

***Annulohyphoxylon* in New Zealand**

P.R. Johnston, July 2024

These notes are based on PDD specimens and ICMP cultures from which DNA sequences were generated in studies by Barbara Paulus (Manaaki Whenua—Landcare Research postdoc project, 2003–2004) and Renee Johansen (Auckland University honours dissertation, 2011).

Annulohyphoxylon species reported from New Zealand include *A. bovei*, *A. moriforme* and *A. nothofagi* (Ju & Rogers 1996), and in addition there are PDD specimens identified as *A. archeri*, *A. bovei* var. *microspora* and *A. cf. truncatum*.

There are no DNA sequences available for *A. nothofagi*.

Of the other species, only *A. bovei* is accepted here as present in New Zealand (Figs 2–4). This is supported by DNA sequences from specimens morphologically and ecologically typical of *A. bovei* sensu Ju & Rogers (1996, as *Hyphoxylon bovei*) (Fig. 1). However, this species was originally described from Argentina, but there are no DNA sequences for this species from southern South America to confirm that the New Zealand fungus matches genetically.

There are several GenBank records from China accessioned as *A. bovei*, but from a BLAST search they match specimens accessioned as *A. bovei* var. *microspora* and *A. areolatum* (regarded as synonyms by Kuhnert et al. 2017).

Specimens from New Zealand originally identified morphologically as *A. moriforme* (including some treated below as species 2 and species 3) and as *A. bovei* var. *microspora* (including some treated below as species 1 and species 4) have DNA sequences that do not match GenBank accessions deposited as these taxa by experts in *Xylariaceae* taxonomy. There is no genetic evidence that these fungi occur in New Zealand.

Some of the New Zealand specimens with stromatal tissue diffusing dark green pigments into KOH have been identified as *A. archeri* or as *A. moriforme*, including some of those treated below as species 2 and species 3. However, based on the descriptions in Ju & Rogers (1996), the New Zealand specimens differ from *A. archeri* in having slightly larger ostiolar discs and from *A. moriforme* in having larger ascospores. *A. archeri*, described originally from Tasmania, has no reliable and authentic sequences in GenBank.

From the DNA sequences available, New Zealand has at least four apparently unnamed species of *Annulohyphoxylon* (Fig. 1). Based on the sequenced specimens, these species can be distinguished morphologically by differences in KOH extractable pigments, degree to which the individual perithecia are exposed, size of the ostiolar discs, size of the ostiole papillae, ascospore size and substrate (whether bark or decorticated wood) (Table 1).

Stadler and Fournier tentatively redetermined two PDD specimens previously identified as *Annulohyphoxylon moriforme* (PDD 73042) and *A. archeri* (PDD 72411) as southern hemisphere *Hyphoxylon cf. truncatum* sensu Ju & Rogers (1996). Morphologically PDD 73042 seems to represent *Annulohyphoxylon* species 2, with perithecia developing on a black plate on the substrate surface and strongly papillate ostioles, although the green pigment changes to blackish and the stromata are on decorticated wood rather than bark. Morphologically, PDD 72411 seems to match species 3, a species with distinctive ostiolar discs that are proportionally larger compared to the perithecial diameter than species 2. A culture has been grown from the ascospores of PDD 72411, this can be used to test this tentative identification.

Table 1. Morphological features of the New Zealand *Annulohyphoxylon* species with DNA sequences.

	<i>A. bovei</i>	Species 1	Species 2	Species 3	Species 4
Perithecial size (mm)	1.3–1.5	0.9–1.3	0.5–0.8	0.5–0.7	1–1.3
Ostiolar disc diam. (cm)	0.6–0.8	0.2–0.32	0.25–0.4	0.3–0.5	0.3–0.5
Ascospore size (µm)	10.5–13(–14) × 5–6.5	8.5–10 × 4–5	9–10 × 4.5–5	9.5–10.5 × 4–5	10–11 × 4.5–5.5
Pigment in 10% KOH	None or slightly blackish	None (rarely faintly green, fading black)	Immediate intense green	Immediate intense green	Slowly faint green, fading blackish
Macroscopic appearance of ascomata	Perithecia widely spaced, often more or less separate, surface with felted brownish appearance	Perithecia widely spaced, sometimes more or less separate, finely papillate ostiole	Perithecia tightly packed, on black plate that extends across substrate, ostiole broadly and strongly papillate	Perithecia tightly packed, ostiolar disc proportionally large compared to perithecial diameter, but margin of disc often poorly defined.	Perithecia strongly exposed forming distinctive separate clusters, surface with felted brownish appearance
substrate	Bark on fallen wood	Decorticated wood, usually blackened	bark on fallen wood.	decorticated wood	Bark on fallen wood

