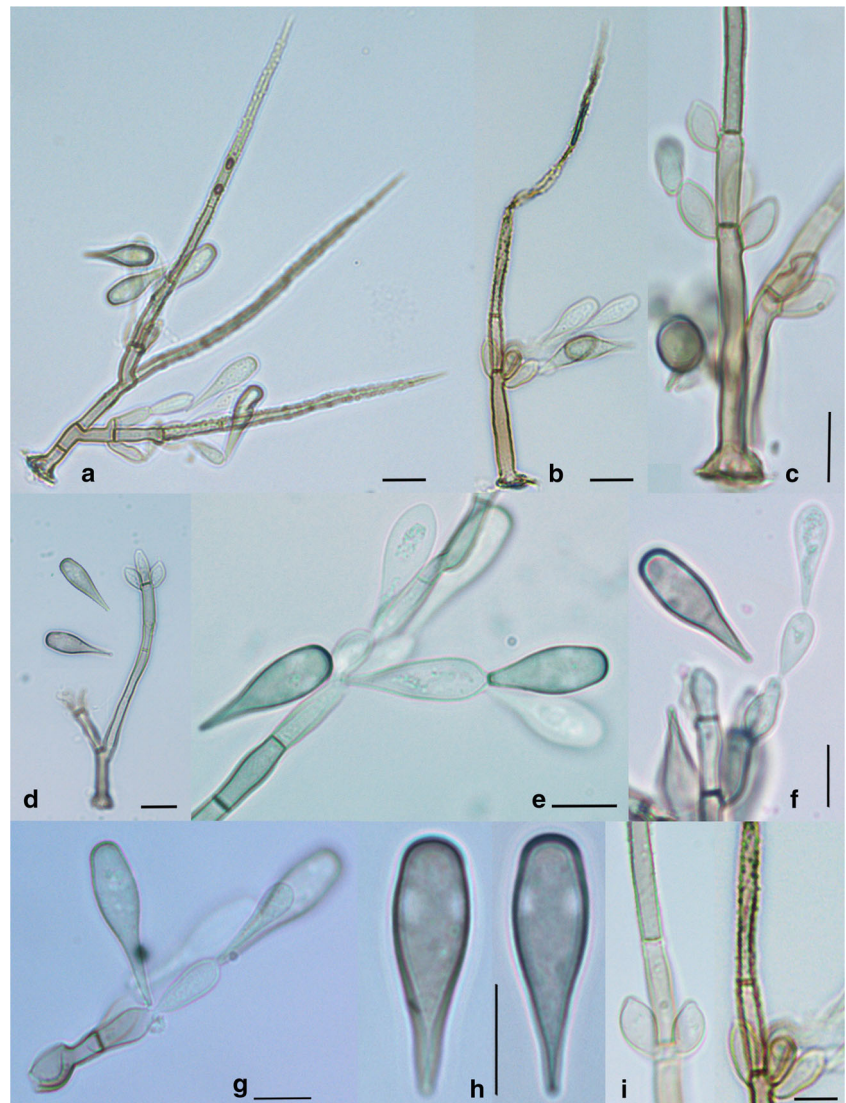


Note: The species concept in *Hemibeltrania* is quite narrow. Taxonomists have traditionally distinguished *Hemibeltrania* species based on conidial shape and size. Rajeshkumar et al. (2016) recently proposed a broader species description for *H. cinnamomi* based on variable conidial shapes and sizes observed on leaves of *Cinnamomum malabattrum*. Conidial shapes range from limoniform, obovate, and broadly ellipsoidal to irregular, and conidia are longer than 10  $\mu\text{m}$ . The symptomatology and other taxonomic characters, however, are identical to the type description given by Pirozynski (1963). Considering this broader species concept for *H. cinnamomi*, the closest species, *H. nectandrae*, may require future examination. In this study, additional isolates of *H. cinnamomi* were sequenced to confirm the position of *Hemibeltrania* in the *Beltraniaceae*. The ITS and LSU phylogenetic analyses establish well-supported, stable and consistent lineages for *Hemibeltrania*, *Pseudobeltrania* and *Porobeltraniella* within the *Beltraniaceae*.

### *Porobeltraniella*

*Porobeltraniella* Gusmão (Fernando and Gusmão 2004), based on *Beltraniella porosa* Piroz. & S. D. Patil (Pirozynski and Patil 1970), was established on the basis of unique characteristics such as several equatorial hyaline pores instead of a continuous band, and sterile setae arising from the conidiogenous apparatus. Kendrick (1980) had noted this configuration earlier, and suggested that this fungus might not be a member of the *Beltrania* group. Therefore, Gusmão (Fernando and Gusmão 2004) reclassified two species of *Beltraniella* in *Porobeltraniella* (for *Beltraniella patilii* see Karandikar and Patwardhan 1992). *Porobeltraniella* is endemic to the Western Ghats of India and known only from the states of Goa and Maharashtra. The isolates of *Porobeltraniella* collected in this study were compared to and verified with AMH 6063 (Ajrekar Mycological Herbarium, MACS ARI, Pune) and the holotype preserved

**Fig. 6** *Porobeltraniella porosa* (AMH 9788). **a–d** Setiform conidiophores. **e–g** Separating cells with conidia development. **h** Conidia. **i** Coarsely verruculose setae. Scale bars 10  $\mu\text{m}$



**Fig. 7** *Porobeltraniella porosa* (holotype) DAOM 128105. **a–d** Setiform conidiophores. **e** Conidia with separating cells. **f–h** Conidia. Scale bars (**a–d**) 50  $\mu$ m; (**e–h**) 10  $\mu$ m



as DAOM 128105 (Agriculture & Agri-Food Canada, Ottawa, Canada), and some amendments are made to broaden the type species concept.

