

## ***Lachnaceae* in New Zealand - Aotearoa**

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### **Introduction**

*Lachnaceae* is a family of small, stalked, usually brightly coloured cup fungi (*Leotiomyces*, the ‘inoperculate discomycetes’), characteristically with their apothecia having hairs. Most species have been described as saprobes on fallen dead plant tissue, but they have also been isolated as endophytes from living leaves and roots (e.g. Johnston et al. 2011). Some species known only from their asexual state have been placed in morphologically similar but phylogenetically distant genera such as the beetle-associated ‘*Chrysosporium*’ *filiforme* (Sigler et al. 1982) that DNA sequences (GenBank AJ131680 ex type specimen) show is a *Lachnellula* sp. (*Lachnaceae*).

Most of the scanty New Zealand literature on morphologically similar fungi has treated species now accepted in *Lachnaceae* as the family *Hyaloscyphaceae* sensu Nannfeldt (1932). The most obvious defining character of this concept of the family is that the apothecia are hairy and the excipular cells rather thin-walled. Raitviir (2004) modified Nannfeldt’s concept of *Hyaloscyphaceae*, raising two subfamilies *Hyaloscyphoideae* and *Lachneae* to the family level as *Hyaloscyphaceae* and *Lachnaceae*. Recent molecular phylogenetic studies have supported this scheme, the two families now known to have a distant relationship within *Helotiales*, and fungi having apothecia with hairs being found scattered across *Helotiales* (e.g. Han et al. 2014). *Lachnaceae* sensu Raitviir (2004) is strongly supported in recent multigene analyses (e.g. Johnston et al. 2019, Johnston & Baschien 2020). In addition, ITS based analyses also generally strongly support this clade (e.g. Guatimosim et al. 2016). ITS-based phylogenies place the genera *Albotricha*, *Brunnipila*, *Capitotricha*, *Dasyscyphella*, *Erioscyphella*, *Incrucipulum*, *Lachnellula*, *Lachnopsis*, *Proliferodiscus*, and *Velebitea* in *Lachnaceae* (Hosoya et al. 2010, Peric & Baral 2014, Guatimosim et al. 2016, Kušan et al. in Phookamsak et al. 2019). All these genera have rough walled hairs. Although well resolved phylogenetically, these genera remain difficult to distinguish morphologically (e.g. Tochiara & Hosoya 2022). Basal to *Lachnaceae* is a clade including several genera with smooth-walled hairs — *Lasiobelonium*, *Solenopezia*, *Trichopeziza*, and *Trichopezizella* — included in *Lachnaceae* by Johnston et al. (2019) but treated as *Solenopeziaceae* by Johnston & Baschien (2020), using a concept of the family modified from the original circumscription of Ekanayaka et al. (2019).

Based on specimens with DNA sequences available (many of these still to be publicly released), species of *Lachnaceae* from New Zealand are known from the genera *Albotricha*, *Capitotricha*, *Erioscyphella*, *Lachnellula*, *Lachnum* sensu stricto, *Lachnopsis*, *Proliferodiscus*, and *Velebitea* (Fig. 1). Based on the same dataset, genera not known to occur in New Zealand include *Brunnipila* and *Incrucipulum*. In contrast, there are some New Zealand species that do not seem to fit phylogenetically into any existing genus, such as ‘*Lachnum*’ *willisii* (e.g. PDD 112217), a morphologically highly divergent *Cudoniella*-like species (e.g. PDD 108691 and related specimens), and a phylogenetically divergent species with delicate, white apothecia on wood (PDD 119495 and related specimens). Most of New Zealand’s species of *Lachnaceae* remain unnamed.

Many specimens of *Lachnaceae* without DNA sequences are in the New Zealand Fungarium/ Te Kohinga Hekaheka o Aotearoa (PDD), and few of these have been critically examined morphologically. It is expected that many additional records of the species discussed below will be found, as well as additional species currently without DNA sequences. For example, in a current ongoing study on the genus *Lachnopsis* in New Zealand 23 species have DNA sequences available,

but there are another four clearly morphologically distinct species that as yet have no DNA sequences (unpubl. data).

## Methods

The trees presented are all based on ITS sequences only. The ML trees were generated using IQTree, with the model SYM+R5. Bootstrap values are provided when >90%. Aotearoa-relevant clade names are provided to the right of the trees, many of these representing informal tag names for unnamed species. *Solenopezziaceae* was used as the outgroup.

PDD numbers refer to dried specimens in the New Zealand Fungarium/ Te Kohinga Hekaheka o Aotearoa and ICMP numbers to living cultures in the International Collection of Microorganisms from Plants/Te Kohinga Hekaheka Ora o Aotearoa. Note that most of the DNA sequences were generated from mycelium from cultures grown from germinated ascospores, but many of these cultures have yet to be formally accessioned into ICMP. Details of most specimens can be found through <https://scd.landcareresearch.co.nz/>, and this web resource also provides images for some of the specimens.

## Results and Discussion

Brief notes are provided below on each of the genera treated in Figs 1–8.

### *Albotricha* (Fig 7)

A single unnamed species known from New Zealand, known also from Australia, with bright yellow apothecia, quite common on dead leaves of large monocots. Forms a clade strongly supported as sister to a clade including specimens from other parts of the world identified as *A. acutipila* and *A. albotestacea*.

### *Asperopilum* (Fig 6)

*Asperopilum* is monotypic, with the type species *A. juncicola*. This fungus is common on *Juncus* in New Zealand and is found also in Australia, although only New Zealand specimens have DNA sequences available. Based on a phylogeny derived from ITS sequences, *A. juncicola* falls in a strongly supported, monophyletic clade within *Lachnum*. Also in the *Asperopilum* clade are specimens identified as *Lachnum controversum*, *L. spartinae* and *L. apalum*, along with three unnamed species from New Zealand. All of the specimens in this clade that were sequenced from apothecia on host plant substrates were collected from monocotyledons, mostly grasses. One of the New Zealand species is also represented by a specimen from Australia (PDD 117545), on a host identified by the collector as *Gahnia*.

