

Overview of *Phacidiales*, including *Aotearoamyces* gen. nov. on *Nothofagus*

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Abstract: The new genus *Aotearoamyces* is proposed to accommodate a single species that was repeatedly collected on fallen wood in *Nothofagaceae* forests of New Zealand and was previously misidentified as a *Claussenomyces* species. This monotypic genus belongs to *Tympanidaceae*, a recently erected family in *Phacidiales*. *Aotearoamyces* is differentiated from other *Tympanidaceae* by phragmospores that do not form conidia either in or outside the asci, an exciple of *textura intricata* with hyphae widely spaced and strongly gelatinized (plectenchyma), and apically flexuous, partly helicoid paraphyses. The asexual morph was studied in pure culture. Phylogenetic analyses of combined SSU, ITS and LSU sequences strongly support a sister relationship between the sexually typified *Aotearoamyces* and the asexually typified “*Collophorina*” *paarla* characterized morphologically by forming endoconidia, a feature not found in the genetically distinct type species of *Collophorina*. Based on our molecular results, we place the genus *Epithamnolia* in the *Mniaecia* lineage within *Phacidiales*.

Key words:

Ascomycota
Claussenomyces
new taxa
Nothofagus
phylogeny
Rhytismatales
Leotiomyces

Article info: Submitted: 20 April 2018; Accepted: 25 October 2018; Published: 30 October 2018.

INTRODUCTION

The taxonomy and classification of the *Leotiomyces* is unsettled with a high proportion of taxa not yet treated using molecular methods (Baral 2016, LoBuglio & Pfister 2010). Consequently, the delimitation of genera, families and orders often changes and the systematic position of taxa is subject to modification, depending on individual researcher's opinions, the impact of the addition of sequences from previously unsampled taxa, opinions about the acceptance of paraphyletic groups, etc. One of the orders recognized in the class is *Phacidiales*. A study of the genera considered to belong in this order provides a good example of the chaotic situation within the class.

The name *Phacidiales* was firstly used by Bessey (1907) and was described as including “true fungi, mostly saprophytic, but sometimes parasitic, with a branching septate mycelium, which bears the mostly open spore fruits (apothecia)”. Three families were included in this initial circumscription, each with two genera (Fig. 1). Ten years later, Höhnelt (1917) used the same ordinal name for six families and 52 genera (Fig. 1): Schizothyriaceae (5 genera), Leptopeltineae (13 genera), Dermopeltineae (10 genera), Phacidiaceae (12 genera), Phacidiostromaceae (4 genera) and Cryptomycetaceae (8 genera). Nowadays the only genus among those listed by Bessey or Höhnelt which remains in the *Phacidiales* is the type genus *Phacidium* (Fig. 1). Most of the genera treated by Höhnelt (1917) are still accepted, but the majority are now distributed across other orders or their position is uncertain because of a lack

of DNA sequence data (Baral 2016, Wijayawardene *et al.* 2017).

How the number of genera and their systematic placement, which reflects changes in morphological concepts, has changed over time is illustrated in Fig. 1. Höhnelt (1917) described the *Phacidiales* as follows: “Discomycetes with superficial or immersed fruitbodies, never erumpent fruitbodies, with or without stroma, excipulum entire or only at the margin, thin and brown or thick and carbonaceous. At maturity it opens very irregularly by a longitudinal split or by several lobes. Rarely the covering layer over the hymenium forms a detaching lid”. After Höhnelt's circumscription, the sexual morph was usually described as a reduced carbonaceous ascoma: black, discoid to hysteriform, frequently immersed in the tissue of the host and with a reduced exciple. The hymenium was described as exposed by a rupture of the upper stromatal layer by one or more slits. The asci were reported as 4–8-spored, thickened apically, with or without an amyloid apical ring. The ascospores were referred to as variable in shape, simple or phragmoseptate, hyaline or rarely brownish, and with or without mucilaginous sheaths or appendages (e.g. Ainsworth & Bisby 1943, 1950, Ainsworth 1961, Korf 1973, Dennis 1978). The concept of the order that developed after Höhnelt generally included three families (Fig. 1): *Cryptomycetaceae*, *Hypodermataceae* or *Rhytismataceae*, and *Phacidiaceae* (e.g. Ainsworth *et al.* 1971, Korf 1973, Dennis 1978, DiCosmo *et al.* 1984).

Between 1983 and 1995 the order *Phacidiales* fell out of use, and the family *Phacidiaceae* was applied in a more restricted sense, including *Phacidium* and two or three other

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Symbols denote current placement

- ★ Asterinales
- ▼ Diaporthales
- ▲ Helotiales
- ◆ Ostropales
- Phacidiales
- Rhytismatales
- ◐ Triblidiales
- ◑ Xylariales
- ? Incertae sedis

Cryptomyceteen	
Colpoma	Sporomega
Cryptomyces	Therrya
Myxophaciella (= Pseudophacidium)	Xylopezia
Myxophacidium (= Pseudophacidium)	Schizothyriaceen
Pleiosictis (= Melittiosporium)	Clypeolum
Phacidiosstromaceen	Microrthyriella (= Schizothyrium)
Aporhytisma (= Diaportha)	Phragmothryiella (= Myriangiella)
Pachyrhytisma (= Rhytisma)	Polyclypeolum (= Schizothyrium)
Phacidiostroma (= Phacidium)	Schizothyrium
Placuntium (= Rhytisma)	Leptopeltineen
Dermopeltineen	Bifusella
Coccomycella (= Coccomyces)	Coccomyces
Hypoderma (= Lophodermium)	Duplicaria
Hypodermella	Entopeltis
Hypodermellina	Haplophyse
Lophodermellina (= Lophodermium)	Leptopeltella (= Leptopeltis)
Macroderma	Leptopeltis
Nymanomyces	Lophodermina (= Lophodermium)
Pseudophacidium	Phacidina
Pseudotrochila	Rhytisma
Xyloma (= Rhytisma)	Schizothyrioma
Phacidiaecen	Thyriopsis
Aldona	Vizella
Coccomycetella	
Cryptomycina	
Hymenobolus	
Hysteropsis	
Lophodermium	
Moutoniella	
Naevia (= Naevia)	
Odontotrema	
Phacidium	
Phaeophacidium	
Sphaeropezia	

Triblidiaaceae
Triblidium
Scleroderis (= Godronia)
Stictidaaceae
Propolis
Stictis
Phacidiaaceae
Phacidium
Rhytisma

Rhytismataceae
Bifusella
Bifusepta
Ceratophacidium
Coccomyces
Colpoma
Davisomycella
Duplicaria
Elytroderma
Hypoderma (= Lophodermium)
Hypodermella
Isthmiella
Lirula
Lophodermella
Lophodermium
Lophomerum
Nymanomyces
Placuntium (= Rhytisma)
Ploioderma
Rhytisma
Solella
Virgella
Cryptomycetaceae
Cryptomyces
Pseudorhytisma
Phacidiaaceae
Cryptomycina
Lophophacidium
Micraspis
Nannfeldtia
Neophacidium
Phacidina
Phacidium
Pseudophacidium
Therrya