*Porobeltraniella porosa* (Piroz. & S.D. Patil) Gusmão, Mycologia 96 (1): 151 (2004) Figs. 6 and 7

≡ *Beltraniella porosa* Piroz. & S.D. Patil, Can. J. Bot. 48(3): 573 (1970)

Colonies epiphyllous, velvety, greyish black to brownish black, mycelium superficial or immersed. Conidiophores setiform, macronematous with radially lobed basal cell, unbranched, verticillate or branched, straight or flexuous, smooth or verruculose, pale brown or dark brown, darker at base, tapering to a pointed tip, up to 300  $\mu$ m long, 4–7  $\mu$ m wide. Conidiogenous cells monoblastic or sympodial, 1–3 denticulate, subhyaline, 8–12  $\times$  4.5–7  $\mu$ m. Separating cells 12–14  $\times$  5.5–7.5  $\mu$ m. Conidia 21.5–28  $\times$  6.5–12  $\mu$ m. Sterile setae 60–195  $\times$  3–4  $\mu$ m.

Colonies on MEA and PDA, medium growth rate, 25 to 35 mm diam at 25  $\pm$  2  $^{\circ}$ C after 7 days, dark olivaceous brown or dark brown (6F6), velutinous, reverse blackish brown to black.

Specimens examined. INDIA. MAHARASHTRA: Poona, on fallen, decaying leaves of *Diospyros embryopteris*, 28 Nov 1965, S.D. Patil (Holotype: DAOM128105); INDIA. MAHARASHTRA, Mulshi, on

leaf litter of *Gnetum ula* (*Gnetaceae*), 10 June 2015, K.C. Rajeshkumar (epitype designated here AMH 9788, MBT372303; cultures ex-epitype NFCCI 3994; additional cultures NFCCI 3995, NFCCI 3996).

Note: In the epitype (AMH 9788), setiform conidiophores are coarsely verruculose at the apex, and conidiophores are up to 195  $\mu$ m long. Conidia observed on the holotype (DOAM 128105) were consistently narrower, up to 8  $\mu$ m, than indicated in the protologue, which stated a width of up to 12  $\mu$ m. In AMH 9788, the conidia measure 6.5–9.5  $\mu$ m wide, consistent with our observations of the holotype. However, the conidial length observed for the epitype fits the range observed on the holotype; 21.5–25.5  $\times$  6.5–9.5  $\mu$ m.

*Porobeltraniella patilii* (K.G. Karand. & Patw.) Gusmão, Mycologia 96 (1): 151 (2004) Fig. 8

≡ *Beltraniella patilii* K.G. Karand. & Patw., Mycotaxon 43: 21 (1992)

Colonies epiphyllous, velvety, brown (5F8) to brownish black; mycelium partly superficial, partly immersed in the substrate. Conidiophores setiform, macronematous, mononematous, arising from elongated, radially lobed basal cells, erect, septate, smooth, up to 240  $\mu$ m high, 3.5–5  $\mu$ m diam at the base, tapering to 1.5–2  $\mu$ m diam near apex. Conidiogenous cells solitary or arranged in verticils of 2–5, ovoid to ampulliform,

**Fig. 8** *Porobeltraniella patilii* (AMH 6063). **a–d** Setiform conidiophores. **e–g** Conidiophore bases. **h** Conidiogenous cells. **i–k** Conidiophore with conidiogenous apparatus. **m–o** Conidia. Scale bars 10  $\mu\text{m}$



continuous, polyblastic, denticulate, subhyaline to pale brown,  $6\text{--}8.5 \times 4.5\text{--}6 \mu\text{m}$ . Sterile setae arising terminally from conidiogenous cells, unbranched, septate, smooth, acute apex, pale brown,  $17\text{--}41.5 \times 1.5\text{--}3 \mu\text{m}$ . Separating cells absent. Conidia aseptate, proximal end rostrate, distal end truncate, turbinate, with circular pores near the broadest part, finely verrucose, pale brown,  $24\text{--}34 \times 7\text{--}10.5 \mu\text{m}$ .

Specimen examined: INDIA. GOA, Anmode Ghat, on fallen, decaying leaves of *Terminalia* sp., 4 Nov 1982, K.G. Karandikar (Holotype: AMH 6063).