*Asperopilum* is morphologically distinct from typical *Lachnum* species in having paraphyses branched and slightly swollen towards the rounded apex, and a red reaction at the ascus apex when treated with Lugol's Iodine (IKI) (no reaction with Melzers reagent). *Lachnum* species typically have lanceolate paraphyses and blue reaction at the ascus apex with both IKI and Melzers reagent. The hairs of *Asperopilum* are roughened but shorter than typical for *Lachnum*.

The other fungi in the clade with *Asperopilum* all have ascus pores reacting blue with iodine and the lanceolate paraphyses typical for *Lachnum*. However, they share with *Asperopilum* excipular cells that are short cylindric to more or less globose, with thickened or slightly thickened walls. Most species of *Lachnum* have excipular cells with thin walls. See:

- [https://scd.landcareresearch.co.nz/Specimen/PDD\\_108737](https://scd.landcareresearch.co.nz/Specimen/PDD_108737) for *Lachnum* cf. *apalum*

- [https://scd.landcareresearch.co.nz/Specimen/PDD\\_42648](https://scd.landcareresearch.co.nz/Specimen/PDD_42648) for *L. controversum* (ex Sydow Mycotheca Germanica No. 1945)
- [https://scd.landcareresearch.co.nz/Specimen/PDD\\_93687](https://scd.landcareresearch.co.nz/Specimen/PDD_93687) for a *L. controversum*-like species from New Zealand (differs phylogenetically from Northern Hemisphere specimens and in ascospore shape)
- [https://scd.landcareresearch.co.nz/Specimen/PDD\\_117544](https://scd.landcareresearch.co.nz/Specimen/PDD_117544) for *Lachnum* sp. "Cortaderia red"
- [https://scd.landcareresearch.co.nz/Specimen/PDD\\_99993](https://scd.landcareresearch.co.nz/Specimen/PDD_99993) for *Asperopilum juncicola*

*Lachnum spartinae* was described as having thin-walled excipular cells, but the images in Cantrell et al. (1996 - Mycotaxon 57: 479-485) seem to show somewhat thickened walls.

The ITS phylogeny places the *Asperopilum* clade within *Lachnum* s.s., making *Lachnum* paraphyletic. A multigene analysis places species representing the *Asperopilum* clade sister to the type species of *Lachnum*, *L. virgineum* (see Johnston 2022, <https://doi.org/10.7931/gx9a-c781>). However, in this analysis *Lachnum* s.s. is represented by only by two *L. cf. virgineum* specimens. Additional multigene data are needed to test whether this relationship is robust, or whether the *Asperopilum* clade will continue to result in a paraphyletic *Lachnum*.

### ***Brunnipila* (Fig 8)**

Four species with sequences in GenBank form a strongly resolved clade in the ITS phylogeny (Fig. 1); no New Zealand specimens that have been sequenced belong in this clade.

### ***Capitotricha* (Fig 7)**

A single unnamed species from New Zealand, with white concolorous, densely hairy apothecia on wood, represented by PDD 104630 and PDD 112210. Phylogenetically this New Zealand species is close to a specimen collected from *Nothofagus* in Chile (PRM 909286, GenBank LT904862), with an ITS match of 97.8%. The Southern Hemisphere species have a strongly supported sister relationship with several *Capitotricha* species from the Northern Hemisphere.

### ***Dasyscyphella* (Fig 5 and Fig 8)**

*Dasyscyphella* is not monophyletic in the ITS tree (Fig. 1). Clades represented by specimens identified as *D. nivea* and *D. montana* are distant within the ITS tree. *D. montana* and *D. nivea* are very similar morphologically, according to Raitviir & Sacconi (1987) they differ in the colour of the dried apothecia and in ascus length.

Specimens from Europe and North America identified as *D. nivea* have an ITS sequence identical to specimens from New Zealand (Fig 8). In New Zealand this species is common on fallen wood; apothecia delicate, concolorous white when fresh, drying yellowish, with distinctive hairs slightly swollen, rough walled near the base and smooth at the tips.

A second, unnamed New Zealand species, represented by PDD 99164 (Fig 5), is phylogenetically close to specimens identified as *D. montana* from Japan (FC-2031, GenBank AB481241, ITS a 97.9% match) and Europe (H.B. 6874, GenBank OM456231, ITS a 96.4% match).

### ***Erioscyphella* (Fig 4)**

The clade here accepted as *Erioscyphella* is strongly supported in the ITS phylogeny (Fig 1), and the same breadth of taxa is supported as monophyletic in a multigene phylogeny with up to 15 genes (Johnston 2020-2022). This clade matches the concept of Tochihara & Hosoya (2022) but includes some additional taxa of particular relevance to New Zealand; '*Arachnopeziza*' *rhopalostylidis*,

'*Lachnum*' *berggrenii*, '*Lachnum*' *nothofagi*, in addition to about six unnamed species (Fig 4). The unnamed species all appear to be host specialised and include two species on the tree fern *Cyathea* (one on *C. smithii*, one on *C. dealbata*), one on *Dracophyllum* (closely related to specimens from Australia also on *Epacridaceae*), another on *Phormium*, and another on *Asplenium*.

Also in New Zealand are the geographically widespread species *Erioscyphella brasiliensis* and *E. abnormis*. The New Zealand specimens referred to '*Lachnum*' *palmae* are on a long branch in the *Erioscyphella* clade. This New Zealand species differs phylogenetically from specimens from Japan (Tochihara & Hosoya 2022) and China (GenBank MG283320), and whether either the Asian or New Zealand specimens represent the same species as the type specimen (from Central America) remains unknown. The New Zealand fungus is characterised by ascospores (45-) 70-80 µm long, with a row of small vacuoles stretching down the whole spore, the excipular tissue comprising long-cylindric, somewhat tangled, thick-walled, forming an agglutinated to gelatinous tissue. The morphologically similar palm-inhabiting species from Australia, *L. pritzelianum*, differs in having shorter spores and lacking gelatinous tissue in the excipulum, based on the description of Spooner (1987).

All of the New Zealand taxa in this clade share gelatinous (or at least thick-walled) excipular cells.