Hypodermataceae
Bifusella
Coccomyces
Colpoma
Hypoderma (= Lophodermium)
Hypodermella
Lophodermium
Rhytisma
Sporomega
Terriera
Cryptomycetaceae
Potebniomyces (= Phacidiopticus)
Cryptomyces
Phacidiaaceae
Cryptomycina
Didymascella
Laquearia
Lasiostrictis (= Naemacyclus)
Melittiosporium
Micraspis
Naemacyclus
Phacidiostroma (= Phacidium)
Phacidium
Pseudophacidium
Rhabdocline
Sphaeropezia
Therrya

Phacidiaaceae
Allantophomopsiella
Allantophomopsis
Bulgaria
Phacidium
Potebniomyces (= Phacidiopticus)
Pseudophacidium

Incertae sedis
Coma
Mniaecia lineage
Mniaecia
?Trizodia
Tympanidaaceae
Claussenomyces
Collophora
Durandiella
Grovesiella
Holwaya
? Micraspis
Myriodiscus
Pragmopora
Tympanis
Helicogoniaceae
Calloropsis
Eleutheromycella
Eleutheromyces
Gelatinipulvinella
Gelatinopsis
Gellingia
Helicogonium
Phacidiaaceae
Allantophomopsiella
Allantophomopsis
Bulgaria
Darkera
? Gremmenia
Lophophacidium
Phacidiopticus
Phacidium
Pseudophacidium
Starbaeckia

Bessey (1907)

Von Höhnel (1917)

Korf (1973)

Dennis (1978)

Crous et al. (2014)

Baral (2016)

genera. Some authors even considered *Phacidiales* as a synonym of *Rhytismatales* (Hawksworth *et al.* 1983). In other cases, *Phacidiaceae*, *Cryptomycetaceae* and *Rhytismataceae* were included in *Rhytismatales* (Hawksworth *et al.* 1983, 1995). *Rhytismataceae* and *Hypodermataceae* included many genera previously placed in *Phacidiales* (Hawksworth *et al.* 1983, 1995). In 1995 the family *Phacidiaceae* contained three genera and was placed by Korf & Lizoň (2000) in *Leotiales*, an invalid name later validated by Korf & Lizoň (2000), and there in *Helotiales*. In 2001 the *Phacidiaceae* still contained only three genera (*Ascocoma*, *Lophophacidium*, and *Phacidium*) and was transferred to *Helotiales*, where it was treated during 2001–2010 (Kirk *et al.* 2001, 2008, Eriksson 2005, 2006, Lumbsch & Hundorf 2007, 2010).

Crous *et al.* (2014) recognized the *Phacidiales* as a monophyletic order distinct from *Helotiales* and included six genera (Fig. 1). Using molecular evidence, these authors expanded the morphological concept of the order by including genera with exposed, cup-shaped apothecia typical of helotiaceous fungi (e.g. *Bulgaria*) as well as genera with immersed ascomata that open by splits across covering stromatic layers, as was characteristic of the concepts of *Phacidiales* of earlier authors. Although DiCosmo *et al.* (1984) reported anamorphs for some members in *Phacidiales*, it was Crous *et al.* (2014) who provided a unified view of the asexual morphs within the order. Previously, the information about asexual morphs was sparse and only a relationship with coelomycetes had been reported (DiCosmo *et al.* 1984).

In the most recent classification of *Leotiomyces* compiled by Baral (2016), the ecology of the order remained the same (saprobic, parasitic), but the morphological concept was expanded and delineated more precisely, including information about the phase during which the apothecia open (prohymenial to mesohymenial), and added features of the living cells, such as the lack of vacuolar bodies in paraphyses, asci with either amyloid or inamyloid apical rings (exceptionally the entire wall is amyloid) and ascospores with variable lipid content. Here the order *Phacidiales* has three families containing about 27 genera, approximately half the number of genera compared to Höhnelt's concept a century ago (Fig. 1). Two to three genera were added to *Phacidiaceae* in addition to those considered by Crous *et al.* (2014): *Darkera*, *Starbaeckia*, and questionably *Gremmenia*. Also, the priority of *Phacidiopycnis* over the sexually typified *Potetniamyces* was indicated. Two new families were included in the order: *Tympanidaceae* and *Helicogoniaceae*. In addition to these three families in *Phacidiales*, Baral included the '*Mniaecia* lineage' with one or two genera (*Mniaecia*, and ?*Trizodia*), and one genus as *incertae sedis* (*Coma* with the sexually typified synonym *Ascocoma*). Subsequently, Suija *et al.* (2017) placed the monotypic genus *Epithamnolia* as *incertae sedis* in *Phacidiales*, due to its phylogenetic and morphological affinities with the asexual morph of *Epiglia* (a

synonym of *Mniaecia* *fide* Van Vooren 2005), thus widening the ecological concept of the order to include lichenicolous fungi.

Taking into consideration the repeated changes within *Phacidiales*, the aim of this research was to enhance and synthesize knowledge of the order. Important results include the erection of a new genus known only from the Southern Hemisphere for a species previously misclassified in *Claussenomyces*, and the observation that the asexual "*Collophorina*" *paarla* is related to it.

MATERIAL AND METHODS

Specimens of the newly described species were collected between 1989 and 2010 in native forests of New Zealand during non-targeted, general collecting expeditions for fungi. All specimens cited are deposited in the PDD fungarium (Manaaki Whenua Landcare Research, Auckland) and living cultures grown from ascospores from the fresh specimens are stored in the ICMP culture collection (Manaaki Whenua Landcare Research, Auckland, www.landcareresearch.co.nz/resources/collections/icmp).

Sections for anatomical examination of ascomata were free-hand sectioned under a Motic stereomicroscope SMZ140 and examined with a Motic B1 light microscope. Microphotographs were taken with an USB Moticam 2500 camera and processed with the software Motic images Plus 2.0. Measurements are given as follows: (smallest single measurement) smallest mean–largest mean (largest single measurement). The small and large means are based on ≥ 10 measurements of individual specimens. No living specimens of the sexual morph were available, and therefore potassium hydroxide at 5 % (KOH) was used to rehydrate herbarium specimens prior to morphological study. Conidia and conidiogenous cells were measured from dried Oatmeal Agar cultures rehydrated in 5 % KOH. The descriptions and abbreviations follow Baral (1992): † = dead state, * = living state; LBs = lipid bodies. Colour coding refers Anonymous (1976).

DNA was extracted from mycelia of cultures grown on agar plates from germinated ascospores from fresh collections, or from dried apothecia taken from fungarium specimens. DNA was extracted and amplified using PCR following the methods of Johnston & Park (2013). Amplification primers used for the ITS1-5.8S-ITS2 region were ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993), for the LSU region were LROR and LR5 (Bunyard *et al.* 1994, Vilgalys & Hester 1990), and for the SSU region were NS1 and NS4 (White *et al.* 1990). Purified PCR products were directly sequenced using the same primer pairs as in the PCR reactions. Partial sequences obtained in sequencing reactions were assembled with Sequencher 4.10.1 (Genecodes Corporation, Ann Arbor, MI). All sequences were deposited in GenBank (Table 1).