### *Pseudobeltrania*

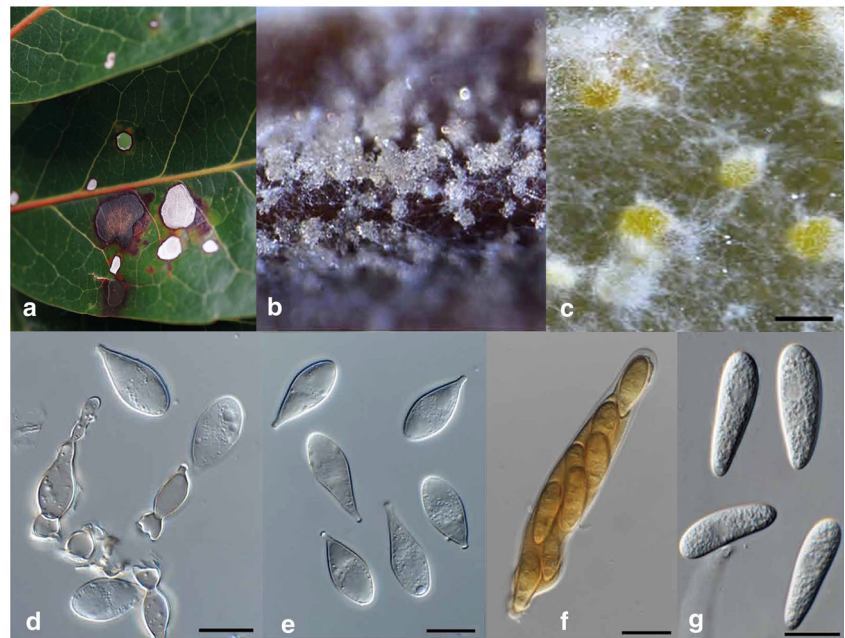
Hennings (1902) added *Pseudobeltrania*, typified by *P. cedrelae*, to this complex based on the absence of setae and separating cells and the presence of conidiophores with radially lobed basal cells and biconic conidia with a hyaline transverse band. Currently, 11

species are included in this genus (MycoBank, accessed 23 July 2016). To establish the phylogenetic placement within the family, a recently described species of *Pseudobeltrania*, which forms both asexual and sexual morphs and for which molecular data are available, is treated here.

***Pseudobeltrania ocoteae*** P.W. Crous & M.J. Wingfield, *Persoonia* 35: 289 (2015) Fig. 9

Mycelium immersed, conidiophores solitary, unbranched, straight to flexuous,  $20\text{--}50 \times 5\text{--}8 \mu\text{m}$ . Conidiogenous cells terminal, integrated, medium-brown, polyblastic,  $12\text{--}40 \times 6\text{--}8 \mu\text{m}$ . Conidia solitary, dry, simple, biconic to pyriform, pale olivaceous, smooth, aseptate,  $(21\text{--})23\text{--}27\text{--}(29) \times (9\text{--})10\text{--}(11) \mu\text{m}$ . Ascospores pale yellow (3A5), solitary to aggregated, ostiolate, up to  $250 \mu\text{m}$  diam. Asci 8-spored,  $70\text{--}90 \times 11\text{--}16 \mu\text{m}$ . Ascospores tri- to multiseriate, obovoid, hyaline, granular, smooth, aseptate with non-persistent mucoid sheath,  $(19\text{--})20\text{--}22\text{--}(24) \times (5.5\text{--})6\text{--}7\text{--}(8) \mu\text{m}$ .

**Fig. 9** *Pseudobeltrania ocoteae* (CBS H-22396). **a, b** Symptomatic leaves. **c** Colony on oatmeal agar (OA). **d, e** Conidiophores and conidia. **f** Ascus in Melzer's reagent. **g** Ascospores. Scale bars (**c**) 250  $\mu\text{m}$ ; (**d–g**) 10  $\mu\text{m}$



Specimen examined: FRANCE. LA RÉUNION: S21°14' 34.7" E55°47'55.9", RN2, on leaf spots of *Ocotea obtusata* (*Lauraceae*), 6 Mar 2014, P.W. Crous & M.J. Wingfield (holotype CBS H-22396, culture ex-type CPC 26219 = CBS 140664)

## Discussion

Nannfeldt (1932) and Barr (1990) established the order *Xylariales* (*Sordariomycetes*, *Xylariomycetidae*), to which they assigned 11 families. *Xylariales* is a large order of perithecial ascomycetes with unitunicate asci, accommodated in subclass *Xylariomycetidae* (Zhang et al. 2006; Maharachchikumbura et al. 2015). *Beltraniaceae* is an old and mostly forgotten family name in the *Xylariales*. Crous et al. (2015) recently emended the *Beltraniaceae* Nann. (1934) and reinstated this family with diagnostic characters. The most recent key to *Beltrania* and similar genera included nine genera in this complex, namely *Beltrania*, *Beltraniella*, *Beltraniomyces*, *Beltraniopsis*, *Hemibeltrania*, *Kiliophora*, *Porobeltraniella*, *Pseudobeltrania* and *Rhombostilbella* (Seifert et al. 2011).