### ***Incrucipilum* (Fig 8)**

Four species with sequences in GenBank form a strongly resolved clade in the ITS phylogeny (Fig. 1); no New Zealand specimens that have been sequenced belong in this clade.

### ***Lachnaceae* gen. nov. 1 (Fig 5)**

The clade referred here to *Lachnaceae* gen. nov. 1 contains a single unnamed species, common in North Island of New Zealand on fallen decorticated wood. Atypical of *Lachnaceae*, this fungus does not have obvious hairs on the apothecia, which macroscopically resemble *Cudoniella*. Its position within *Lachnaceae* has been confirmed with a phylogeny based on a set of 15 genes extracted from a genome generated from PDD 119490 (Johnston 2020–2022). The excipulum comprises tangled hyphae with gelatinous walls. The outside of the receptacle has scale-like clumps of tissue that comprise loose, cylindric, smooth, thin-walled cells, that could be interpreted as reduced hairs.

### ***Lachnaceae* gen. nov. 2 (Fig 5)**

A single unnamed New Zealand species, common on fallen wood of *Nothofagus*, with a sister species in Australia, again on *Nothofagus* wood. Morphologically these species recall *Cyathicula* macroscopically, and have a heavily gelatinised excipulum, but the excipular elements comprise tangled rather than parallel hyphae. There is a poorly developed layer of flexuous and partly tangled hairs on the receptacle, these having roughened walls.

### ***Lachnaceae* on *Carex* (Fig 7)**

Represented by a single sequenced specimen (PDD 61822), the ITS sequence is close to a species on *Nothofagus* wood, but with poor support for a phylogenetic relationship.

### ***Lachnaceae* on *Nothofagus* wood (Fig 7)**

A unnamed New Zealand species, represented by PDD 82928 and PDD 110298, has white, densely hairy apothecia on wood. It is phylogenetically closest to a Norwegian specimen identified as '*Lachnum*' *rhytismatis* (note that this specimen was on *Vaccinium* leaves, while the type specimen is on leaves of *Acer* leaves but it matches closely a specimen from Switzerland from *Acer*, TNS-F-65645, GenBank LC424835).

### ***Lachnaceae* on *Weinmannia* (Fig 5)**

Represented by a single sequenced specimen (PDD 93937), the ITS sequence provides no support for a relationship to a named genus within the family.

### ***Lachnellula* (Fig 8)**

There are two sequenced species in New Zealand, one of these tentatively identified morphologically as *L. resinaria*. Represented by two specimens and known only from pine, this species is assumed to be exotic. Based on accessions in GenBank and UNITE, there are two genetic concepts of *L. resinaria*, one from Europe (UNITE UDB07672759, TUF139151; GenBank MN719894, G.M. 2015-08-02), the other from North America and Japan (GenBank AB481246, TNS-F-16450 (FC-2304); GenBank MT913605, MICH 340549). The New Zealand specimen has an ITS sequence with a 97% match to the European concept of *L. resinaria*, about 91% to the North American concept.

The second New Zealand species is unnamed and has been found once, on *Nothofagus* wood.

### ***Lachnopsis* (Figs 2 and 3)**

DNA sequencing and morphological examination has revealed at least 23 *Lachnopsis* species in New Zealand. Only three of these can be unequivocally linked to named species, '*Lachnum*' *pteridophyllum*, '*Lachnum*' *filiceum* and '*Lachnum*' *pteridicola*. Note that specimens with sequences in GenBank that have been identified as *Lachnum pteridophyllum* represent two distinct species, one from China is an *Erioscyphella* (Perić & Baral 2014), the other from Puerto Rico is basal within *Lachnopsis* (Guatimosim et al. 2016). Biogeographically, neither is likely to represent the same species as the type of *L. pteridophyllum*, originally described from Tasmania. Although Tasmania material has not been available to sequence, several specimens from New Zealand that morphologically match *L. pteridophyllum* as described by Spooner (1987) are within *Lachnopsis* but phylogenetically distinct from the species reported as this species from Puerto Rico. Based on these data, reports of this species being widely distributed across tropical regions are doubtful.

With few exceptions globally, species in *Lachnopsis* inhabit ferns. In contrast, few species in other genera of *Lachnaceae* inhabit ferns. For example, of the named and unnamed New Zealand *Lachnaceae* species in genera apart from *Lachnopsis* that have DNA sequences available, only three of the approximately 47 species (two in *Erioscyphella*, one in *Lachnum*) are found on ferns.

Two of the *Lachnopsis* species on substrates other than ferns (PDD 91757 on *Beilschmiedia tawa* and PDD 93117 on *Leucopogon fasciculatus*) are morphologically unusual for the genus in having asci thin-walled at the apex and with no amyloid reaction. In a traditional morphological sense, these species match *Perottia* sensu Spooner (1987), a genus he discussed as being close to *Lachnellula*. Although the lack of an amyloid ascus pore is a distinctive and easily observed feature, Baral & Matheis (2000) regarded it as taxonomically uninformative and they reject this as a character useful for distinguishing *Perottia* from *Lachnellula*. Our two *Perottia*-like fungi are deep within the *Lachnopsis* clade and phylogenetically distant from specimens accepted as *Lachnellula*. These *Perottia*-like fungi have excipular tissue that is less gelatinous than typical for *Lachnellula*.

### ***Lachnum* (Fig 6)**

The ITS tree resolves a strongly supported *Lachnum* s.s. clade, but included in this clade is the monotypic *Asperopilum juncicola*. Although *A. juncicola* is morphologically divergent from most *Lachnum* species, this phylogenetic relationship is supported by a multi-gene analysis (see notes above under *Asperopilum*).

Most of the New Zealand species of *Lachnum* are found on dead leaves of monocotyledons, with a few *L. virgineum*-like species on wood. Based on small differences in ITS sequences, there appear to be a complex of wood-inhabiting species close to *L. virgineum* and another complex of monocotyledon-inhabiting species close to *L. hyalopus* and *L. apalum*.