Fig. 1. Historical survey of systematic concepts of *Phacidiales*. Only information about the authors that accepted *Phacidiales* as an order is included. For each concept of the order, families are included in a black box and genera in a grey box, names in red are currently not accepted. Symbols at the right side of the box indicate the current ordinal placement of each genus according to Index Fungorum (2018) and Baral (2016), see explanation of symbols above.

Table 1. Specimens used in this study with family information and GenBank accession numbers. Sequences of the new species are indicated in **bold**.

Species	Family	GenBank number		
		ITS	LSU	SSU
<i>Sarcoleotia globosa</i> 1	<i>Geoglossaceae</i>	AY789410	AY789409	
<i>Sarcoleotia globosa</i> 2	<i>Geoglossaceae</i>	AY789300	AY789299	AY789298
<i>Epithamnolia</i> on <i>Candelaria</i> (HA90)	<i>incertae sedis</i> s. Suija et al. (2017)	KY814532	KY814513	KY814524
<i>Epithamnolia</i> on <i>Lecanora</i> (HA92)	<i>incertae sedis</i> s. Suija et al. (2017)	KY814526	KY814508	KY814519
<i>Mniaecia jungermaniae</i>	<i>Mniaecia</i> lineage	EU940185	EU940109	EU940036
<i>Mniaecia nivea</i>	<i>Mniaecia</i> lineage	EU940188	EU940115	EU940042
<i>Mniaecia gloeocapsae</i>	<i>Mniaecia</i> lineage	EU940204	EU940128	EU940055
<i>Trizodia acrobia</i> 1	<i>Mniaecia</i> lineage	EU940190	EU940113	EU940040
<i>Trizodia acrobia</i> 2	<i>Mniaecia</i> lineage	EU940191	EU940114	EU940041
<i>Bulgaria inquinans</i>	<i>Phacidiaceae</i>	KJ663831	DQ470960	DQ471008
<i>Phacidium lacerum</i>	<i>Phacidiaceae</i>	KJ663841	DQ470976	DQ471028
<i>Phacidiopycnis pyri</i>	<i>Phacidiaceae</i>	DQ491510	DQ470949	DQ470997
<i>Allantophomopsis lunata</i>	<i>Phacidiaceae</i>	KR873229	KR873263	
<i>Phacidium lauri</i>	<i>Phacidiaceae</i>	KJ663850	KJ663891	
<i>Geltingia associata</i> 1	<i>Helicogoniaceae</i>	KJ559540	KJ559562	KJ559584
<i>Geltingia associata</i> 2	<i>Helicogoniaceae</i>		KJ559576	KJ559580
<i>Eleutheromyces subulatus</i> 1	<i>Helicogoniaceae</i>	NR145309	EU754162	EU754063
<i>Eleutheromyces subulatus</i> 2	<i>Helicogoniaceae</i>	KJ710468	KJ710444	
<i>Eleutheromyces subulatus</i> 3	<i>Helicogoniaceae</i>		EU754161	EU754062
<i>Collophorina africana</i> 1	<i>Tympanidaceae</i>	GQ154570	GQ154609	GQ154630
<i>Collophorina africana</i> 2	<i>Tympanidaceae</i>	GQ154571	GQ154610	GQ154631
<i>Collophorina paarla</i> 1	<i>Tympanidaceae</i>	GQ154586	GQ154613	GQ154634
<i>Collophorina paarla</i> 2	<i>Tympanidaceae</i>	GQ154575	GQ154611	GQ154632
<i>Collophorina rubra</i>	<i>Tympanidaceae</i>	GQ154547	GQ154606	GQ154627
<i>Holwaya mucida</i> 1	<i>Tympanidaceae</i>	KT225524	AY544680	AY544729
<i>Holwaya mucida</i> 2	<i>Tympanidaceae</i>	DQ257357	DQ257356	DQ257355
<i>Myriodiscus sparassoides</i>	<i>Tympanidaceae</i>	JX219379	JX219381	JX219377
<i>Claussenomyces prasinulus</i>	<i>Tympanidaceae</i>		KX090815	KX090866
<i>Aotearomyces nothofagi</i> ICMP 21969	<i>Tympanidaceae</i>	KM677201	MG807387	MG807391
<i>Aotearomyces nothofagi</i> ICMP 21968	<i>Tympanidaceae</i>	KM677202	MG807386	MG807390
<i>Aotearomyces nothofagi</i> PDD 106298	<i>Tympanidaceae</i>	MG807392	MG807388	MG807389

Phylogenetic analyses

An analysis using three different rDNA regions (SSU, ITS, LSU) for the representative members of *Phacidiales* was performed. This includes taxa from three families: *Phacidiaceae* (5 seq.), *Helicogoniaceae* (5 seq.) and *Tympanidaceae* (12 seq.). Also, five sequences of the *Mniaecia* lineage were included, and two representing the genus *Epithamnolia*, which was recently placed in *Phacidiales* as *incertae sedis* (Suija et al. 2017). Thirty-one taxa were used for the molecular analysis (Table 1). The sequences were aligned using the L-INS-i algorithm for the ITS region, and G-INS-i algorithm for SSU & LSU (Katoh & Toh 2008) with MAFFT v7.017 (Katoh et al. 2002). The

program Gblocks v. 0.91b was used to identify and eliminate ambiguously aligned regions (Castresana 2000), using the following relaxed settings (Talavera & Castresana 2007): minimum number of sequences for a conserved or flanking position= 16; maximum number of contiguous non-conserved position= 10; minimum length of a block= 5; and gaps in an alignment column allowed in up to half the number of included sequences. The analyses were performed using the optimal model of nucleotide substitution identified with JModeltest (Posada 2008; <http://darwin.uvigo.es>), based on the Akaike information criterion (Akaike 1974). Maximum likelihood (ML) and Bayesian Inference (BI) analyses were performed using

