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 Tympaniaceae, a recently erected family in Phacidiales. Aotearoamyces is differentiated from other Tympaniaceae by phragmospores that do not form conidia either in or outside the ascii, 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 Mniaecea lineage within Phacidiales.

Key words: Ascomycota Claussenomyces new taxa Nothofagus phylogeny Rhytismatales Leotiomyces

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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, Höhnel (1917) used the same ordinal name for six families and 52 genera (Fig. 1): Schizothryiaceae (5 genera), Leptopeltineaceae (13 genera), Dermopeltineaceae (10 genera), Phacidicaeaceae (12 genera), Phacidostromaceae (4 genera) and Cryptomyceteaceae (8 genera). Nowadays the only genus among those listed by Bessey or Höhnel which remains in the Phacidiales is the type genus Phacidium (Fig. 1). Most of the genera treated by Höhnel (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. Höhnel (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 Höhnel’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 excipule. 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 Höhnel generally included three families (Fig. 1): Cryptomyceteaceae, Hypodermataceae or Rhytismataceae, and Phacidiaeaceae (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 Phacidiaeaceae was applied in a more restricted sense, including Phacidium and two or three other
genera. Some authors even considered Phacidiaceae as a synonym of Rhytismales (Hawksworth et al. 1983). In other cases, Phacidiaceae, Cryptomycetaceae and Rhytismataceae were included in Rhytismatales (Hawksworth et al. 1983, 1995). Rhytismatales and Hypodermales included many genera previously placed in Phacidiaceae (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 (Ascosoma, 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 Phacidiaceae 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 Phacidiaceae of earlier authors. Although DiCosmo et al. (1984) reported anamorphs for some members in Phacidiaceae, 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 Leotiomycetes 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 Phacidiaceae 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, Starbaekia, and questionably Gremmenia. Also, the priority of Phacidiopycnis over the sexually typified Potebniamycies was indicated. Two new families were included in the order: Tympaniaceae and Helicogoniaceae. In addition to these three families in Phacidiaceae, 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 Ascosoma). Subsequently, Suija et al. (2017) placed the monotypic genus Epithecium as incertae sedis in Phacidiaceae, due to its phylogenetic and morphological affinities with the asexual morph of Epigilia (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 Phacidiaceae, 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 “Collopohrina” paarta 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 (Manuaki Whenua Landcare Research, Auckland) and living cultures grown from ascospores from the fresh specimens are stored in the ICMP culture collection (Manuaki 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 cultures grown from ascospores from the fresh 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. 1993), for the SSU region were NS1 and NS4 (White et al. 1990), and for the LSU region were ITS1F and ITS4 (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 Phacidiaceae. Only information about the authors that accepted Phacidiaceae 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       |
|----------------------------------------------|-------------------------------------|----------------------|
| Sarcoleotia globosa 1                        | Geoglossaceae                       | AY789410 AY789409    |
| Sarcoleotia globosa 2                        | Geoglossaceae                       | AY789300 AY789299 AY789298 |
| Epithamnolia on Candelaria (HA90)             | incertae sedis s. Suija et al. (2017) | KY814532 KY814513 KY814524 |
| Epithamnolia on Lecanora (HA92)              | incertae sedis s. Suija et al. (2017) | KY814526 KY814508 KY814519 |
| Mniaceia jungermaniae                        | Mniaceia lineage                    | EU940185 EU940109 EU940036 |
| Mniaceia nivea                               | Mniaceia lineage                    | EU940188 EU940115 EU940042 |
| Mniaceia gloeocapsae                         | Mniaceia lineage                    | EU940204 EU940128 EU940055 |
| Trizodia acrobia 1                           | Mniaceia lineage                    | EU940190 EU940113 EU940040 |
| Trizodia acrobia 2                           | Mniaceia lineage                    | EU940191 EU940114 EU940041 |
| Bulgaria inquinans                           | Phacidiaceae                        | KJ663831 DQ470960 DQ471008 |
| Phacidium lacerum                            | Phacidiaceae                        | KJ663841 DQ470976 DQ471028 |
| Phacidopycnis pyri                           | Phacidiaceae                        | DQ491510 DQ470949 DQ470997 |
| Allantophomopsis lunata                      | Phacidiaceae                        | KR873229 KR873263    |
| Phacidium lauri                              | Phacidiaceae                        | KJ663850 KJ663891    |
| Geltingia associata 1                         | Helicogoniaceae                     | KJ559540 KJ559562 KJ559584 |
| Geltingia associata 2                         | Helicogoniaceae                     | KJ559576 KJ559580    |
| Eleutheromyces subulatus 1                   | Helicogoniaceae                     | NR145309 EU754162 EU754063 |
| Eleutheromyces subulatus 2                   | Helicogoniaceae                     | KJ710468 KJ710444    |
| Eleutheromyces subulatus 3                   | Helicogoniaceae                     | EU754161 EU754062    |
| Collophorina africana 1                      | Tympanidaceae                       | GQ154570 GQ154609 GQ154630 |
| Collophorina africana 2                      | Tympanidaceae                       | GQ154571 GQ154610 GQ154631 |
| Collophorina paarla 1                        | Tympanidaceae                       | GQ154586 GQ154613 GQ154634 |
| Collophorina paarla 2                        | Tympanidaceae                       | GQ154575 GQ154611 GQ154632 |
| Collophorina rubra                           | Tympanidaceae                       | GQ154547 GQ154606 GQ154627 |
| Holwaya mucida 1                              | Tympanidaceae                       | KT225524 AY544680 AY544729 |
| Holwaya mucida 2                              | Tympanidaceae                       | DQ257357 DQ257356 DQ257355 |
| Myriodiscus sparassoides                     | Tympanidaceae                       | JX219379 JX219381 JX219377 |
| Claussenomyces prasinulus                    | Tympanidaceae                       | KX090815 KX090866    |
| Aotearoamyces nothofagi ICMP 21969            | Tympanidaceae                       | KM677201 MG807387 MG807391 |
| Aotearoamyces nothofagi ICMP 21968            | Tympanidaceae                       | KM677202 MG807386 MG807390 |
| Aotearoamyces nothofagi PDD 106298            | Tympanidaceae                       | 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 Mniaceia 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 (Katoth & Toh 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
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 ≥75 % and with PP≥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 embedded in an olive-brown gelatinous matrix.