These taxa need to be carefully studied in detail using both morphological and molecular data but some tentative notes are included here. *Lachnum hyalopus* (a *Phormium* specialist), *Lachnum apalum* var. *beatonii* (a *Juncus* specialist), and unnamed *Lachnum* species on *Carex* (PDD 117556), *Astelia* (PDD 71062) and *Cyathea* (PDD 112178) are phylogenetically very close. Collectively, their ITS sequences have a 98.7% match and the various species lack unique sets of consistent nucleotide changes. However, backing up the host-related species structure are morphological differences, especially in ascospore size and shape of the excipular cells. *L. apalum* var. *beatonii* has longer ascospores than *L. hyalopus* (40–65  $\mu\text{m}$  versus 30–45  $\mu\text{m}$ ), short-cylindric, more or less square, excipular cells versus long-cylindric excipular cells, and hairs undifferentiated compared to slightly swollen at the tips. The species on *Astelia* has ascospores 15–20  $\times$  2.5  $\mu\text{m}$ , short and broad excipular cells and hairs slightly swollen at the tips. The species on *Cyathea* has ascospores 55–75  $\times$  2  $\mu\text{m}$  and long-cylindric excipular cells.

### ***'Lachnum' willisii* (Fig 8)**

Originally described from Australia, specimens matching morphologically and ecologically (growing on leaf tomentum of living leaves of *Asteraceae*) occur also in New Zealand. The dark brown, thick-walled hairs of this species are distinctive for *Lachnaceae*, and in the ITS tree it appears to be basal within *Lachnaceae*, but with low levels of support meaning that this position needs confirming with additional genes.

### ***Neodasyscypha* (Fig 8)**

Sequenced specimens from New Zealand closely match specimens identified as *N. cerina* from North America and Europe, confirming genetically the broad geographic distribution of this wood-inhabiting species.

### ***Perrotia* (Fig 7)**

Two GenBank accessions identified as the type species *Perrotia flammea*, one from Europe and one from North America, represent different species, although forming a monophyletic clade in an ITS based phylogeny. No New Zealand sequences fall into this clade.

Several *Perrotia* species have been reported from New Zealand, but whether they belong in the genus phylogenetically is not known. There are some New Zealand species of *Lachnopsis* with a *Perrotia*-like morphology (see notes above) and it is possible that several phylogenetically distant fungi have been placed in *Perrotia* because of their distinctive ascus morphology. The specimens in the *P. flammea* clade are phylogenetically distant to a specimen identified in GenBank as *Perrotia distincta* (Fig 5).

### ***Proliferodiscus* (Fig 5)**

Specimens morphologically typical of *Proliferodiscus dingleyae* are common on *Nothofagus* bark in New Zealand, and occasionally also on other woody substrates, and this fungus is sometimes also isolated as an endophyte from living leaves of *Nothofagus* (Fig 5). These specimens fall in a clade in the ITS tree with several specimens identified as *Proliferodiscus* from other parts of the world. However, based on variation in ITS sequences, the New Zealand specimens appear to represent a complex of perhaps four separate species. To keep in mind is the possibility of unusual variation

within ITS sequences across a single species. From one of the collections sequenced (PDD 59362 ex *Ulex*), divergent sequences were generated from two different apothecia, each sequence from a very clear .abi file.

Also in this clade are specimens identified as *Perrotia distincta* (phylogenetically distant from the type of the genus, *Perrotia flammea*).

### ***Velebitea* (Fig 8)**

One New Zealand specimen collected from decorticated wood (PDD 108745) is an unnamed species phylogenetically close to the monotypic *Velebitea chrysotexta*. Although *V. chrysotexta* was recombined in *Dasyscyphella* by Baral & Quijada (2020), Kušan et al. in Phookamsak et al. (2019) discuss the reasons they did not use this genus for their new species, including lack of DNA sequence data for the type species of *Dasyscyphella*, the apparently polyphyletic nature of *Dasyscyphella* based on GenBank accessions (see notes above), and morphological differences between *V. chrysotexta* and the type species of *Dasyscyphella*, *D. cassandrae*. Phylogenetically, *Velebitea* forms a clade isolated within Lachnaceae, distant to any sequenced specimens identified as *Dasyscyphella*.

The New Zealand species has a 98% match to *V. chrysotexta*, and they share many micromorphological features; morphology of the hairs, paraphyses, ascospores, etc. Macroscopically the New Zealand species and *V. chrysotexta* are quite distinct.