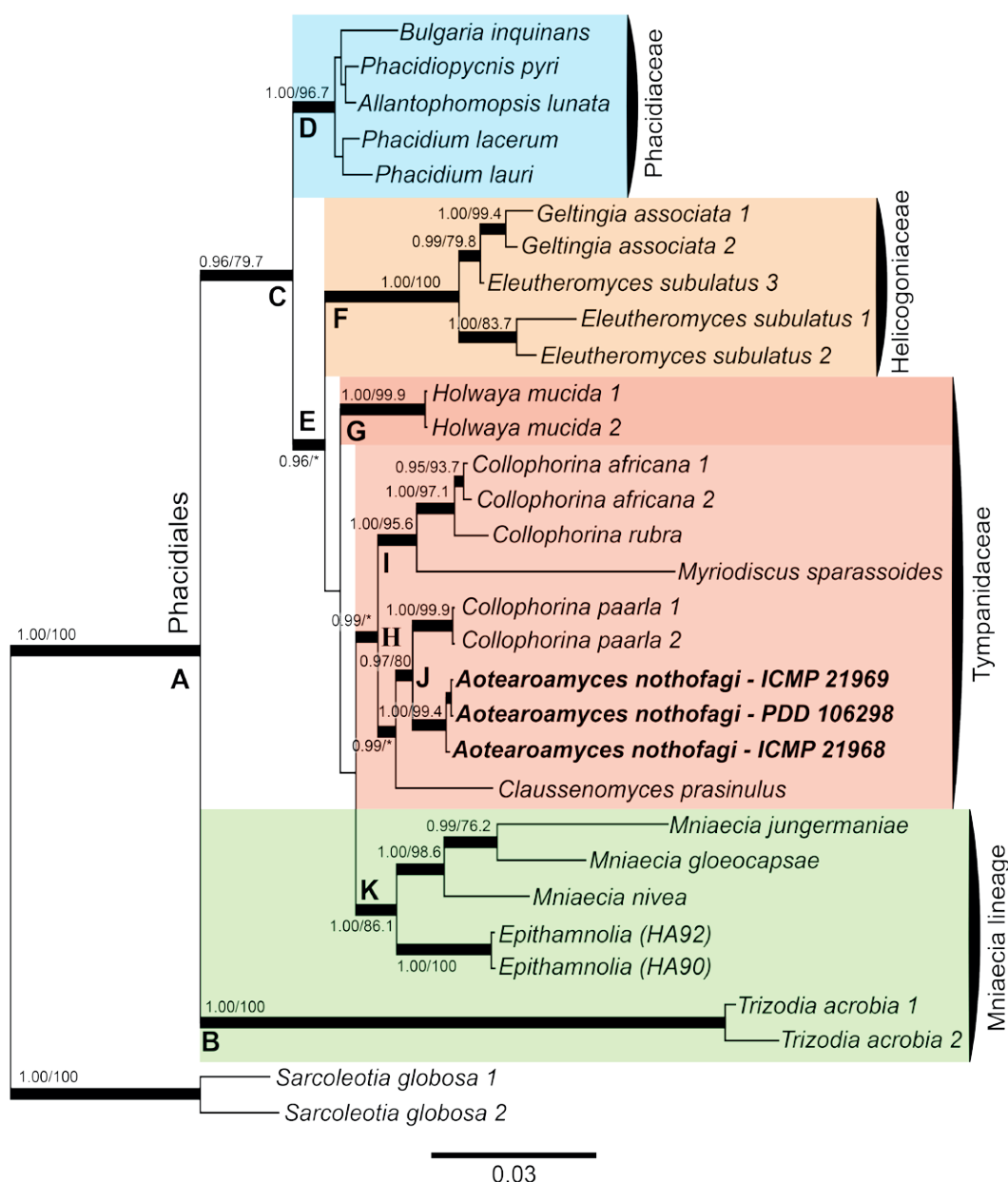


Fig. 2. Bayesian majority-rule consensus tree based on concatenated SSU, ITS, and LSU sequences. Bold branches are those which were well supported (see Methods) by ML/BI methods. Asterisks indicate a branch supported by only Bayesian methods.

Geneious v.6.1.7. Bayesian inference analyses followed Quijada *et al.* (2014), only varying in the number of starting trees (10 million generations) and the tree sampling (every 1000th generation) for BI analysis. Branch support in ML was inferred from 1000 rounds of bootstrap. We only considered supported clades for ML those with bootstraps values $\geq 75\%$ and with $PP \geq 0.95$ (strongly supported) for BI. Phylogenetics trees were drawn with Geneious and artwork was prepared in Adobe Illustrator CS5.

RESULTS

Relationships among the members of *Phacidiales* were investigated for three regions (SSU, ITS, and LSU). The final alignment used for the phylogenetic analyses contained 3015 bp, with 599 variable and 405 parsimony-informative

positions. The analyses identified at least 11 strongly supported clades (Fig. 2, clades A-K). *Phacidiales* (clade A: 1.00 BIPP, 100 MLBS) includes two main subclades: clade B (*Trizodia*, previously tentatively placed in the *Mniaecia* lineage; Baral 2016) and clade C (*Mniaecia* lineage; *sensu* Baral (*loc.cit.*) p.p., *Tympanidaceae*, *Phacidiaceae*, and *Helicogoniaceae*). The monophyletic clade K (1.00 BIPP, 86.1 MLBS) contains two genera (*Epithamnolia* and *Mniaecia*). Clade E (0.96 BIPP, 47.4 MLBS) contains *Helicogoniaceae*, *Tympanidaceae* and the *Mniaecia* lineage. *Phacidiaceae* (clade D: 1.00 BIPP, 96.7 MLBS) and *Helicogoniaceae* (clade F: 1.00 BIPP, 100 MLBS) are monophyletic. *Tympanidaceae* is paraphyletic. *Holwaya* appears supported in a different clade (clade G: 100 BIPP, 99.9 MLBS) with respect to the other genera in *Tympanidaceae* (clade H: 0.99 BIPP, 51.7 MLBS). The genus *Collophorina* is paraphyletic and its members are in two clades of *Tympanidaceae*. *Collophorina*

rubra and *C. africana* are together with *Myriodiscus* (clade I: 1.00 BIPP, 95.6 MLBS), and *Aotearoamyces* appears as a supported monophyletic clade in a sister relationship to *Collophorina paarla* (clade J: 0.97 BIPP, 80 MLBS).

TAXONOMY

***Aotearoamyces* P.R. Johnst., J.A. Cooper & Quijada, gen. nov.**

Mycobank MB825175

Etymology: The generic name refers to the indigenous name of New Zealand (*Aotearoa*) and the Greek name for fungi (*myces*).

Diagnosis: The sexual morph of *Aotearoamyces* resembles *Holwaya mucida*, but the apothecia are turbinate with the disc plane or slightly convex. Ascus and ascospore shape are similar to species of *Claussenomyces*, but without production of conidia or ascoconidia directly from the ascospores. *Durandiella* and *Tympanis* have similar exciples, but *Aotearoamyces* differs in hyphae that are strongly spaced and gelatinized. It differs from all the others members in *Tympanidaceae* by the curved or helicoid paraphyses. The asexual morph lacks endoconidia in the vegetative hyphae; conidiophores occur in well-developed synnemata; and conidia are small, 0-septate, hyaline and curved, formed by phialidic conidiogenesis.

Type species: *Aotearoamyces nothofagi* P.R. Johnst. et al. 2018

Classification: *Tympanidaceae*, *Phacidiales*, *Leotiomyces*, *Pezizomycotina*, *Ascomycota*, *Fungi*.

Description: *Ascomata* apothecia, black, erumpent, short to medium long stipitate (to 1 mm tall), pulvinate-discooid to turbinate, solitary, or more commonly clustered in groups and arising from a common gelatinous stromatic base. *Asci* 8-spored, slightly thick-walled towards apex, inamyloid, arising from croziers. *Ascospores* cylindric-fusoid to fusoid-clavate and phragmoseptate, rarely with a longiseptum. *Paraphyses* apically flexuous to helicoid or curving downwards (hooked) and embedded in an olive-brown gelatinous matrix. *Exciple* of *textura intricata*, innermost layer of the exciple composed of a loose network of narrow hyphae, widely spaced and embedded in an abundant light brown gelatinous matrix (plectenchyma); outer ectal exciple with pustules composed of closely septate, prismatic to angular cells, dark brownish, cells more densely packed than in the inner ectal exciple, cells covered by a dark brown pigmented exudate. *Asexual morph* in culture with short-cylindric, curved, 0-septate, hyaline conidia formed at a single, apical conidiogenous locus on flask-shaped, phialidic conidiogenous cells. *Conidiogenous cells* solitary or with several cells held on a

single, short, cylindric basal cell, on hyphae grouped into ropey, synemmatous structures.