The families and genera of the *Xylariales* have been traditionally segregated based on morphology of sexual morphs, and the species are recorded as plant pathogens, saprobes, endophytes or epiphytes (Zhang et al. 2006; Kirk et al. 2008). Several different morphological classification systems have been proposed for *Xylariales* (Munk 1953; Müller and von Arx 1973; Barr 1990; Hawksworth et al. 1995; Eriksson et al. 2003), with different interpretations of the morphology of stromata, perithecia, ascospore characters and, to a lesser

extent, asexual morphs. Although several researchers have further attempted to define phylogenetic family relationships within the *Xylariales*, those attempts were often hampered by a lack of reliable reference strains and sequence data (Eriksson et al. 2003; Smith et al. 2003; Zhang et al. 2006; Tang et al. 2009; Triebel et al. 2005). Jaklitsch et al. (2016) most recently revised *Xylariales* and accepted 16 families viz. *Amphisphaeriaceae*, *Apiosporaceae*, *Beltraniaceae*, *Cainiaceae*, *Coniocessiaceae*, *Diatrypaceae*, *Hyponectriaceae*, *Lopadostomataceae*, *Melogrammataceae*, *Microdochiaceae*, *Phlogicylindriaceae*, *Pseudomassariaceae*, *Requienellaceae*, *Sporocadaceae*, *Vialaeaceae* and *Xylariaceae*, but excluded the *Clypeosphaeriaceae*. Likewise, Maharachchikumbura et al. (2015) revisited *Xylariales* based on multigene sequence data, and expanded the order by adding two new families, *Lopadostomataceae* and *Pseudomassariaceae*. Senanayake et al. (2015) also reviewed the order using a combined ITS and LSU analysis by redefining *Xylariomycetidae*, and resurrected the order *Amphisphaeriales*. Based on morphology and DNA sequence data (LSU, ITS, *tub2* and *rpb2*), Hernández-Restrepo et al. (2016) included *Amphisphaeriaceae*, *Apiosporaceae*, *Clypeosphaeriaceae*, *Diatrypaceae*, *Hyponectriaceae*, *Microdochiaceae* and *Xylariaceae* in the *Xylariales*. Daranagama et al. (2016) showed 11 distinct families in *Xylariales* and highlighted the complexity of familial relationships, as several families were not strongly supported. The authors also noted that much of the data publicly available consist of only a few gene sequences for a limited number of authentic strains of *Xylariales*. An even smaller number of sequence data is available for protein coding genes such as *rpb2* or *tub2*. Thus the

interpretation of phylogenetic relationships in *Xylariales* is not yet sufficiently supported, and several lineages remain unresolved (Jaklitsch and Voglmayr 2012). Our analyses of the LSU data shows that the resulting phylogeny is quite sensitive to the selection of sequences/species and the phylogenetic method being employed; caution should therefore be taken in the interpretation of results based predominantly on LSU sequences.

Although phylogenetic studies focusing on other family lineages of *Xylariales* are available, *Beltraniaceae* is often under-represented and not thoroughly analysed in the context of the other accepted families. Shirouzu et al. (2010), while establishing phylogenetic relationships of *Beltraniella botryospora* and allied fungi, estimated the phylogenetic relationships of beltranioid fungi in *Xylariales* and suggested that *Beltraniella* is polyphyletic. Although Jaklitsch et al. (2016) recognised *Beltraniaceae*, and recombined *Pseudomassaria carolinensis* in *Beltraniella*, genera such as *Hemibeltrania*, *Pseudobeltrania*, *Porobeltraniella*, *Subramaniomyces*, and *Parapleurotheciopsis* were not included in their phylogeny. The present study analysed the phylogenetic position of the genera *Beltrania*, *Beltraniella*, *Beltraniopsis*, *Hemibeltrania*, *Porobeltraniella* and *Pseudobeltrania*, confirmed their position in the *Beltraniaceae*, and preliminarily confirmed the monophyly of all of the recognized genera.

The emended diagnosis of the *Beltraniaceae* presently includes only asexual characters (Crous et al. 2015). Two sexual morphs are thus far reported among genera of *Beltraniaceae*: *Beltraniella carolinensis* (M.E. Barr & Hodges) Jaklitsch & Voglmayr (Jaklitsch et al. 2016), formerly *Pseudomassaria carolinensis* M.E. Barr & Hodges (Hodges and Barr 1971), and *Pseudobeltrania ocoteae* Crous & M.J. Wingf. (Crous et al. 2015). They are rather different, with pale brown perithecia of *B. carolinensis* embedded in leaf tissue, asci with a refractive apical annulus containing 4–8 hyaline, and 1-septate ascospores, whereas the pale yellow perithecia of *P. ocoteae* were observed only in culture, have asci that lack an apical ring, and hyaline aseptate ascospores with a sheath. These sexual characters indicate different genera, and provide a preliminary view of how sexual characters might be added to the family diagnosis. The association of the sexual fungus *Pidoplitchkoviella terricola* with the family by some authors is at odds with its dark brown perithecia, evanescent asci, and triangular, pale brown ascospores (Kirilenko 1975).