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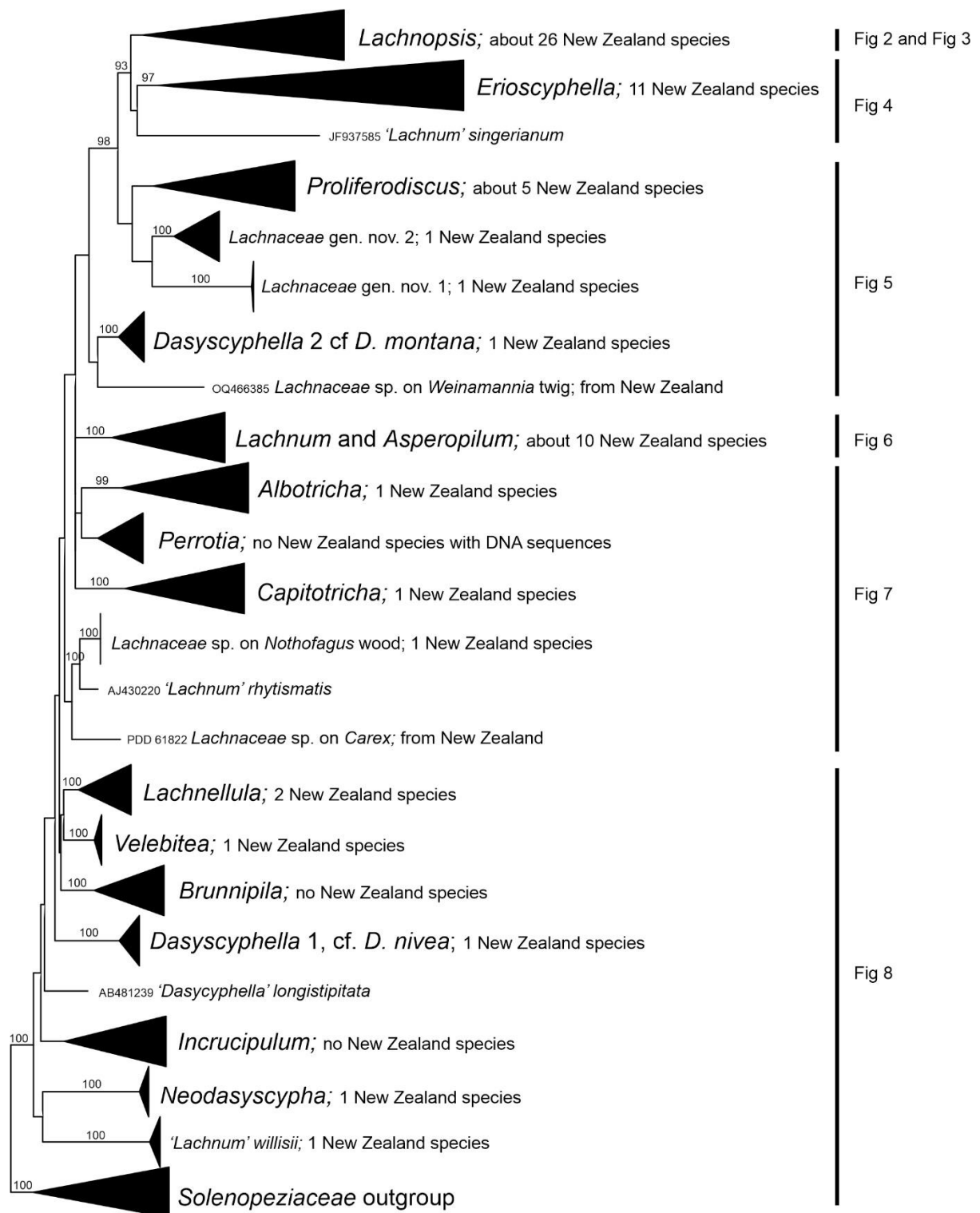