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

**Classification:** Tympanidaceae, Phacidiaceae, Leotiomycetes, Pezizomycotina, Ascomycota, Fungi.

**Description:** Ascomata apothecia, black, erumpent, short to medium long stipitate (to 1 mm tall), pulvinate-discid to turbinate, arising from a common gelatinous stromatic base. Asci †83–124 × 10.5–14.5 µm, 8-spored, inamyloid, arising from croziers. Ascospores cylindric-fusoid to fusoid-clavate, 7–16 phragmospore rarely with a longiseptum. Paraphyses apically up to †1.5–(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 synnematus structures, forming consistently curved vermisform conidia. Conidiomata not observed in cultures.

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

**Description:** Apothecium pulvinate-discid 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 †(63–)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 single, short, cylindric basal cell, on hyphae grouped into ropey, synnematus 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-discid 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 phragmospore rarely with a longiseptum. Paraphyses apically up to †1.5–(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 synnematus structures, forming consistently curved vermisform conidia. Conidiomata not observed in cultures.
narrow, hyaline hyphae, distantly septate, strongly spaced, ± vertically oriented and embedded in an abundant, light brown gelatinous matrix; outermost layer †(5.5–)7–17.5 µm thick at margin, †(16.5–)24.5–37.5(–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 †6–10 × 2.5–3 µm at margin, †(4–6)–7.5(–9) × (1.5–)2.5–3(–4) µm at lower flank and base, cell walls †0.5–1 µm thick. Medullary exciple indistinctly differentiated from the ectal exciple and progressively changing toward the hymenium the hyphae becoming more closely spaced, hyphae †(0.5)1–1.5 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 †(3–)4–7.5(–8.5) × (0.5–)1(–1.5) µm, formed on flask-shaped conidiogenous cells †(4–)5.5–7(–9) × (1.5–)2(–3.5) µm, conidiogenous cells sometimes in groups of 3–4 held on a simple basal conidiophore of †(4–)5–9(–11) × (1.5–)2.5(–3) µm, conidiosgenesis phialidic without collarette.

