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 asci, an exciple of textura intricata with hyphal 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” paarlensis 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.

Introductions

The taxonomy and classification of the Leotiomycetes 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): Schizothyriaceae (5 genera), Leptopeltineae (13 genera), Dermopectineae (10 genera), Phacidiaeae (12 genera), Phacidiosormaeae (4 genera) and Cryptomycteeae (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 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 Höhnel generally included three families (Fig. 1): Cryptomyctaceae, Hypodermataceae or Rhytismataceae, and Phacidiaeae (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 Phacidiaeae was applied in a more restricted sense, including Phacidium and two or three other...
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 Hypodermales were included as two genera previously placed in Phacidiales (Hawksworth et al. 1983, 1995). In 1995 the family Phacidiaceae contained three genera and was placed by Korf & Lizoń (2000) in Leotiaceae, an invalid name later validated by Korf & Lizoń (2000), and there in Helotiaceae. In 2001 the Phacidiaceae still contained only three genera (Ascocoma, Lophophacidium, and Phacidium) and was transferred to Helotiaceae, 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 Helotiaceae 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 ascocoma 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, ascii 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 Höhnel’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 Phacidopycnis over the sexually typified Potebiarmycies was indicated. Two new families were included in the order: Tympinaceae 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 Ascocoma). Subsequently, Suíja et al. (2017) placed the monotypic genus Epithamnolla 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 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” 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 PDG 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/resourc e s/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 & Brun 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). 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).

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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**.

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**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 Mniacea 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 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://darwing.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 hypheae 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 hypheae; 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, Leotiomycetes, Pezizomycotina, Ascomycota, Fungi.

Description: Ascomata apothecia, black, erumpent, short to medium long stipitate (to 1 mm tall), pulvinate-discoid 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 longisep tum. 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 synnematous structures, forming consistently curved vermiform conidia. Conidiomata not observed in cultures.

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

Description: Apothecia pulvinate-discoid 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. Ascii †83–124 × 10.5–14.5 µm, 8-spored, inamyloid, arising from croziers. Ascospores cylindric-fusoid to fusoid-clavate and phragmoseptate, rarely with a longisep tum. 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 hypheae, 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, synnemmatous 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-discoid 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 longisep tum. 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 synnematous structures, forming consistently curved vermiform conidia. Conidiomata not observed in cultures.

Description: Apothecia pulvinate-discoid 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. Ascii †83–124 × 10.5–14.5 µm, 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, slightly or slightly curved, with (7–)13–14(–16) transversal septa (rarely 1 longisep tum), 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, ± vertically oriented and embedded in an abundant, light brown gelatinous matrix; outermost layer †(5.5–)7–13(–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 rosy 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–)2.5(–3) µm, conidiogenous cells sometimes in groups of 3–4 held on a simple basal conidiophore of †(4.5–)6(–8.5) × (1.5–)2.5(–3) µm, conidio genesis 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 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 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. 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. Aoteaeroxymes 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 Aoteaeroxymes nothofagi are held on well-developed synnematous conidiophores bearing conidia that are consistently curved.

The sexual morph of Aoteaeroxymes 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, cylindric-fusoid to fusiform-clavate (Fig. 5: A5–H5); (3) the paraphyses are usually agglutinated and embedded in a dark amorphous exudate (Fig. 5, A5–H5). However, Aoteaeroxymes 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 Claussenomyces species (Fig. 5, B3). Claussenomyces jahnianus, lacking reports of conidia formed on ascospores, can be differentiated from Aoteaeroxymes by the acicular ascospores and apically moniliform, closely septate paraphyses (Quijada 2015). The exciple of Aoteaeroxymes, 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 Aoteaeroxymes (Fig. 5, A5). The genera Myriodiscus (Fig. 5, H2), Durandiella (Fig. 5, D2), and Aoteaeroxymes have a similar plectenchymatous exciple. Durandiella differs in the morphology of the paraphysis apex.
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.

REFERENCES