Fig 1

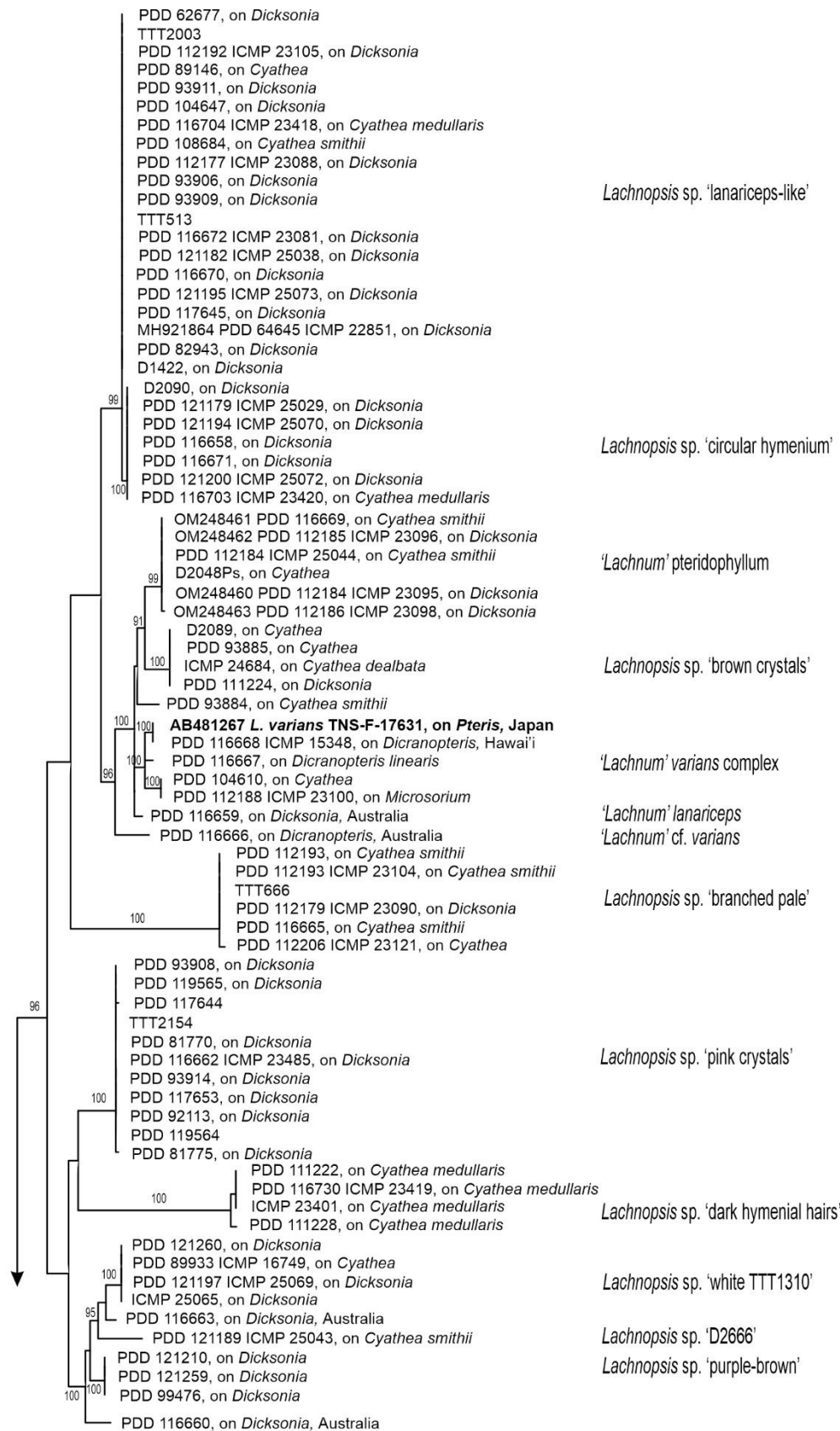


Fig 2

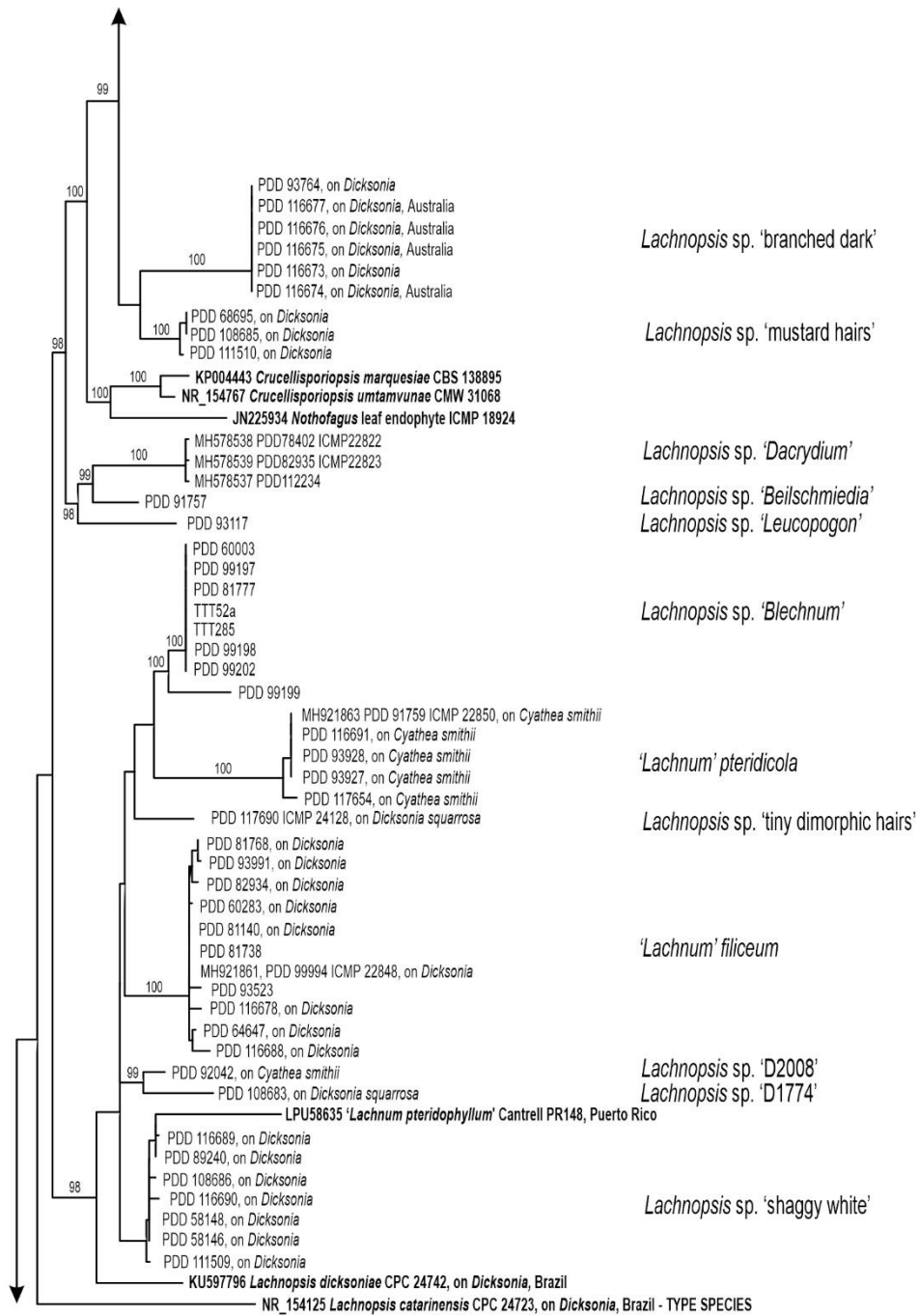


Fig 3