***Aotearoamyces nothofagi* P.R. Johnst., J.A. Cooper & Quijada, sp. nov.**

Mycobank MB825176

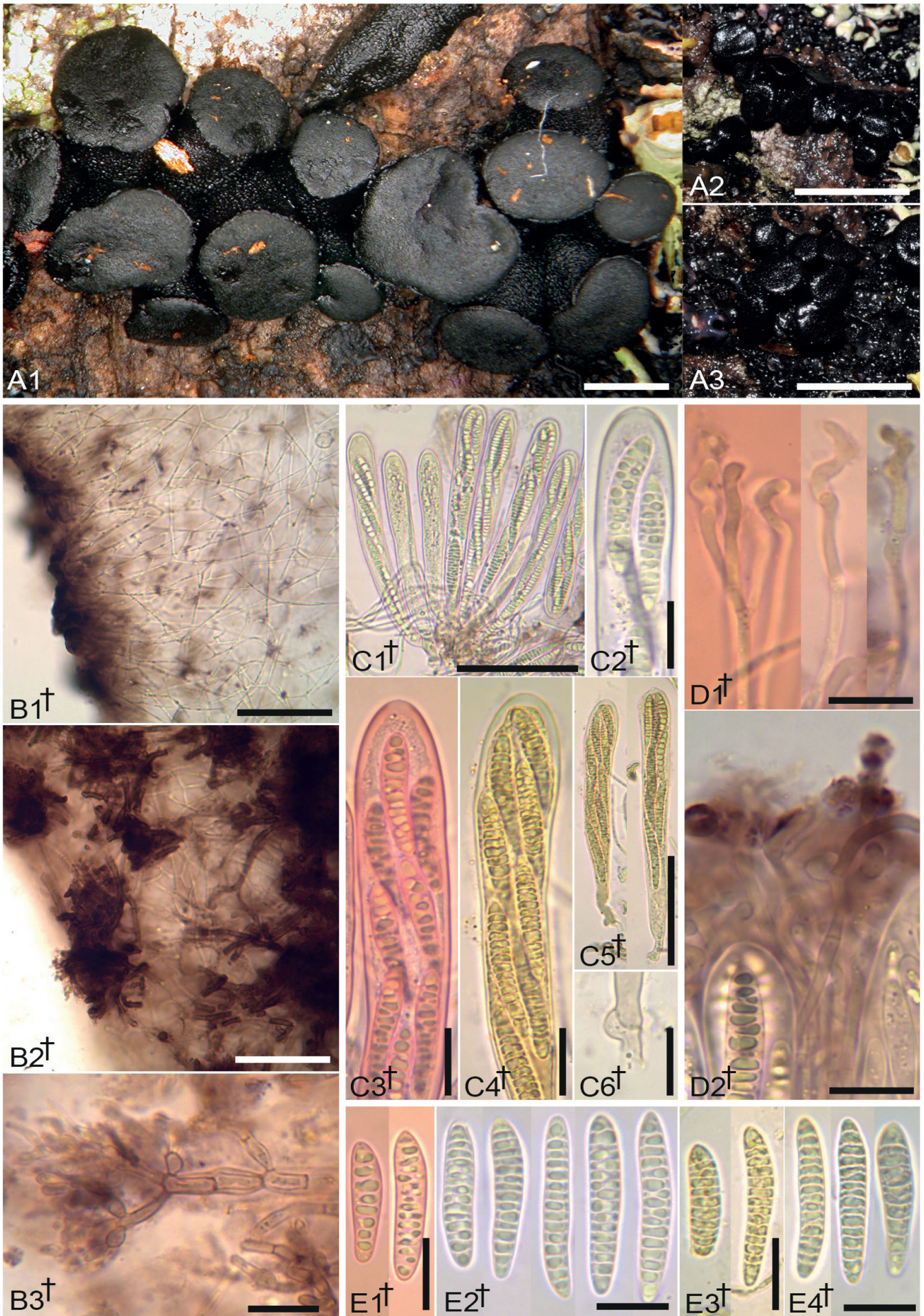
Etymology: The specific epithet refers to the generic name of the host plant in the holotype (*Nothofagus*).