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 Höhnel (1917), who expanded the order to include 52 genera in six families. In the 1970s (e.g. Korf 1973, Dennis 1978) the rhizomataceous 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 **Epithammnalia**, 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 **Leotiomycetes** 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. palida**, Damm et al. (2010) said that “**C. paarla** and **C. palida** 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 synnematos clonidiophores bearing conidia that are consistently curved.

The sexual morph of **Aotearoamyces** shares several morphological traits with **Tympaniaceae** (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, cylindric-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, C3 and E3), which allows it to be distinguished from **Holwaya**, **Tympanis** and most **Clausenomyces** species (Fig. 5, B3). **Clausenomyces jahnianus**, 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. prisatica**) and **Pragmopora** (Fig. 5, G2: **t. obilita**); 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 plecenchymatous exciple. **Durandiella** differs in the morphology of the paraphysis apex

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**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 ascii (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 *Aoteaeroamyces* 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.

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**REFERENCES**

Ainsworth GC, Bisby GR (1950) *A Dictionary of the Fungi*. Kew: Imperial Mycological Institute.

Ainsworth GC, Bisby GR (1950) *A Dictionary of the Fungi*. 3rd edn. Kew: Commonwealth Mycological Institute.

Ainsworth GC (1961) *Ainsworth & Bisby’s Dictionary of the Fungi*. 5th edn. Kew: Commonwealth Mycological Institute.

Ainsworth GC, James PW, Hawksworth DL (1971) *Ainsworth & Bisby’s Dictionary of the Fungi*. 6th edn. Kew: Commonwealth Mycological Institute.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.

Anonymous (1976) ISCC-NBS Color-name Charts illustrated with centroid colors. Washington DC: Inter-Society Color Council, National Bureau of Standards.

Baral HO (1992) Vital versus herbarium taxonomy: morphological differences between living and dead cells of ascomycetes, and their taxonomic implications. *Mycotaxon* 44: 339–390.

Baral HO (2016) Inoperculate discomycetes. In: *Syllabus of Plant families*. A. Engler’s *Syllabus der Pflanzenfamilien*. Vol. 1(2), Ascomycota (Jaklitsch W, Baral HO, Lücking R, Lumbsch T, eds): 55–221. 13th edn. Stuttgart: Borntraeger Science Publishers.

Bessey CE (1907) A synopsis of plant phyla. *University of Nebraska Studies* 7: 275–373.

Bunyard BA, Nicholson MS, Royse DJ (1994) A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. *Mycologia* 86: 762–772.

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular and Evolution* 17: 540–552.

Crous PW, Quaedvlieg W, Hansen K, Hawksworth DL, Groenewald JZ (2014) *Phacidiales* and *Ceuthospora* (*Phacidiaceae*) are congeneric: taxonomic and nomenclatural implications. *IMA Fungus* 5: 173–193.

Damm U, Faurie PH, Crous PW (2010) *Coniochaeta* (*Lecythophora*), *Collophora* gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. *Persoonia* 24: 60–80.

Dennis RWG (1978) *British Ascomycetes*. 2nd edn. Vaduz: J. Cramer..

DiCosmo F, Nag Raj TR, Kendrick WB (1984) A revision of the *Phacidiaceae* and related anamorphs. *Mycotaxon* 21: 1–234.

Eriksson OS (2005) Outline of *Ascomycota* – 2005. *Myconet* 11: 1–113.

Eriksson OS (2006) Outline of *Ascomycota* – 2006. *Myconet* 12: 1–82.

Hawksworth DL, Kirk PM, Sutton BC, Pegler DN (1995) *Ainsworth & Bisby’s Dictionary of the Fungi*. 8th edn. Wallingford: CAB International.

Hawksworth DL, Sutton BC, Ainsworth GC (1983) *Ainsworth & Bisby’s Dictionary of the fungi (including the lichens)*. 7th edn. Kew: Commonwealth Mycological Institute.

Höhnel F (1917) System der *Phacidiales* v. H. *Berichte der Deutschen Botanischen Gesellschaft* 34: 416–422.

