

## Aotearoamyces, a native saprobe on Nothofagaceae in New Zealand related to South African pathogens in the genus Collophorina

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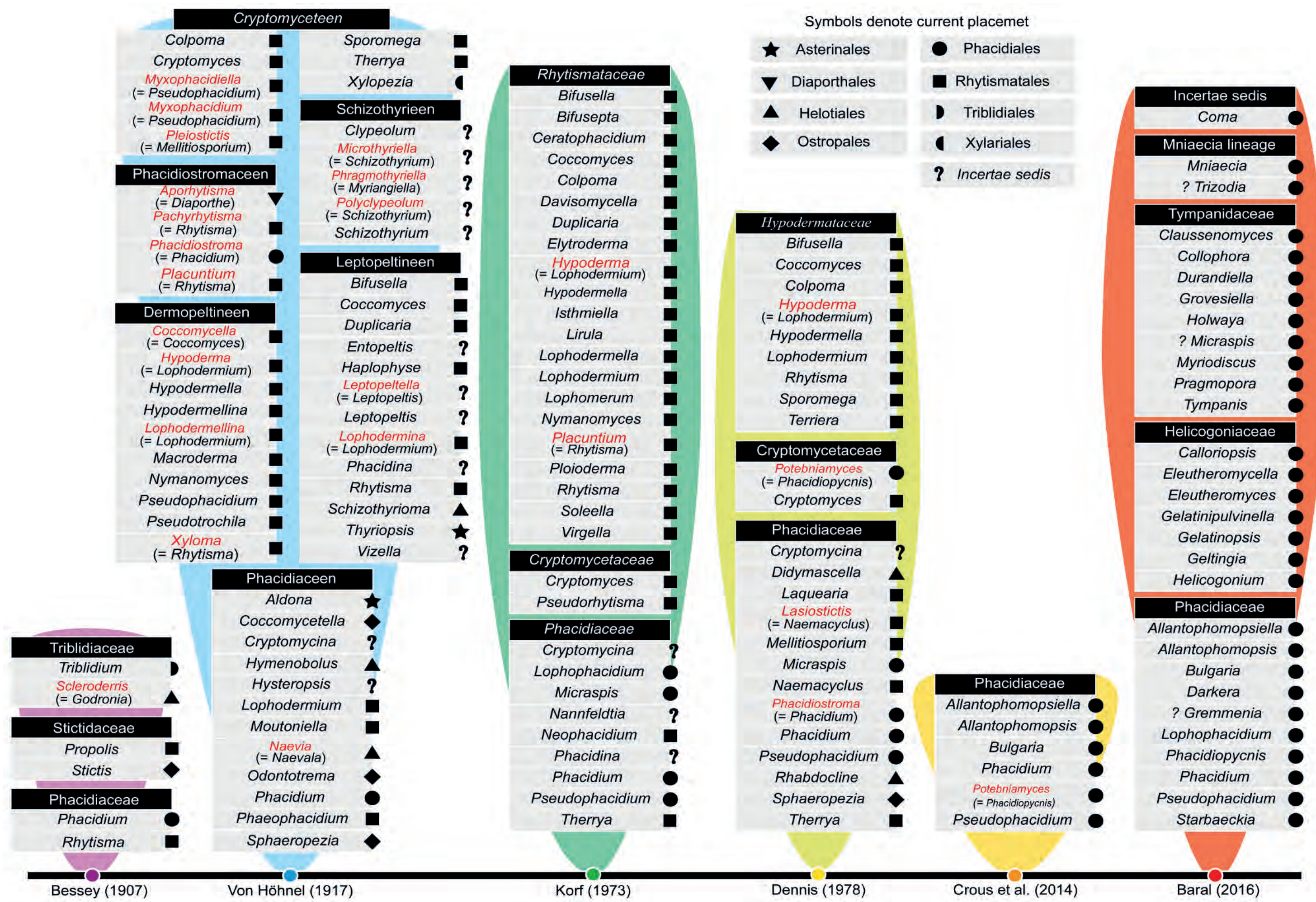
*Aotearoamyces nothofagi* P.R. Johnst., J.A. Cooper & Quijada

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

**Species diagnosis:** Apothecia black, up to 1 mm, erumpent, short to medium long stipitate, pulvinate-discoid to turbinate, arising from a common gelatinous stromatic base. Asci  $\times 83\text{--}124 \times 10.5\text{--}14.5 \mu\text{m}$ , octosporous, inamyloid, arising from croziers. Ascospores  $\times 17.6\text{--}31 \times 3.2\text{--}4.9 \mu\text{m}$ , cylindrical-fusoid-clavate, 7–16 phragmo-septate rarely with longiseptum. Paraphyses apically up to  $\times 1.5\text{--}2(2.5) \mu\text{m}$ , flexuous to helicoid or curving downwards (hooked) and embedded in an olive-brownish gelatinous matrix. Conidia produce on phialides not as endoconidia, the conidiogenous cells are held on well-developed conidiophores arranged in small synnemata structures, forming consistently curved vermiform conidia. Conidiomata not observed in cultures.