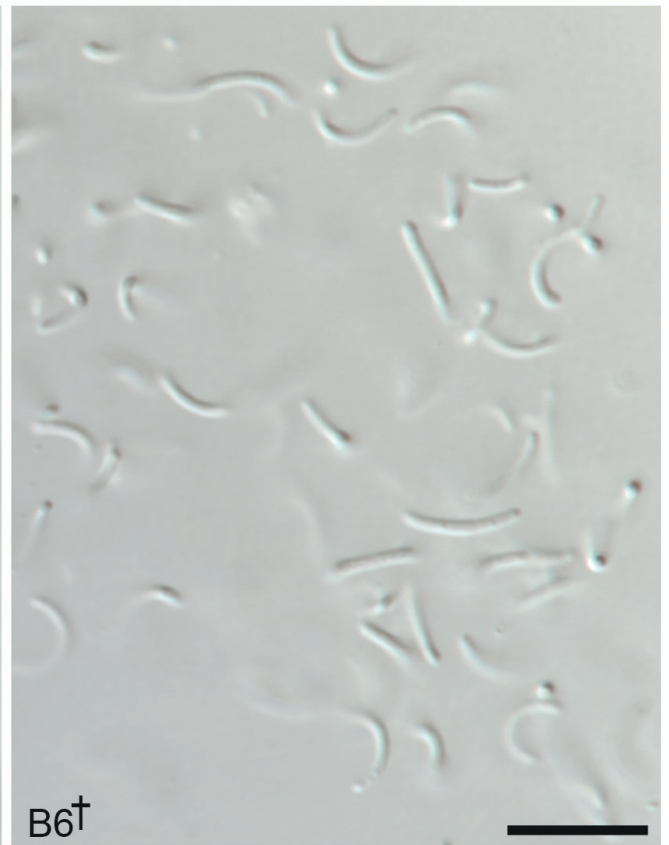
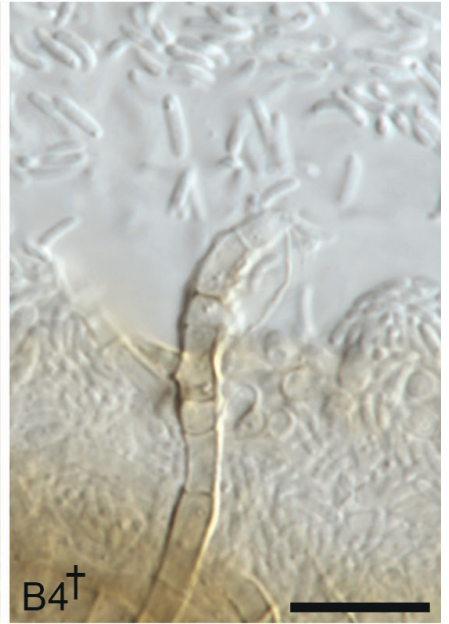
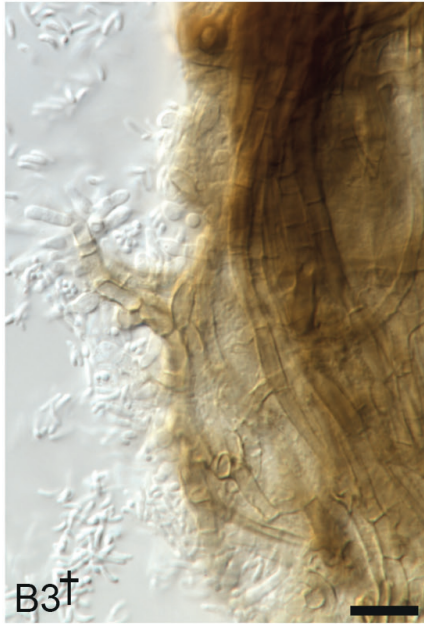
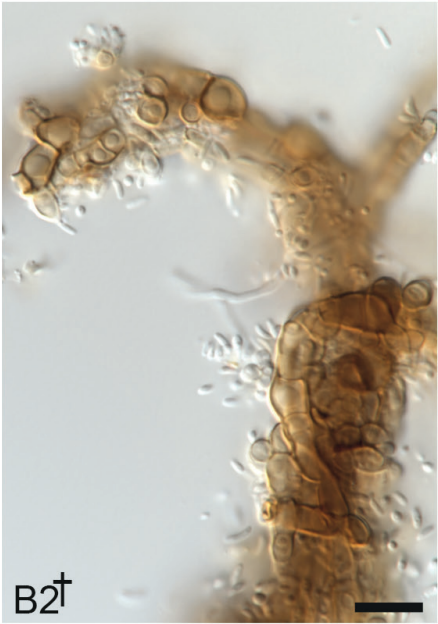
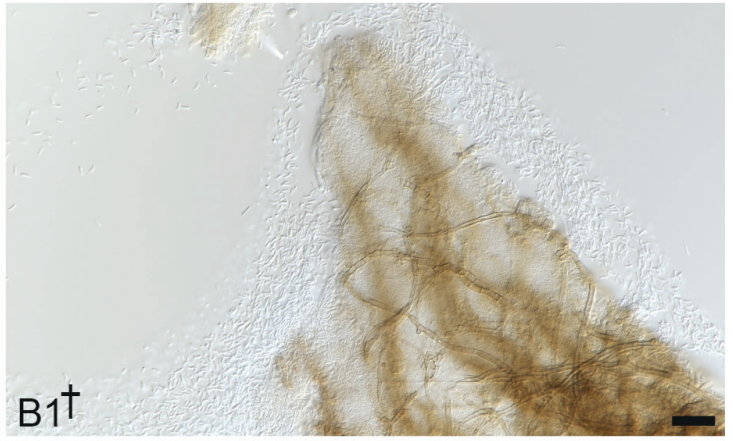
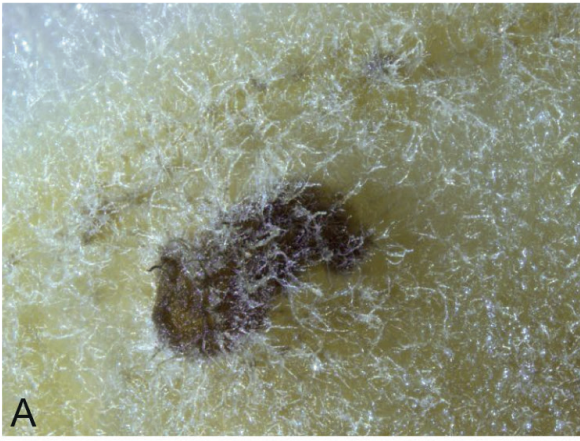
Diagnosis: *Apothecia* black, to 1 mm diam and height, erumpent, short to medium long stipitate, pulvinate-discooid to turbinate, arising from a common gelatinous stromatic base. *Asci* †83–124 × 10.5–14.5 μm, 8-spored, inamyloid, arising from croziers. *Ascospores* †17.5–31 × 3–5 μm, cylindric-fusoid-clavate, 7–16 phragmoseptate rarely with a longiseptum. *Paraphyses* apically up to †1.5–2(2.5) μm wide, flexuous to helicoid or curving downwards (hooked) and embedded in an olive-brownish gelatinous matrix. *Conidia* observed in culture, produced from phialides. *Conidiogenous cells* held on well-developed conidiophores arranged in small synnematos structures, forming consistently curved vermiform conidia. *Conidiomata* not observed in cultures.

Type: **New Zealand:** *South Island:* Craigiebrun, on *Nothofagus solandri*, 7 May 2010, N. Siegel (PDD 95741 – holotype).

Description: *Apothecia* pulvinate-discooid to turbinate, 0.4–1 mm diam, strongly gelatinous, erumpent from bark, disc plane to slightly convex when fresh, round or somewhat irregular when crowded; margin thin, distinct, slightly lacerate, short to medium long stipitate (0.2–0.7 mm diam), stipe tapering downward, apically almost as broad as disc; in groups, rarely solitary, arising from a common gelatinous stromatic base; black (267.Black) to deep greyish blue (187.d.gy.B), shiny when moist, shrinking on drying to ± half the size; exterior strongly roughened. *Asci* †(83–)101.5–109.5(–124) × (10.5–) 12–13(–14.5) μm, cylindric-clavate, 8-spored, inamyloid, apex hemispherical, spores 2–3-seriate, arising from croziers; ascus wall at apex and partly also laterally slightly thickened in dead state to †0.5–1.5(–2) μm. *Ascospores* †(17.5–)23.5–25.5(–31) × (3–)3.5–4(–5) μm, cylindric-fusoid to fusoid-clavate, ends obtuse to subacute, hyaline, straight or slightly curved, with (7–)13–14(–16) transversal septa (rarely 1 longiseptum), each cell with one refractive lipid guttule (LBs, tested in KOH), never seen to form conidia on the spores. *Paraphyses* filiform, apex cylindrical to slightly clavate, flexuous to helicoid or curving downwards (hooked), agglutinated and intertwined among each other, embedded in an olive-brownish gelatinous matrix, terminal cell †(7–)11–17(–27.5) × 1.5–2(–2.5) μm, cell below †(10–) 13–16.5(–19.5) × (1–)1.5–2 μm, frequently branched at apex, cells ± equidistantly septate but terminal cell slightly shorter than lower cells. *Ectal exciple* †150–600 μm thick, inner layers of *textura intricata* composed of a loose net of

Fig. 3. Morphological features of *Aotearoamyces nothofagi* (PDD 95741, 80575). **A.** Apothecia in fresh state. **B.** Exciple: **B1–2.** section at flank, **B3.** Ectal exciple cells at flank. **C.** Asci. **D.** Paraphyses. **E.** Ascospores. Dead state, mounted in: CR = C3, D1, E1; KOH = B1-3, C1-2, C6, D2, E2, E4; MLZ = C4-5, E3. Bars: A1 = 500 μm; A2-3 = 2 mm; B1-2, C1, C5 = 50 μm; B3, C2-4, C6, D1-2, E1-4 = 10 μm.