Our analyses of the ITS and LSU sequence data (Figs. 1 and 2) confirmed the close association of *Beltrania*, *Beltraniella*, *Porobeltraniella*, *Pseudobeltrania* and *Hemibeltrania*. Isolates that are listed in the ITS phylogeny as *B. rhombica* and *B. querna* appear to be intermingled. Further study using sequences from type material and multiple loci are needed to determine whether this represents a species complex with numerous cryptic species, or only one species with significant sequence variation. Interestingly, many of the

ITS sequences downloaded from GenBank and included in the phylogenetic study were labelled as “sp.” by the depositors and were tagged as either being endophytic or isolated from leaf litter from across the globe. This hints at a much wider distribution of this group of fungi, with many potential new species awaiting discovery.

The phylogenetic position of *Pidoplitchkoviella terricola* and *Parapleurotheciopsis inaequiseptata* seems to be subject to the number and diversity of included sequences and the kind of phylogenetic method used; in some cases these species were associated with *Beltraniaceae*, and at other times they were not. Our study placed *Pidoplitchkoviella terricola* and *Parapleurotheciopsis inaequiseptata* as *incertae sedis*; resolving the correct phylogenetic position of these species would require their inclusion in a more solid multigene phylogenetic study. *Subramaniomyces fusisaprophyticus* was always associated with the *Beltraniaceae* in all of the LSU phylogenies performed in the course of this study, and can best be classified in this family. Maharachchikumbura et al. (2016) considered *Parapleurotheciopsis* and *Subramaniomyces* as part of the *Beltraniaceae*, but the authors considered *Pidoplitchkoviella* as *incertae sedis*. The genus *Kiliophora* was studied using small subunit (SSU) sequences and placed in the family *Amphisphaeriaceae* (Hidayat et al. 2014); a revision based on ITS and LSU analysis may be needed to verify its family-level association with the *Beltraniaceae*. Other genera such as *Beltraniomyces* and *Rhombostilbella* are as yet unstudied with molecular data, and their inclusion in *Beltraniaceae* remains to be established.

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## References

- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 25:4692–4693. doi:10.1093/nar/25.22.4692
- Barr ME (1990) Prodomus to nonlichenized, pyrenomycetous members of the class *Hymenoascomycetes*. *Mycotaxon* 39:43–184
- Batista AC, Bezerra JL (1960) *Beltraniopsis*—Novo gênero de fungos dematiaceae. *Inst Micol Univ Recife Publicaçõ* 296:1–13
- Castlebury LA, Rossman AY, Jaklitsch WJ, Vasilyeva LN (2002) A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences. *Mycologia* 94(6):1017–1031. doi:10.2307/3761867
- Castlebury LA, Rossman AY, Gi-Ho SU, Hyten AS, Spatafora JW (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. *Mycol Res* 108(08):864–872. doi:10.1017/S0953756204000607