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

The Tympanidaceae has been placed in the order Phacidiales within the Leotiomyces (Baral 2016). The taxonomy and classification of the class 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 change 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 Leotiomyces. How the number of genera and their systematic placement, which reflects changes in morphological concepts, has changed over time is illustrated in the Figure below. 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 *Knauzenomyces*, and the observation that the asexual *Collophorina paarla* is related to it.



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 to indicate the placement of each genus and its current order according to Index Fungorum (2018) and Baral (2016), note key to symbols above.

**Material and Methods** - The specimens were collected between 1989 and 2010 in native forests of New Zealand during non-targeted, general collecting expeditions for fungi. All the dried specimens cited are deposited in the PDD fungarium. Macro- and micromorphology studies follow Baral (1992) and Quijada (2015). DNA was extracted and amplified using PCR following the methods of Johnston and Park (2013). An analysis using three different rDNA regions (SSU, ITS, LSU) for the representative members of Phacidiales was performed. This includes taxa from three families: Phacidaceae (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). The methods for ML and BI analyses follow Quijada (2015).

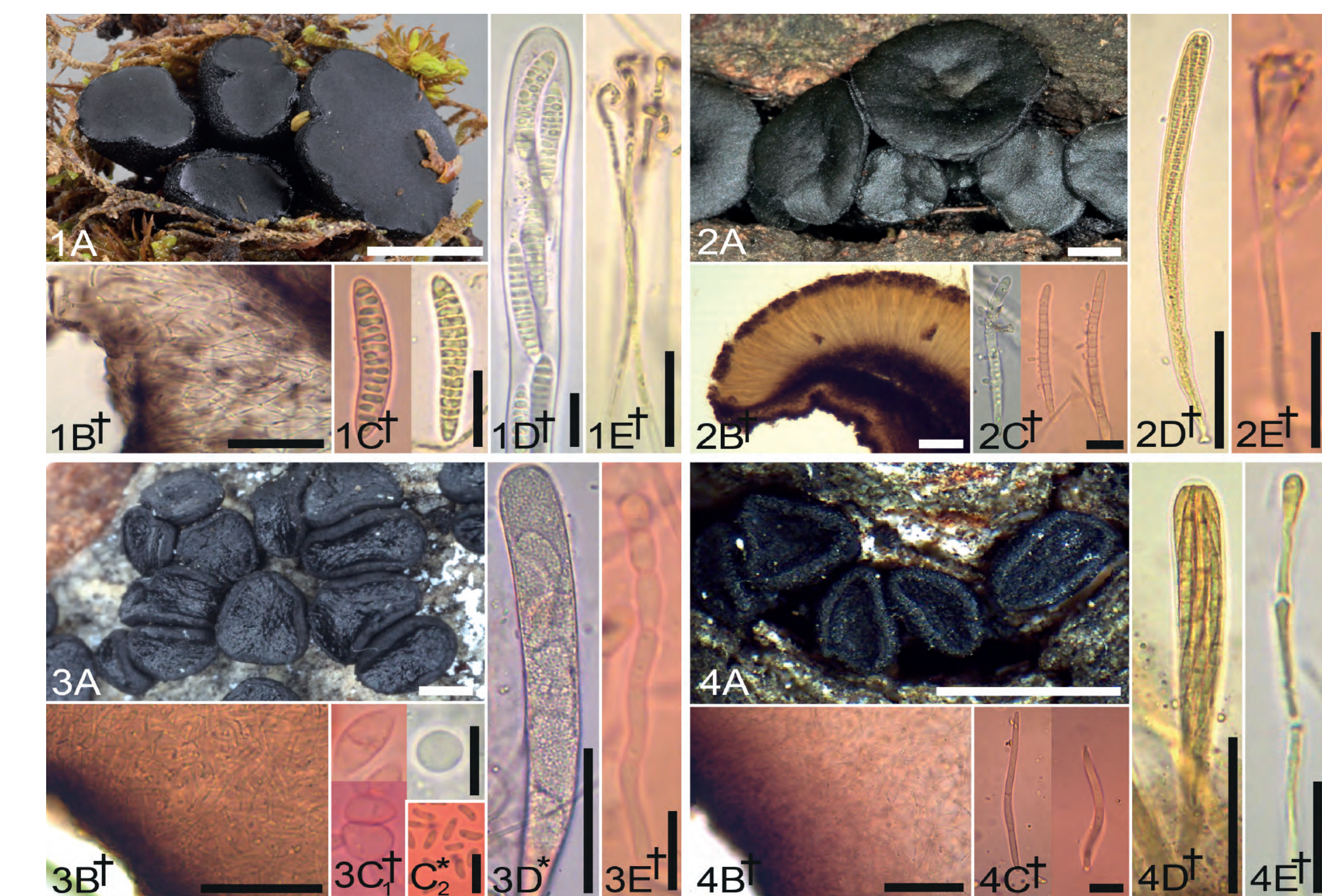
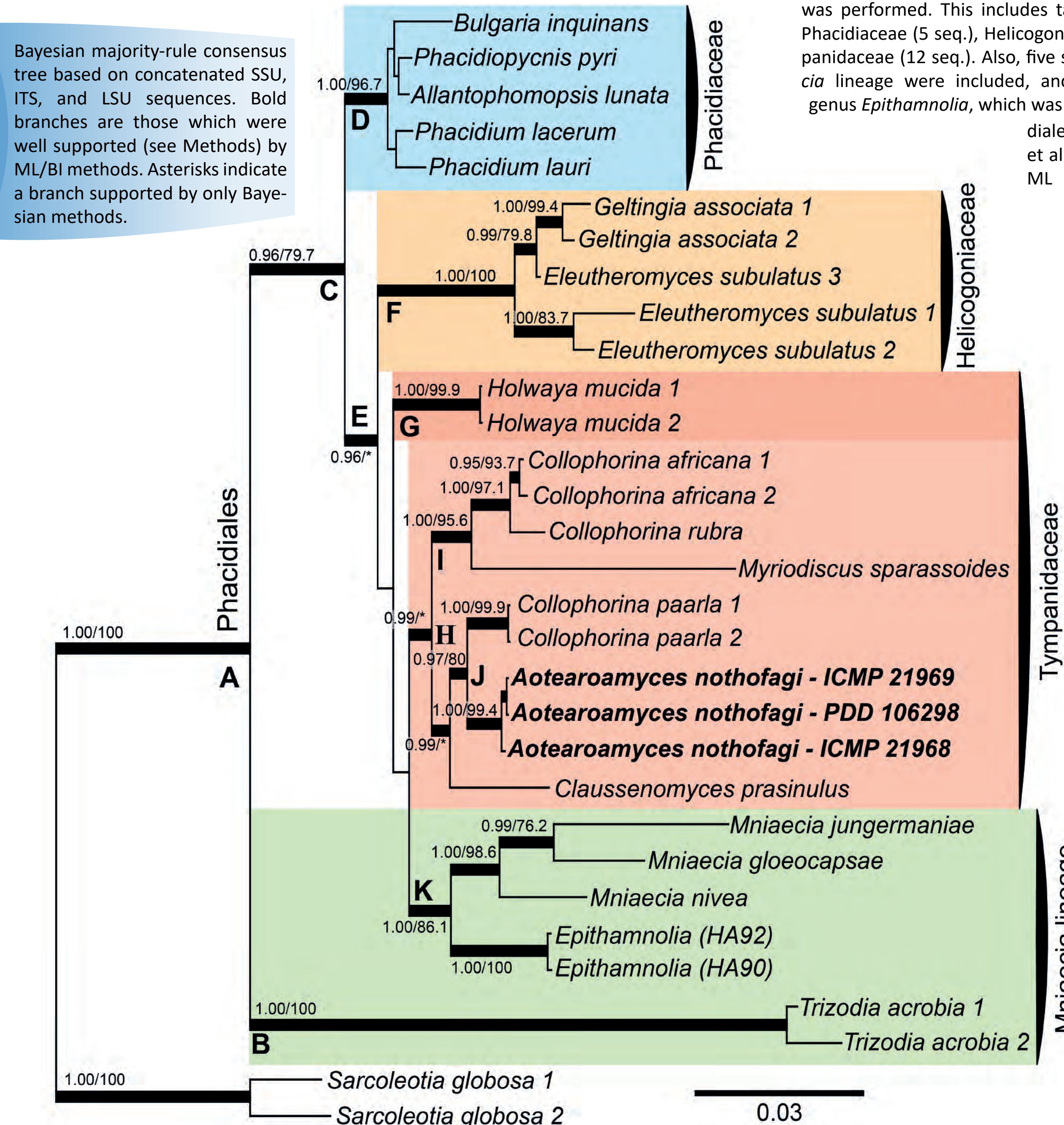