Fig 4

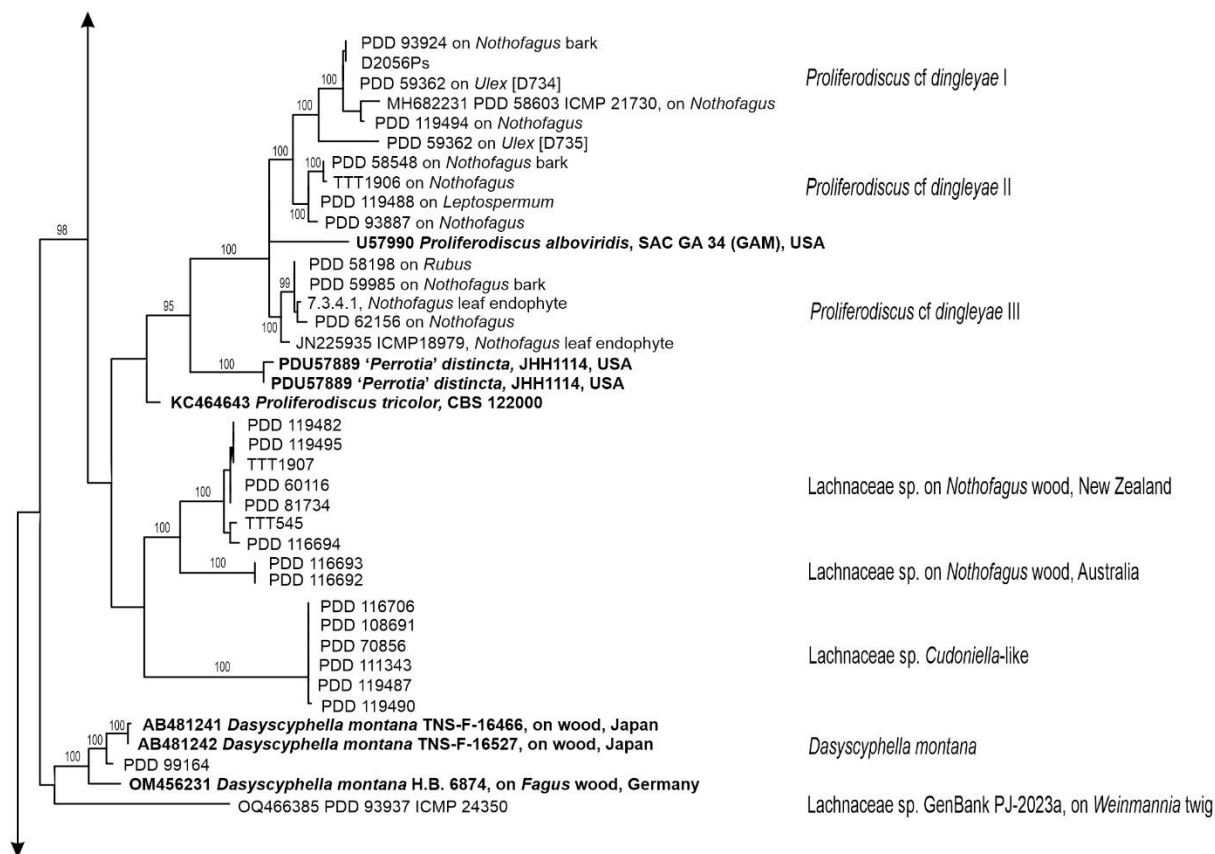


Fig 5

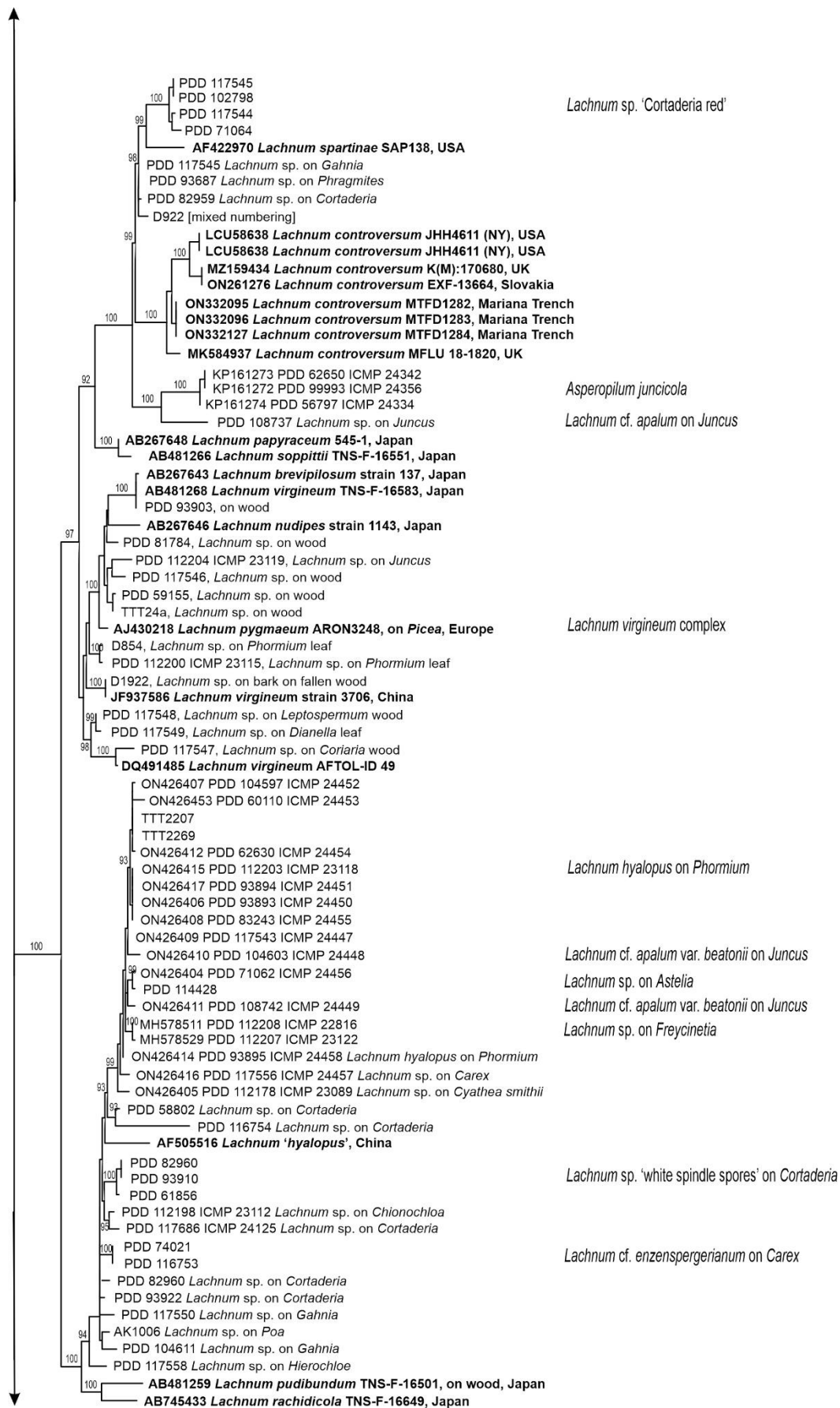