- Chen C, Verkley GJ, Sun G, Groenewald JZ, Crous PW (2015) Redefining common endophytes and plant pathogens in *Neofabraea*, *Pezicula*, and related genera. *Fungal Biol*. doi:10.1016/j.funbio.2015.09.013
- Crous PW, Braun U, Schubert K, Groenewald JZ (2007) Delimiting *Cladosporium* from morphologically similar genera. *Stud Mycol* 58:33–56. doi:10.3114/sim.2007.58.02
- Crous PW, Groenewald JZ (2013) A phylogenetic re-evaluation of *Arthrimum*. *IMA fungus* 4(1):133–154. doi:10.5598/ima fungus.2013.04.01.13
- Crous PW, Shivas RG, Quaedvlieg W, Van der Bank M, Zhang Y, Summerell BA, Guarro J, Wingfield MJ, Wood AR, Alfenas AC, Braun U (2014) Fungal Planet description sheets: 214–280. *Persoonia* 32(1):184–306. doi:10.3767/003158514X682395
- Crous PW, Slippers B, Wingfield MJ, Rheeder J, Marasas WFO, Phillips AJL, Alves A, Burgess T, Barber P, Groenewald JZ (2006) Phylogenetic lineages in the Botryosphaeriaceae. *Stud Mycol* 55: 235–253. doi:10.3114/sim.55.1.235
- Crous PW, Summerell BA, Shivas RG, Romberg M, Mel'nik VA, Verkley GJ, Groenewald JZ (2011) Fungal Planet description sheets: 92–106. *Persoonia* 27(1):130–162. doi:10.3767/003158511X617561
- Crous PW, Summerell BA, Shivas RG, Carnegie AJ, Groenewald JZ (2012) A re-appraisal of *Harknessia* (Diaporthales), and the introduction of *Harknessiaceae* fam. nov. *Persoonia* 28(1):49–65. doi:10.3767/003158512X639791
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (2009) Fungal biodiversity. CBS Laboratory Manual Series, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands
- Crous PW, Wingfield MJ, Le Roux JJ, Richardson DM, Strasberg D, Shivas RG, Alvarado P, Edwards J, Moreno G, Sharma R, Sonawane MS, Tan YP, Altés A, Barasubiye T, Barnes CW, Blanchette RA, Boertmann D, Bogo A, Carlavilla JR, Cheewangkoon R, Daniel R, de Beer ZW, de Jesús Yá-ez-Morales M, Duong TA, Fernández-Vicente J, Geering ADW, Guest DI, Held BW, Heykoop M, Hubka V, Ismail AM, Kajale SC, Khemmuw W, Kolařík M, Kurlí R, Lebeuf R, Lévesque CA, Lombard L, Magista D, Manjón JL, Marinowitz S, Mohedano JM, Nováková A, Oberlies NH, Otto EC, Paguigan ND, Pascoe IG, Pérez-Butrón JL, Perrone G, Rahi P, Raja HA, Rintoul T, Sanhueza RMV, Scarlett K, Shouche YS, Shuttleworth LA, Taylor PWJ, Thorn RG, Vawdrey LL, Vidal RS, Voitek A, Wong PTW, Wood AR, Zamora JC, Groenewald JZ (2015) Fungal planet description sheets: 371–399. *Persoonia* 35:264–327. doi:10.3767/003158515X690269
- Daranagama DA, Camporesi E, Liu XZ, Bhat DJ, Chamyuang S, Bahkali AH, Stadler M, Hyde KD (2016) *Tristatiperidium* microsporium gen. et sp. nov. (Xylariales) on dead leaves of *Arundo plinii*. *Mycol Prog* 15:1–8. doi:10.1007/s13225-015-0329-6
- Endo M, Hatakeyama S, Harada Y, Tanaka K (2008) Description of a coelomycete *Ciliochorella castaneae* newly found in Japan, and notes on its distribution and phylogeny. *Nippon Kingakukai Kaiho* 49(2):115–120 (In Japanese)
- Eriksson OE, Baral HO, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe T (2003) Outline of Ascomycota. *Myconet* 9:1–89
- Fernando L, Gusmão P (2004) *Porobeltraniella* gen. nov. to accommodate two species of *Beltraniella*. *Mycologia* 96(1):150–153. doi:10.2307/3761996
- García-Laviña CX, Bettucci L, Tiscornia S (2016) Fungal communities associated with *Eugenia uruguayensis* (Myrtaceae) leaf litter in early stages of decomposition in Uruguay. *Sydowia* 68:139–150
- Hawksworth DL, Kirk P, Sutton BC, Pegler DN (1995) Ainsworth & Bisby's dictionary of the fungi, 8th edn. CAB International, Oxon
- Hennings P (1902) *Fungi S. Paulenses II. a cl. Puttemans collecti*. *Hedwigia* 41:295–311