***Annulohyphoxylon* species 1** (Figs 5–10)

This species usually has no KOH extractable pigment with one exception, PDD 83620 produces a small amount of green pigment, that quickly fades to blackish. *Annulohyphoxylon* sp. 4 and *H. bovei* have a similar behaviour but have slightly larger ascospores, larger ostiolar discs, and are found on bark attached to dead wood rather than decorticated wood (Table 1).

***Annulohyphoxylon* species 2** (Figs 11–16)

This species is morphologically close to *A. archeri*, sharing the reddish colour to the stroma, intense green KOH extractable pigments and ascospore size. It differs in having an ostiolar ring slightly larger than reported for the type specimen of *A. archeri*, 0.25–0.4 mm as opposed to less than 0.2 mm discs for *A. archeri*.

Annulohyphoxylon sp. 3, also produces an intense, green KOH extractable pigment but the sequenced specimens differ in having a more finely papillate ostiole and being found on decorticated wood rather than dead bark. Its ostiolar disc also covers more than half of the surface of the perithecia, proportionally larger than those of species 2.

***Annulohyphoxylon* species 3** (Figs 17–18)

Annulohyphoxylon sp. 3 and *Annulohyphoxylon* sp. 2 both produce an intense, green KOH extractable pigment. The sequenced specimens of species 3 differ in having a less coarsely papillate ostiole, the size of the ostiolar disc compared with the perithecial size is proportionally larger, and the sequenced specimens were found on decorticated wood rather than dead bark.

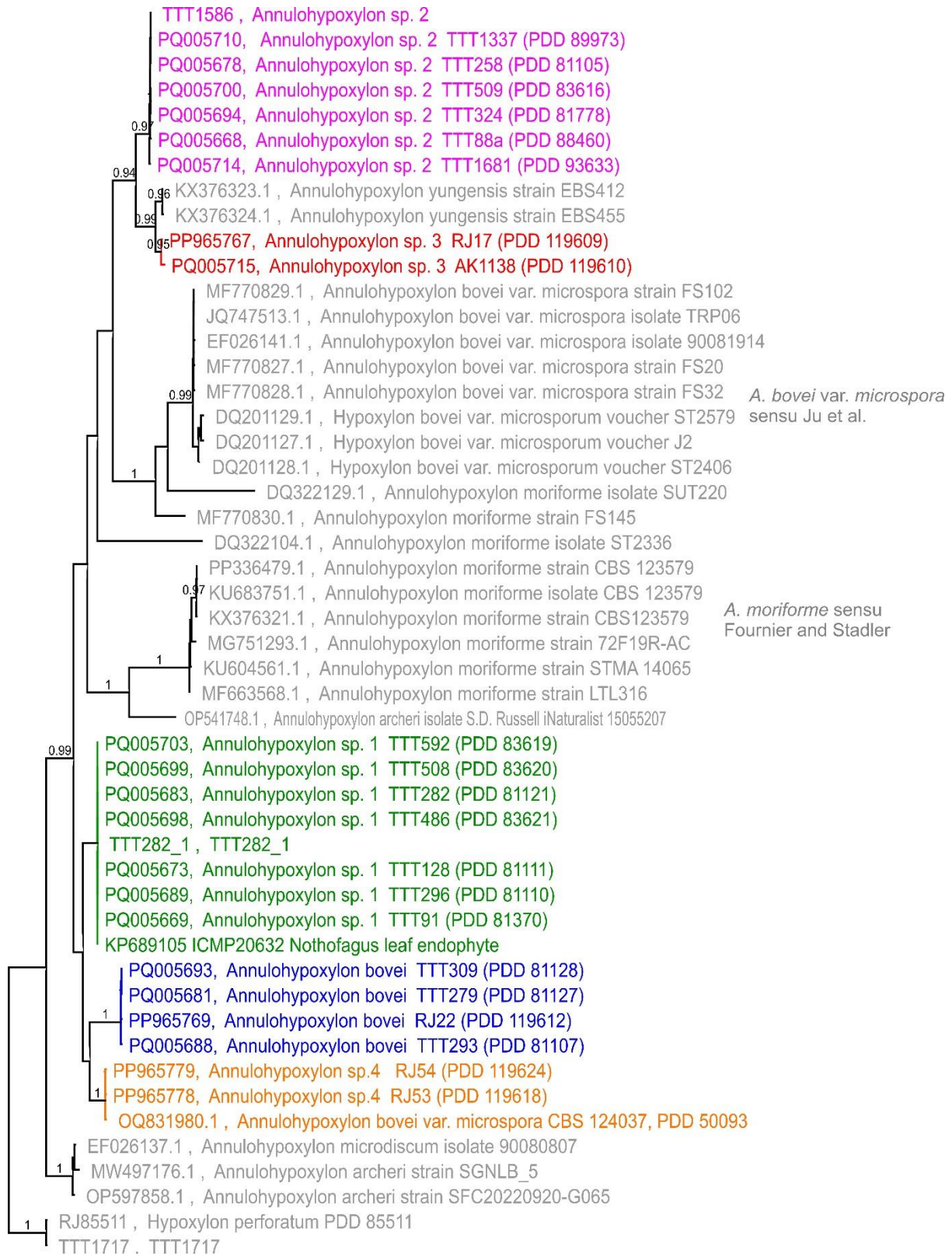
Based on ITS sequences, this species has a sister relationship with the southern South American species, *A. yungensis* (Fig. 1). Based on the description in Kuhnert et al. (2017), *A. yungensis* has smaller ostiolar discs and ascospores, and brownish rather than green pigments in KOH.