Fig 6

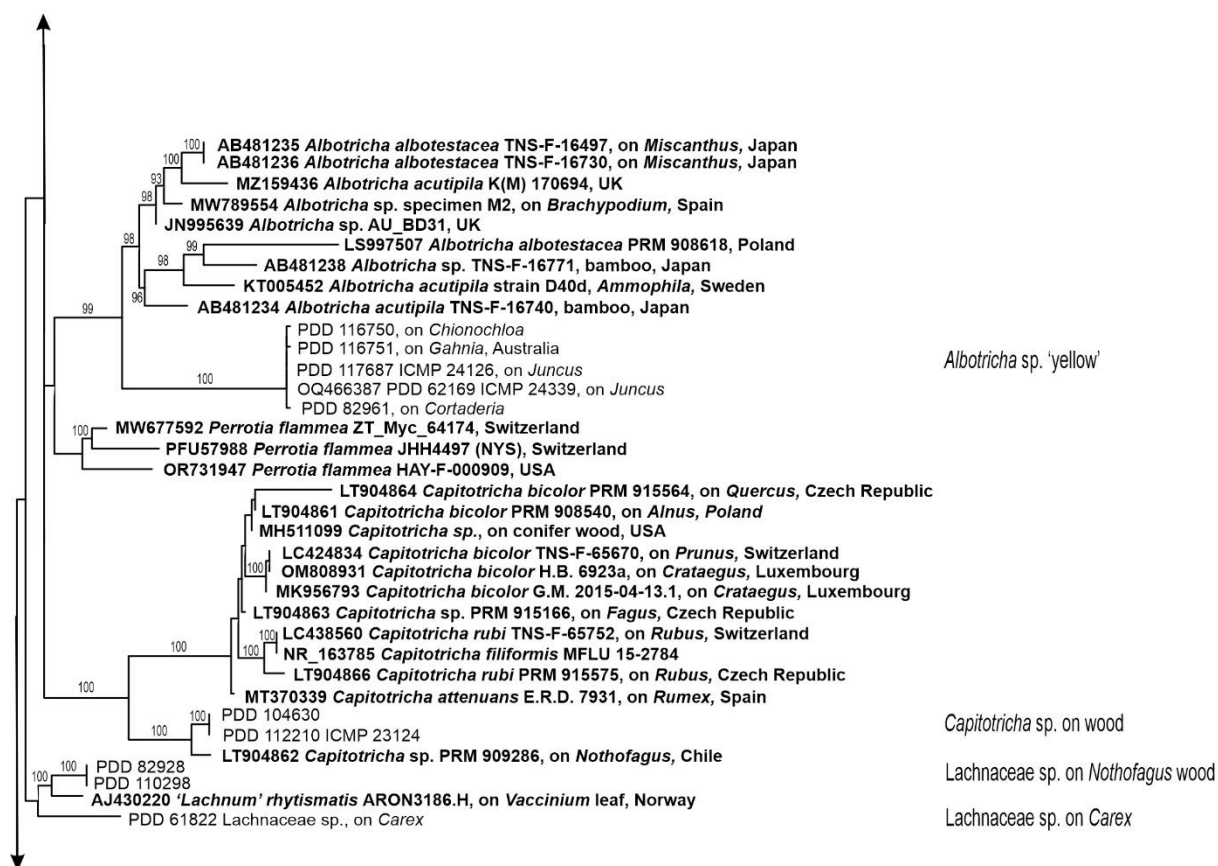


Fig 7



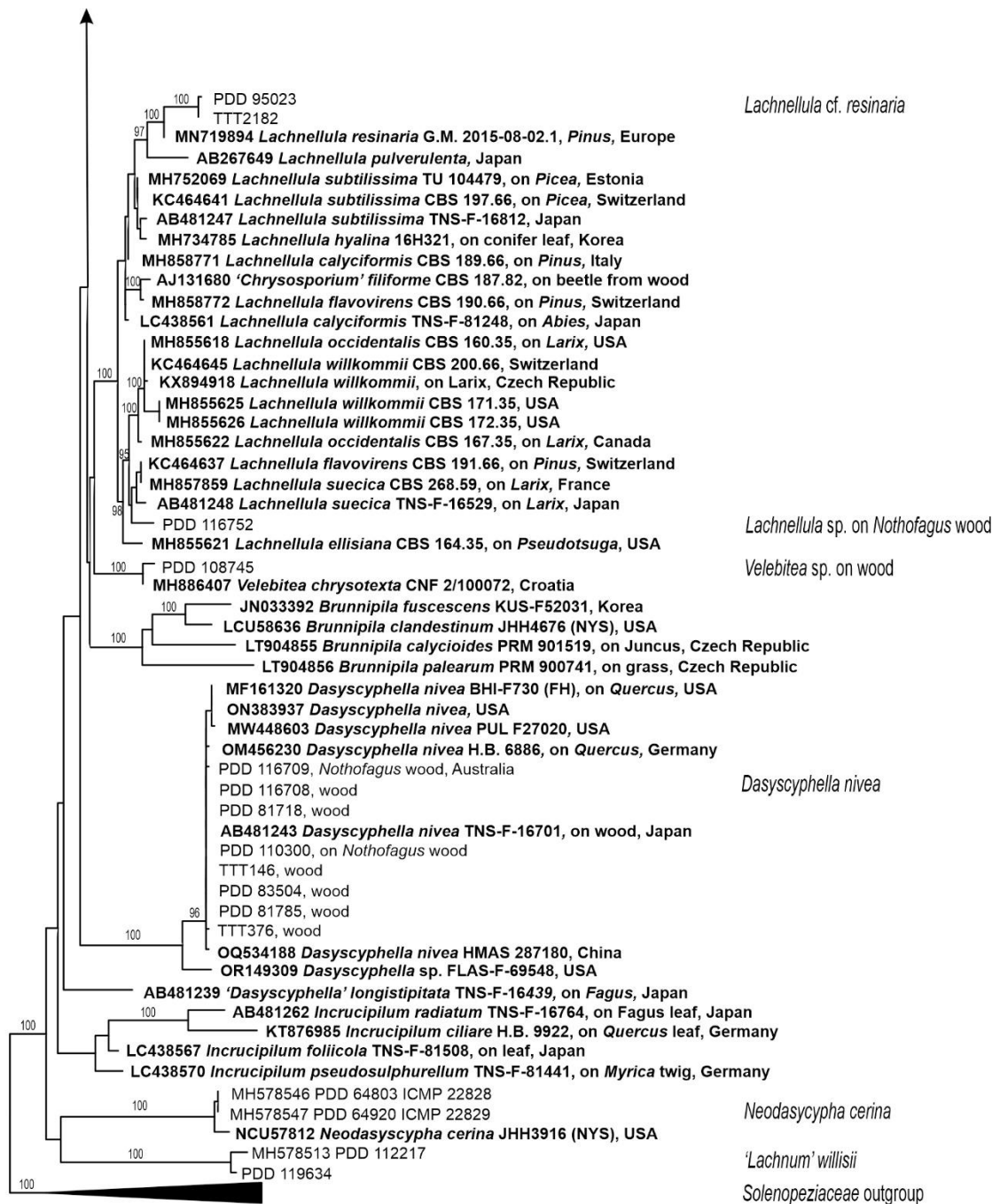


Fig 8