- Hernández-Restrepo M, Groenewald JZ, Crous PW (2016) Taxonomic and phylogenetic re-evaluation of *Microdochium*, *Monographella* and *Idriella*. *Persoonia* 36:57–82. doi:10.3767/003158516X688676
- Hidayat I, Harahap I, Rahayu G (2014) The first phylogenetic study of *Kiliophora* (Fungi, Anamorphic Xylariales). *Mycosphere* 5:78–84. doi:10.5943/mycosphere/5/1/3
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol* 42:182–192
- Hirooka Y, Kobayashi T, Ono T, Rossman AY, Chaverri P (2010) *Verrucostoma*, a new genus in the Bionectriaceae from the Bonin Islands, Japan. *Mycologia* 102(2):418–429. doi:10.3852/09-137
- Hodges CS, Barr ME (1971) A new species of *Pseudomassaria* and its *Beltraniella* conidial state. *Mycologia* 63:562–566
- Huhndorf SM, Miller AN, Fernández FA (2004) Molecular systematics of the Sordariales: the order and the family Lasiosphaeriaceae redefined. *Mycologia* 96(2):368–387. doi:10.2307/3762068
- Jaklitsch WM, Fournier J, Rogers JD, Voglmayr H (2014) Phylogenetic and taxonomic revision of *Lopodostoma*. *Persoonia* 32:52–82. doi:10.3767/003158514X679272
- Jaklitsch WM, Voglmayr H (2012) Phylogenetic relationships of five genera of *Xylariales* and *Rosasphaeria* gen. nov. (*Hypocreales*). *Fungal Divers* 52:75–98. doi:10.1007/s13225-011-0104-2
- Jaklitsch WM, Gardinnet A, Voglmayr H (2016) Resolution of morphology-based taxonomic delusions: *Acrocordiella*, *Basiseptospora*, *Bligiascospora*, *Clypeosphaeria*, *Hymenoplella*, *Lepteutypa*, *Pseudapiospora*, *Requienella*, *Seiridium* and *Strickeria*. *Persoonia* 37:82–105. doi:10.3767/003158516X690475
- Jeewon R, Edward CY (2003) Molecular systematics of the Amphisphaeriaceae based on cladistic analyses of partial LSU rDNA gene sequences. *Mycol Res* 107(12):1392–1402. doi:10.1017/S095375620300875X
- Karandikar KG, Patwardhan PG (1992) Two new hyphomycetes from India. *Mycotaxon* 43:21–24
- Kendrick B (1980) The generic concept in Hyphomycetes, a reappraisal. *Mycotaxon* 11(1):339–364
- Kirilenko ST (1975) *Pidoplitchkoviella terricola* – a new ascomycete. *Mykrobiol Zhurnal* 37:603–605
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the fungi, 10th edn. CABI, Wallingford
- Kornerup A, Wanscher JH (1978) *Methuen handbook of colour*, 3rd edn. Methuen, London
- Lee S, Crous PW, Wingfield MJ (2006) Pestalotioid fungi from Restionaceae in the Cape floral kingdom. *Stud Mycol* 55:175–187. doi:10.3114/sim.55.1.175
- Liu JK, Hyde KD, Jones EG, Ariyawansa HA, Bhat DJ, Boonmee S, Maharachchikumbura SS, McKenzie EH, Phookamsak R, Phukhamsakda C, Shenoy BD (2015) Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. *Fungal Divers* 72(1):1–97. doi:10.1007/s13225-015-0324-y
- Lumbsch HT, Wirtz N, Lindemuth R, Schmitt I (2002) Higher level phylogenetic relationships of Euscomycetes (Pezizomycotina) inferred from a combined analysis of nuclear and mitochondrial sequence data. *Mycol Prog* 1(1):57–70. doi:10.1007/s11557-006-0005-z
- Maharachchikumbura SS, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014) *Pestalotiopsis* revisited. *Stud Mycol* 79:121–186. doi:10.1016/j.simyco.2014.09.005
- Maharachchikumbura SS, Hyde KD, Jones EBG, McKenzie EHC, Huang SK, Abdel-Wahab MA, Daranagama DA, Dayarathne M, D'souza MJ, Goonasekara ID, Hongsanan S, Jayawardena RS, Kirk PM, Konta S, Liu JK, Liu ZY, Norphanphoun C, Pang KL, Perera RH, Senanayake IC, Shang Q, Shenoy BD, Xiao YP, Bahkali AH, Kang JC, Somrothipol S, Suetrong S, Wen TC, Xu JC (2015) Towards a natural classification and backbone tree for Sordariomycetes. *Fungal Divers* 72:199–301. doi:10.1007/s13225-015-0331-z