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

Johnston PR, Park D (2013) The phylogenetic position of *Lanzia* *berggrenii* and its sister species. *Mycosystema* 32: 366–385.

Katoch K, Misawa K, Kuma K, Miyata T (2002) MAFFT, a novel method for rapid multiple sequence alignment base don fast Fourier transform. *Nucleic Acid Research* 30: 3059–3066.

Katoch K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9: 212.

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) *Ainsworth & Bisby’s Dictionary of the Fungi*. 9th edn. Wallingford: CAB International.

Kirk PM, Cannon PF, Minter DW, Stalper JA (2008) *Ainsworth & Bisby’s Dictionary of the fungi*. 10th edn. Wallingford: CAB International.

Korf RP (1973) *Discomycetes and Tuberales*. In: *The Fungi: an advanced treatise*. Vol. IVA. *A Taxonomic Review with keys: Ascomycetes and Fungi Imperfecti* (Ainsworth GC, Sparrow FK, Sussman AS, eds): 249–319. New York: Academic Press.

Fig. 5. Morphological features of *Aoteaeroamyces nothofagi* compared to those of other genera in *Tympanidaceae*: *A. Aoteaeroamyces nothofagi* (PDD 95741, 80575). B. *Holwaya mucida* (Dragisa Savic herb. without number; CUP 60122, 2006) C. *Tympanis* spp. (NYBG 423829, 1168034, 1168030; TAAM 165632) D. *Durandellia gallica* (S F196603, Baral herb. HB 9244). E. *Clausenomyces* spp. (TFCMic. 23325, 23950, 24008, 24011; CUP 3938). F. *Grovesiella abieticina* (DAVFP 23014; NYBG 1293222). G. *Pragmopora ambiphila* (TFCMic. 23861, 23922, 23726). H. *Myriodiscus* *sparassoides* (TFCMic. 24582). Morphological features compared: 1. Apathecia, 2. section of the exciple, 3. ascospores (with or without conidia or ascoconidia), 4. ascii, and 5. paraphyses. Mounted in: water = C4, D2, G2–G4, KOH = A2, A4, C2, E3, G5, H2, H5, CR = A3, B3, B5, C3, C5, C3, E2, E3, E5, F2–F3, 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, C3, D3, E3, F3–H3, A4, E4–H4, A5–C5, E5–H5; 5 = 10 µm.
Korf RP, Lizoň P (2000) Validation of Nannfeldt’s ordinal name Helotiales. Mycotaxon 75: 501–502.
LoBuglio KF, Pfister DH (2010) Placement of Medeolaria farlowii in the Leotiomycetes, and comments on sampling within the class. Mycological Progress 9: 36–368.
Lumbsch HT, Huhndorf SM (2007) Outline of Ascomycota – 2007. Myconet 13:1–58.
Lumbsch HT, Huhndorf SM (2010) Outline of Ascomycota – 2009. Myconet 14:1–40.
Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25: 1253–1256.
Quijada L, Baral HO, Jaen-Molina R, Weiss M, Caujapé-Castells J, Beltrán-Tejera E (2014) Phylogenetic and morphological circumscription of the Orbilia aurantiorubra group. Phytotaxa 175: 1–18.
Quijada L (2015) Estudio de los órdenes Helotiales s.l. y Orbiliales (Ascomycota, Fungi) en la Isla de Tenerife. PhD thesis, Universidad de La Laguna.
Suija A, van den Boom P, Zimmermann E, Zhurbenko MP, Diederich P (2017) Lichenicolous species of Hainesia belong to Phacidiales (Leotiomycetes) and are included in an extended concept of Epithamnion. Mycologia 109: 882–899.
Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56: 564–577.
Van Vooren N (2005) Mniaecia gloeocapsae (Boud.) van Vooren. Bulletin Mensuel de la Société Linnéenne de Lyon 74: 19.
Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246.
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications (Innis MA, Gelfand DH, Sninsky JJ & White TJ, eds): 315–322. San Diego: Academic Press.
Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, Madrid H, et al. (2017) Notes for genera Ascomycota. Fungal Diversity 86: 1–594.