narrow, hyaline hyphae, distantly septate, strongly spaced, \pm vertically oriented and embedded in an abundant, light brown gelatinous matrix; outermost layer $\dagger(5.5\text{--})7\text{--}13\text{--}(17.5)$ μm thick at margin, $\dagger(16.5\text{--})24.5\text{--}37.5\text{--}(64)$ μm thick at flanks, with pustules of closely septate, prismatic to angular cells, dark brownish, thick-walled and frequently branched, covered with a dark brown pigmented exudate, individual cells $\dagger6\text{--}10 \times 2.5\text{--}3$ μm at margin, $\dagger(4\text{--})6\text{--}7.5\text{--}(9) \times (1.5\text{--})2.5\text{--}3\text{--}(4)$ μm at lower flank and base, cell walls $\dagger0.5\text{--}1$ μm thick. *Medullary exciple* indistinctly differentiated from the ectal exciple and progressively changing toward the hymenium the hyphae becoming more closely spaced, hyphae $\dagger(0.5)1\text{--}1.5$ μm wide. *Tissues* releasing a yellowish pigment in KOH. *Culture* from germinated ascospores about 40 mm diam after 4 wk, aerial mycelium sparse, grouped in ropey strands on which the conidia are formed, colonies dark olivaceous to dark reddish brown. *Asexual morph in culture* with curved, 0-septate, hyaline conidia $\dagger(3\text{--})4\text{--}7.5\text{--}(8.5) \times (0.5\text{--})1\text{--}(1.5)$ μm , formed on flask-shaped conidiogenous cells $\dagger(4\text{--})5.5\text{--}7\text{--}(9) \times (1.5\text{--})2\text{--}(3.5)$ μm , conidiogenous cells sometimes in groups of 3–4 held on a simple basal conidiophore of $\dagger(4.5\text{--})6\text{--}(8.5) \times (1.5\text{--})2.5\text{--}(3)$ μm , conidiogenesis phialidic without collarete.

Other specimens examined: New Zealand: South Island: Abel Tasman National Park, on unidentified wood, 14 May 2004, P.R. Johnston D1844 (PDD 80575, ICMP 21037); Arthur's Pass National Park, on unidentified wood, 5 May 1989, P.R. Johnston D368, G.L. Barron, P.K. Buchanan & M. Rajchenberg (PDD 55517, ICMP 21038); Otago Lakes, Routeburn Track carpark, on unidentified fallen wood in Nothofagaceae forest, 7 May 2016, S. McMullan-Fisher (PDD 110269).

DISCUSSION

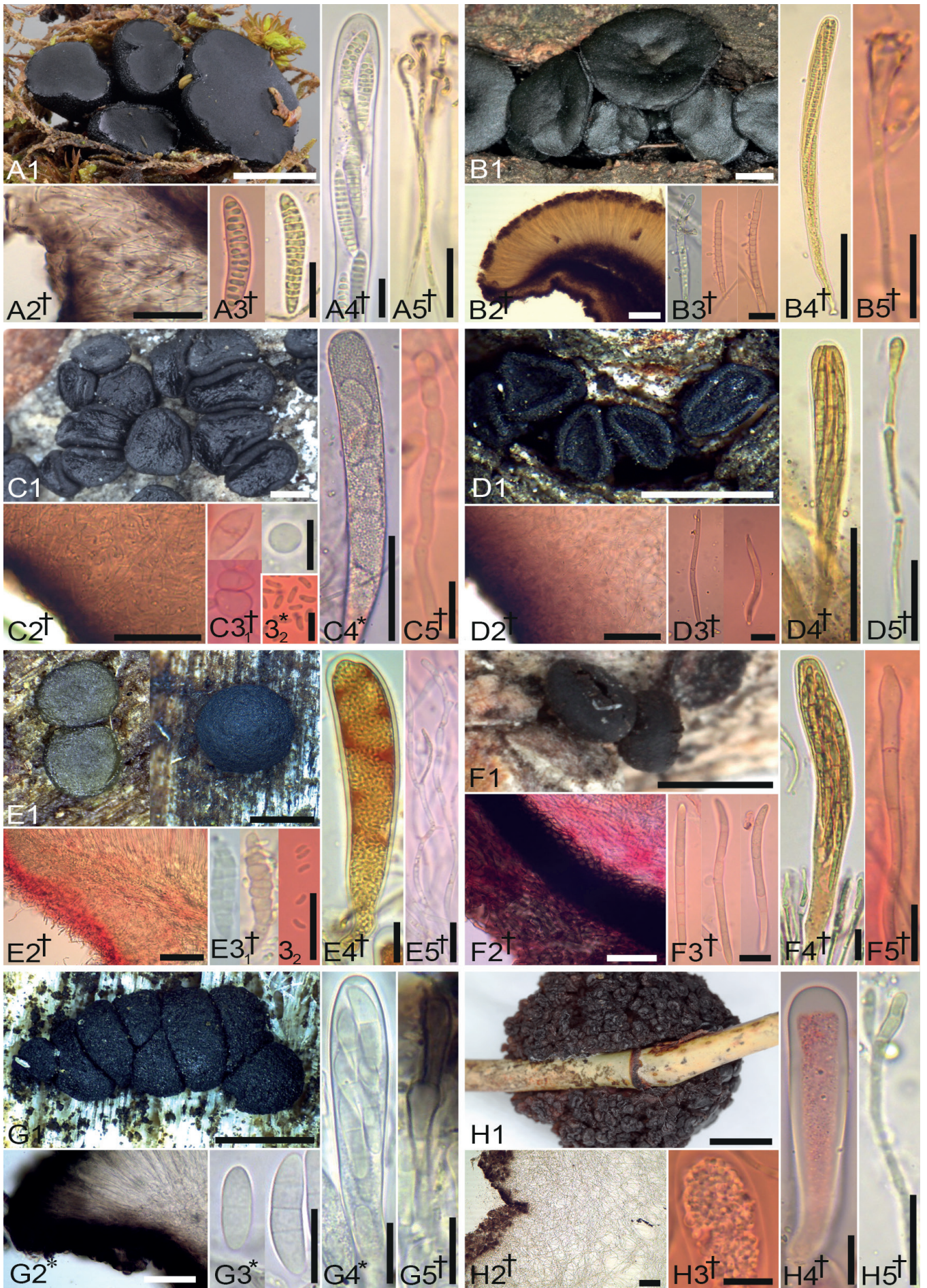
Throughout its history, the number of species, genera and families in the order *Phacidiales* has changed considerably (Fig. 1). The order as circumscribed by Bessey (1907), who included six genera and three families, was differently conceived by Höhnelt (1917), who expanded the order to include 52 genera in six families. In the 1970s (e.g. Korf 1973, Dennis 1978) the rhytismataceous fungi were often included in *Phacidiales*, although today they are placed in the separate order *Rhytismatales*. The most current classification of *Phacidiales* includes about 29 genera, most of them distributed across three families and one informal taxonomic lineage (Crous *et al.* 2014, Baral 2016, Suija *et al.* 2017). These changing concepts reflect the changes in emphasis placed on macro- and micromorphological features, as well as the impact of molecular phylogenetics. Molecular studies have allowed genera known only from an asexual morph, such as *Collophorina*, to be placed in *Phacidiales* (Baral *loc. cit.*). Our phylogenetic analyses allowed placement of *Epithamnolia*, a conidial fungus previously reported as *incertae sedis* in *Phacidiales* (Suija *et al.* 2017), in the *Mniaecia* clade for the first time.