- Maharachchikumbura SS, Hyde KD, Jones EBG, McKenzie EHC, Bhat JD, Dayaratne MC, Huang SK, Norphanphoun C, Senanayake IC, Perera RH, Shang QJ, Xiao Y, D'Souza MJ, Hongsan S, Jayawardena RS, Daranagama DA, Konta S, Goonasekara ID, Zhuang WY, Jeewon R, Phillips AJL, Abdel-Wahab MA, Al-Sadi AM, Bahkali AH, Boonmee S, Boonyuen N, Cheewangkoon R, Dissanayake AJ, Kang J, Li QR, Liu JK, Liu XZ, Liu ZY, Luangsa-ard JJ, Pang KL, Phookamsak R, Promputtha I, Suetrong S, Stadler M, Wen T, Wijayawardene NN (2016) Families of sordariomycetes. *Fungal Divers* 79(1):1–317
- Manoharachary C, Agarwal DK, Krishna Rao N (2003) *Beltraniomyces*, a new genus of dematiaceous hyphomycetes from India. *Indian Phytopathol* 56(4):418–421
- Müller E, von Arx JA (1973) Pyrenomycetes: meliolales, coronophorales, sphaeriales. In: Ainsworth GC, Sparrow FK, Sussman AS (eds) *The fungi, an advanced treatise*, vol 4A. Academic Press, New York, pp 87–132
- Munk A (1953) The system of the pyrenomycetes. *Dansk Bot Ark* 15(2): 1–163
- Nannfeldt JA (1932) Studien über die Morphologie und Systematik der nichtlichenisierten inoperculaten Discomyceten. *Nova Acta Regiae Soc Sci Ups* 8(2):1–368
- Nannizzi A (1934) Repertorio sistematico dei miceti dell' uomo e degli animali 4:1–557
- Penzig AGO (1882) *Beltrania*, un nuovo genere di ifomiceti. *Nuovo Giornale Bot Ital Boll Soc Bot Ital* 14:72–75
- Pereira JS, Costa RR, Nagamoto NS, Forti LC, Pagnocca FC, Rodrigues A (2016) Comparative analysis of fungal communities in colonies of two leaf-cutting ant species with different substratum preferences. *Fungal Ecol* 21:68–75. doi:10.1016/j.funeco.2016.03.004
- Pirozynski KA (1963) *Beltrania* and related genera. *Mycol Pap* 90:1–37
- Pirozynski KA, Patil SD (1970) Some setose Hyphomycetes of leaf litter in south India. *Can J Bot* 48:567–581. doi:10.1139/b70-079
- Quaedvlieg W, Verkley GJ, Shin HD, Barreto RW, Alfenas AC, Swart WJ, Groenewald JZ, Crous PW (2013) Sizing up Septoria. *Stud Mycol* 75:307–390. doi:10.3114/sim0017
- Rajeshkumar KC, Marathe SD, Madhusudhanan K, Castañeda-Ruiz RF (2016) Taxonomic re-evaluation and phylogenetic position of *Hemibeltrania cinnamomi* within *Xylariales*. *Mycotaxon* 131(1): 87–94. doi:10.5248/131.87
- Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycol Res* 98:625–634. doi:10.1016/S0953-7562(09)80409-7
- Robert V, Stegehuis G, Stalpers J (2005) The MycoBank engine and related databases. <http://www.mycobank.org>
- Saccardo PA (1886) *Sylloge fungorum omnium hucusque cognitorum IV*. Padua, Italy
- Saccardo PA (1889) *Sylloge fungorum omnium hucusque cognitorum XIV*. Padua, Italy
- Seifert K, Morgan-Jones G, Gams W, Kendrick B (2011) The genera of hyphomycetes. CBS biodiversity series no. 9. CBS-KNAW Fungal Biodiversity Centre, Utrecht
- Senanayake IC, Maharachchikumbura SSN, Hyde KD, Bhat JD, Jones EBG, Mckenzie EHC, Dai DQ, Daranagama DA, Dayaratne MC, Goonasekara ID, Konta S, Li WJ, Shang QJ, Stadler M, Wijayawardene NN, Xiao YP, Norphanphoun C, Li QR, Liu XZ, Bahkali AH, Kang JC, Wang Y, Wen TC, Xu JC, Camporesi E (2015) Towards unraveling relationships in Xylariomycetidae (*Sordariomycetes*). *Fungal Divers* 73:73–144. doi:10.1007/s13225-015-0340-y
- Shirouzu T, Hirose D, Tokumasu S, To-Anun C, Maekawa N (2010) Host affinity and phylogenetic position of a new anamorphic fungus *Beltraniella botryospora* from living and fallen leaves of evergreen oaks. *Fungal Divers* 43:85–92
- Smith GJD, Liew ECY, Hyde KD (2003) The Xylariales: a monophyletic order containing 7 families. *Fungal Divers* 13:185–218
- Spatafora JW, Sung GH, Johnson D, Hesse C, O'Rourke B, Serdani M, Spotts R, Lutzoni F, Hofstetter V, Miadlikowska J, Reeb V (2006) A five-gene phylogeny of Pezizomycotina. *Mycologia* 98(6):1018–1028. doi:10.3852/mycologia.98.6.1018
- Stevens FL (1917) Porto Rican fungi, old and new. *Trans Ill. Acad Sci* 10: 162–218
- Subramanian CV (1952) Fungi imperfecti from Madras—III. *Beltraniella* gen. nov. *Proc Indian Acad Sci Section B* 36:223–228
- Suh SO, Blackwell M (1999) Molecular phylogeny of the cleistothecial fungi placed in Cephalothecaceae and Pseudeurotiaceae. *Mycologia* 91(5):836–848. doi:10.2307/3761537
- Sullivan R, Bergen MS, Patel R, Bills GF, Alderman SC, Spatafora JW, White JF Jr (2001) Features and phylogenetic status of an enigmatic clavicipitalean fungus *Neoclaviceps monostipa* gen. et sp. nov. *Mycologia* 1:90–99. doi:10.2307/3761608
- Tanaka K, Endo M, Hirayama K, Okane I, Hosoya T, Sato T (2011) Phylogeny of *Discosia* and *Seimatosporium*, and introduction of *Adisciso* and *Immersidiscosia* genera nova. *Persoonia* 26(1):85–98. doi:10.3767/003158511X576666
- Tang AMC, Jeewon R, Hyde KD (2007) Phylogenetic utility of protein (RPB2,  $\beta$ -tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of Sordariomycetes (Ascomycota, Fungi). *A Van Leeuw* 91(4):327–349. doi:10.1007/s10482-006-9120-8
- Tang AMC, Jeewon R, Hyde KD (2009) A re-evaluation of the evolutionary relationships within the *Xylariaceae* based on ribosomal and protein-coding gene sequences. *Fungal Divers* 34:127–155
- Thomas DC, Vandegrift R, Ludden A, Carroll GC, Roy BA (2016) Spatial ecology of the fungal genus *Xylaria* in a tropical cloud forest. *Biotropica* 48:381–393. doi:10.1111/btp.12273
- Triebel D, Peršoh D, Wollweber H, Stadler M (2005) Phylogenetic relationships among *Daldinia*, *Entonaema* and *Hypoxylon* as inferred from ITS nrDNA analyses of *Xylariales*. *Nova Hedwigia* 80:25–43
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 172:4238–4246
- White TJ, Bruns T, Lee J, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic, San Diego, pp 315–322. doi:10.1016/B978-0-12-372180-8.50042-1
- Zhang N, Castlebury LA, Miller AN, Huhndorf SM, Schoch CL, Seifert KA, Rossman AY, Roger JD, Kohlmeyer J, Sung GH (2006) An overview of the systematics of the *Sordariomycetes* based on a four-gene phylogeny. *Mycologia* 98:1076–1087