Figure 2. Morphological features of *Aotearoamyces nothofagi* compared to those of other genera in Tympanidaceae: 1. *Aotearoamyces nothofagi*, 2. *Holwaya mucida*, 3. *Tympanis* spp., 4. *Durandiella gallica*. Morphological features compared: a. Apothecia, b. section of the excipulum, c. ascospores (with or without conidia or ascoconidia), d. asci, and e. paraphyses. Scale bars: 500  $\mu\text{m}$  = 1a-4a; 100  $\mu\text{m}$  = 2b; 50  $\mu\text{m}$  = 1b, 3b-4b, 2d, 3d-4d, 4e; 10  $\mu\text{m}$  = 1c-2c, 3c1, 4c, 1d, 1e-3e; 5  $\mu\text{m}$  = 3c2.

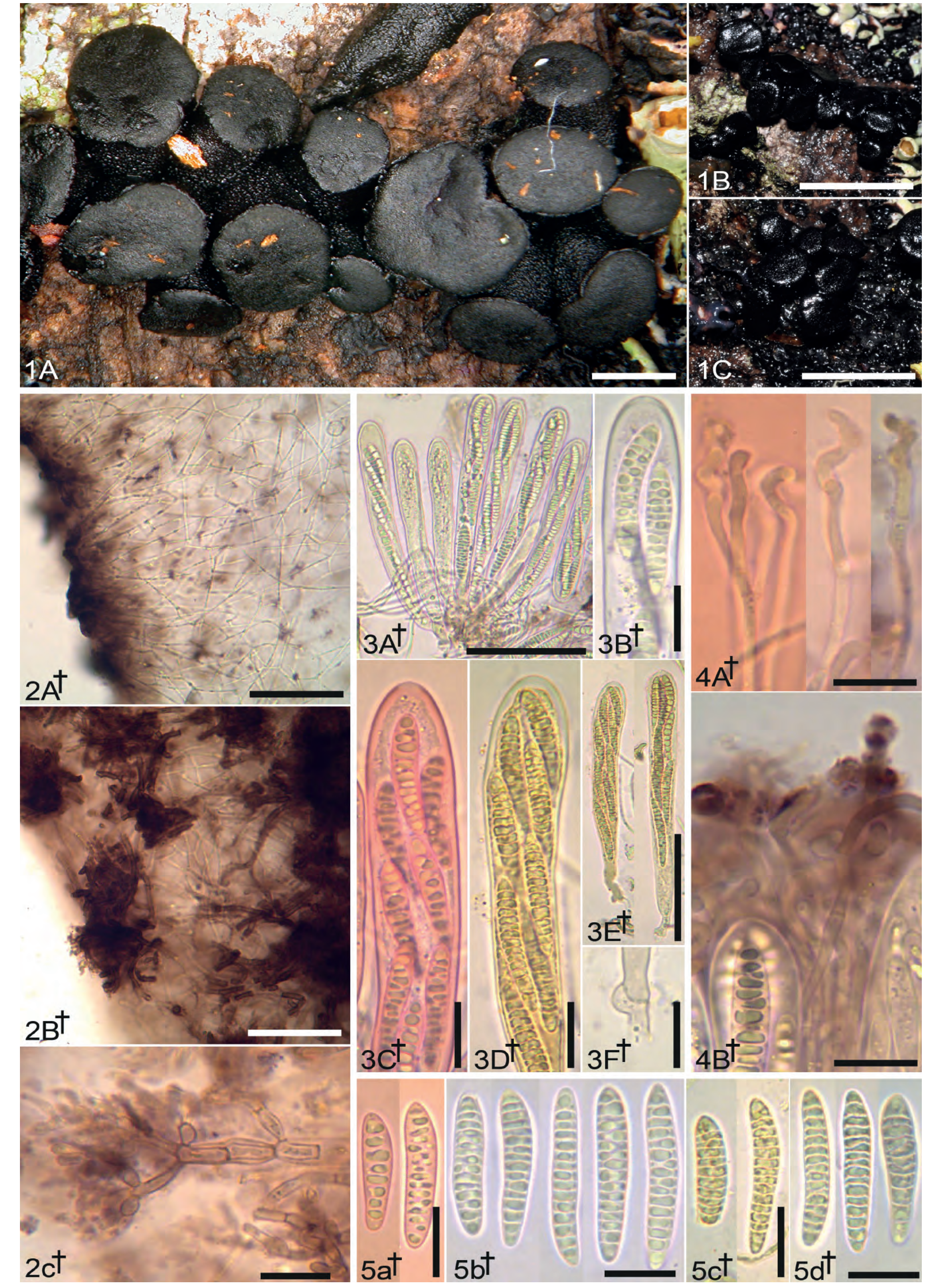


Figure 1. Morphological features of *Aotearoamyces nothofagi*. 1. Apothecia in fresh state. 2. Excipulum: 2a-b. section at flank, 2c. ectal excipulum cells at flank. 3. Asci. 4. Paraphyses. 5. Ascospores. Scale bars: 2 mm = 1b-c, 500  $\mu\text{m}$  = 1a; 50  $\mu\text{m}$  = 2a-b, 3a, 3e; 10  $\mu\text{m}$  = 2c, 3b-d, 3f, 4a-b, 5a-d.

**Discussion** - Throughout its history, the number of species, genera and families in the order Phacidiales has changed considerably. The order as circumscribed by Bessey (1907), who included six genera and three families, was differently conceived by Höhnell (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 the Phacidiales, although today they are placed in a separate order Rhytismatales. The most current classification of the 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 macromorphology and micromorphological features, as well as the impact of molecular phylogenetics. Our phylogenetic analyses has allowed the placement of *Epithamnolia*, a conidial fungus, in the *Mniaecia* clade for the first time.