Five species, known only from asexual morphs that were isolated from woody necroses in peach and nectarine, were included when Damm *et al.* (2010) erected the genus *Collophora* with *C. africana*, *C. capensis*, *C. paarla*, *C. pallida*, and *C. rubra*, the type species). Since that name was illegitimate as a later homonym of *Collophora* Mart. 1830, *Apocynaceae*, the species were recombined into the new genus *Collophorina*, and the number of species reduced from seven to five due to synonymy of *C. capensis* with *C. africana*, and *C. pallida* with *C. paarla* (Wijayawardene *et al.* 2017). Damm *et al.* (2010) placed the genus in *Leotiomyces* as *incertae sedis*. In the same work, the authors remarked “although these species form two clades in the LSU phylogeny, they are placed in one genus, because of their similar morphological features and the lack of morphological characters distinguishing the two clades”. In our analyses, the genus is also paraphyletic in agreement with Damm *et al.* (2010) (Fig. 2): *Collophorina paarla* belongs in one supported clade (Fig. 2, Clade J), and *C. africana* and the type species *C. rubra* in a different strongly supported clade.

In the discussion about *C. pallida*, Damm *et al.* (2010) said that “*C. paarla* and *C. pallida* are the only *Collophora* species for which endoconidia have been observed”. This morphological feature could be used to support the splitting of *Collophorina* into at least two genera. *Aotearoamyces* is most closely related to the clade containing the *Collophorina* species with endoconidia, but we did not see any endoconidia form in our culture studies. Compared to Damm *et al.*'s illustrations and descriptions, the conidiogenous cells of *Aotearoamyces nothofagi* are held on well-developed synnematosus conidiophores bearing conidia that are consistently curved.

The sexual morph of *Aotearoamyces* shares several morphological traits with *Tympanidaceae* (Fig. 5): (1) the asci are inamyloid, apically and/or laterally thick-walled and arising from croziers (Fig. 5, A4–H4); (2) the ascospores are phragmosporous, cylindrical-fusoid to fusiform-clavate (Fig. 5, A3–H3); and (3) the paraphyses are usually agglutinated and embedded in a dark amorphous exudate (Fig. 5, A5–H5). However, *Aotearoamyces* also differs in many aspects: conidia are not present inside the asci or attached to ascospores (Fig. 5, C3₂ and E3₂), which allows it to be distinguished from *Holwaya*, *Tympanis* and most *Claussenomyces* species (Fig. 5, B3). *Claussenomyces jahniianus*, lacking reports of conidia formed on ascospores, can be differentiated from *Aotearoamyces* by the acicular ascospores and apically moniliform, closely septate paraphyses (Quijada 2015). The exciple of *Aotearoamyces*, of *textura intricata* with widely spaced hyphae immersed in gel (Fig. 5, A2), differs completely from the exciple in *Grovesiella* (Fig. 5, F2: *textura angularis* to *t. prismatica*) and *Pragmopora* (Fig. 5, G2: *t. oblita*); these genera also differ in the paraphyses never being helicoid or hooked at the apex as those in *Aotearoamyces* (Fig. 5, A5). The genera *Myriodiscus* (Fig. 5, H2), *Durandiella* (Fig. 5, D2), and *Aotearoamyces* have a similar plectenchymatous exciple. *Durandiella* differs in the morphology of the paraphysis apex

Fig. 4. Cultural features of *Aotearoamyces nothofagi* (PDD 95741, 55517; ICMP 21037, 21038). **A.** A part of an apothecium in culture. **B1–4.** Vegetative hyphae. **B5.** Conidiogenous cells. **B6.** Conidia. All in dead state (mounted in KOH). Bars: B1 = 20 μm ; B2–6 = 10 μm .



(Fig. 5, D5: straight vs. 1e: curved to helicoid) and ascospores (Fig. 5, D3: acicular-fusiform to falcate vs. A3: cylindrical-fusoid to fusoid-clavate); and *Myriodiscus* differs in having polysporous asci (Fig. 5, H3) and in macroscopic appearance (Fig. 5, H1: discoid apothecia aggregated in a subglobose fructification vs. A1: turbinate apothecia sharing a stromatic base). Given the above, we concluded that *Aotearomyces* is a new monotypic genus in *Phacidiales*, phylogenetically related to "*Collophorina*" *paarla* and morphologically sharing several features with other genera of *Tympanidaceae* that have a sexual morph.

ACKNOWLEDGEMENTS

L.Q. thanks the "Fundación Ramón Areces" for support. This study is part of the project "DNA barcoding for plant-pathogens diagnostic and monitoring: Forest diseases and turbo-taxonomy in *Tympanidaceae* as a case of study", and also the fellowship programme Becas Fundación Ramón Areces para Estudios Postdoctorales, XXIX Convocatoria para Ampliación de Estudios en el Extranjero en Ciencia de la Vida y de la Materia. P.R.J. and J.A.C. were supported with funding from the Science and Innovation Group of the New Zealand Ministry of Business, Innovation and Employment through the Manaaki Whenua Landcare Research Systematics Portfolio. Also, we would like to thank Hans-Otto Baral for revising the manuscript.

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Fig. 5. Morphological features of *Aotearomyces nothofagi* compared to those of other genera in *Tympanidaceae*: **A.** *Aotearomyces nothofagi* (PDD 95741, 80575). **B.** *Holwaya mucida* (Dragisa Savic herb. without number; CUP 60122, 2006) **C.** *Tympanis* spp. (NYBG 423829, 1168034, 1168036; TAAM 165632) **D.** *Durandiella gallica* (S F196603, Baral herb. HB 9244). **E.** *Claussenomyces* spp. (TFCMic. 23325, 23950, 24408, 24011; CUP 3938). **F.** *Grovesiella abieticola* (DAVFP 23014; NYBG 1293222). **G.** *Pragmopora amphibola* (TFCMic. 23861, 23922, 23726). **H.** *Myriodiscus sparassoides* (TFCMic. 24582). Morphological features compared: 1. Apothecia, 2. section of the exciple, 3. ascospores (with or without conidia or ascoconidia), 4. asci, and 5. paraphyses. Mounted in: water = C4, D2, G2–G4, KOH = A2, A4, C2, E3₁, G5, H2, H5; CR = A3, B3, B5, C3₁₋₂, C5, D3, E2, E3₂, E5, F2–3, F5, H3–4; MLZ = A3, B2, B4, D4–5, E4, F4. Bars: A1–H1 = 500 µm; B2, H2 = 100 µm; A2, C2–G2, B4, C4–D4, D5 = 50 µm; A3–B3, C3₁, D3, E3₁, F3–H3, A4, E4–H4, A5–C5, E5–H5; 5 = 10 µm.

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