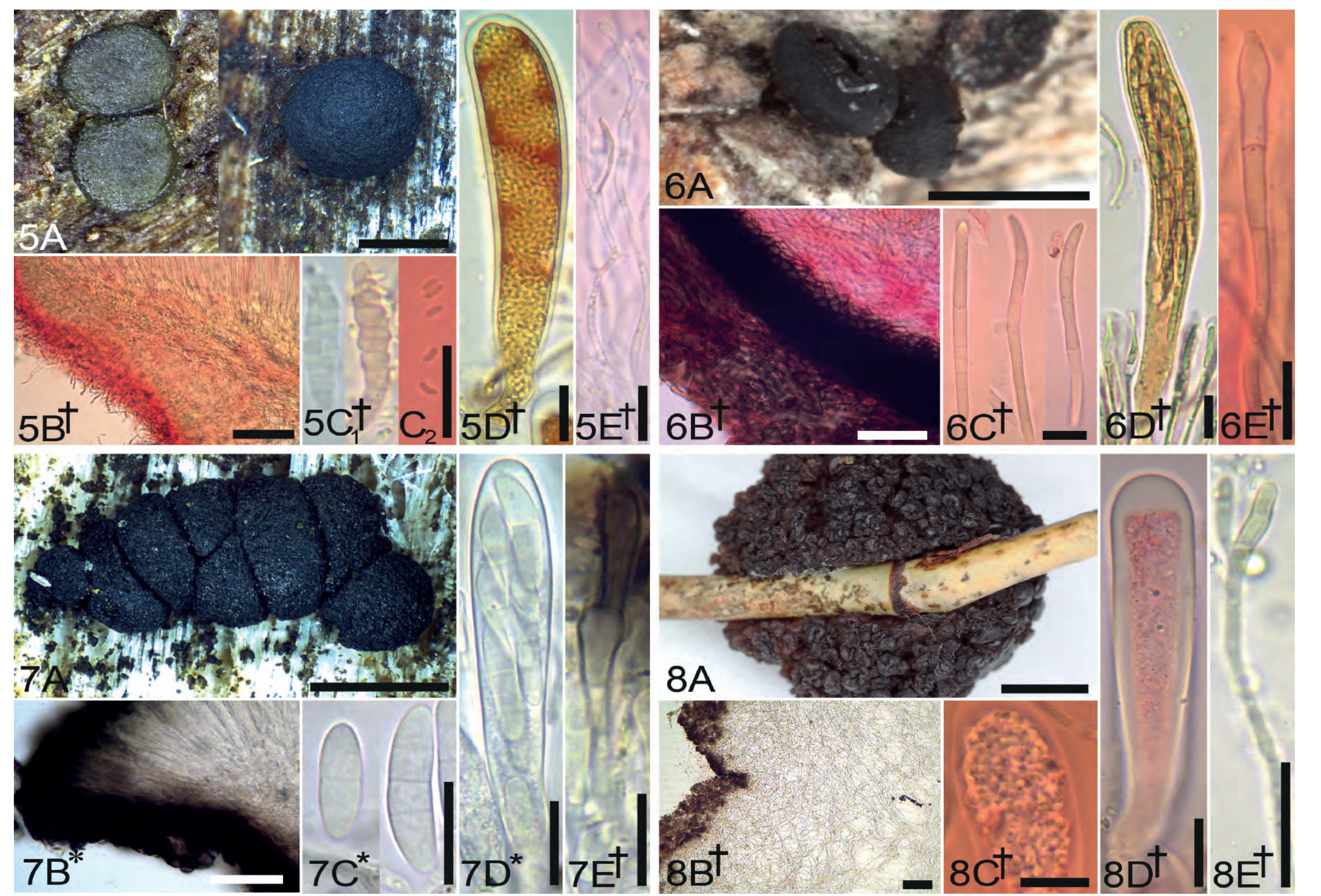


Figure 3. Morphological features of *Aotearoamyces nothofagi* compared to those of other genera in Tympanidaceae: 5. *Claussenomyces* spp., 6. *Grovesiella abieticola*, 7. *Pragmopora* sp. and 8. *Myriodiscus sparassoides*. Morphological features compared: a. Apothecia, b. section of the excipulum, c. ascospores (with or without conidia or ascoconidia), d. asci, and e. paraphyses. Scale bars: 500  $\mu\text{m}$  = 5a-8a; 100  $\mu\text{m}$  = 8b; 50  $\mu\text{m}$  = 5b-7b; 10  $\mu\text{m}$  = 5c1, 6c-8c, 5d-8d, 5e-8e; 5  $\mu\text{m}$  = 5c2.

*Aotearoamyces* is most closely related to the clade containing the *Collophorina* species with endoconidia, but there is no sign of endoconidia formation in our culture studies. Compared to Damm et al.'s (2010) illustrations and descriptions, the conidiogenous cells of *Aotearoamyces nothofagi* are held on more well-developed conidiophores aggregated on small synnemata structures, and the conidia are consistently curved. This character of conidial shape is shared with other genera in Tympanidaceae like *Claussenomyces* or *Holwaya* (Baral 2016). The sexual morph of *Aotearoamyces* shares several morphological traits with Tympanidaceae (Fig. 2-3): (1) the asci are inamyloid, apically and/or laterally thick-walled and arising from croziers (Fig. 2-3, 1d-8d), (2) the ascospores are phragmo-sporous, cylindrical-fusoid to fusiform-clavate (Fig. 2-3, 1c-8c), and (3) the paraphyses are usually agglutinated and embedded in a dark amorphous exudate (Fig. 2-3, 1e-8e). However, *Aotearoamyces* also differs in many aspects: conidia are not present inside the asci or attached to ascospores (Fig. 2-3, 3C2 and 5C2), which allows it to be distinguished from *Holwaya*, *Tympanis* and most *Claussenomyces* spp. (Fig. 2, 2c). *Claussenomyces jahnius*, lacking ascoconidia, can be differentiated from *Aotearoamyces* by its acicular ascospores and moniliform, closely septate paraphyses.

The excipulum of *Aotearoamyces*, which is strongly gelatinized and appears as network of loose hyphae (Fig. 2, 1b), differs completely from the excipulum in *Grovesiella* (Fig. 3, 6b: textura angularis to prismatica) and *Pragmopora* (Fig. 3, 7b: t. oblita); these genera also differ in the paraphyses never being helicoid or hooked at apex like those in *Aotearoamyces* (Fig. 2, 2e). The genera *Myriodiscus* (Fig. 3, 8b), *Durandiella* (Fig. 2, 4b), and *Aotearoamyces* have a similar plectenchymatous excipulum. *Durandiella* differs in the morphology of paraphysis apex (Fig. 2, 4d: straight vs. 1e: curved to helicoid) and ascospores (Fig. 2, 4c: acicular-fusiform to falcate vs. 1c: cylindrical-fusoid to fusoid-clavate); and *Myriodiscus* differs in having polysporous asci (Fig. 3, 8c) and in macroscopic appearance (Fig. 3, 8a: discoid apothecia aggregated in a subglobose fructification vs. 1a: turbinate apothecia sharing a stromatic base). Given the above, we concluded that *Aotearoamyces* 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 state.

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