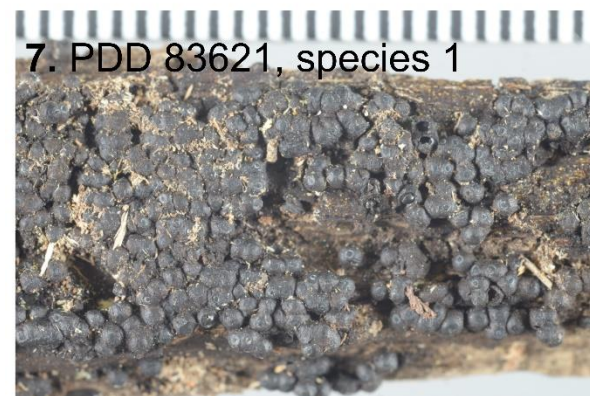
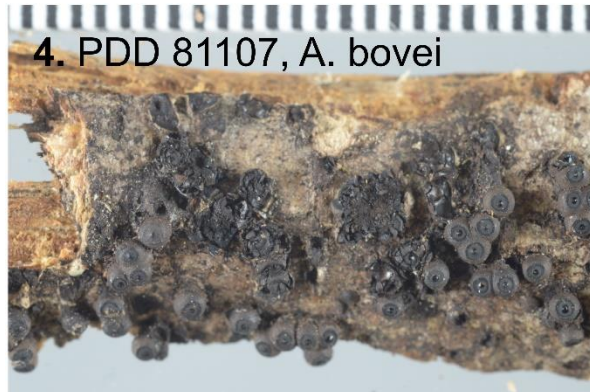
***Annulohyphoxylon* species 4** (Figs 19–20)

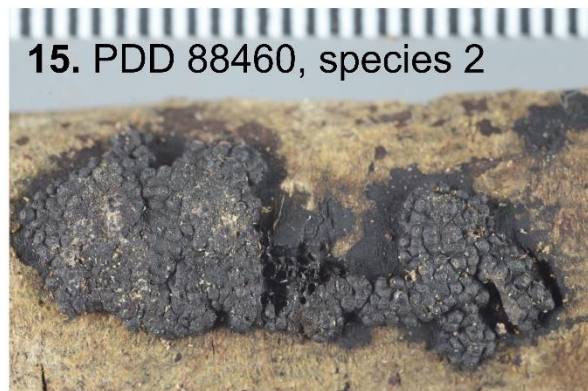
The three sequenced PDD specimens had all been incorrectly identified as *A. bovei* var. *microspora*, morphologically similar to this species. It has ascospores slightly larger than the other unnamed species and slightly smaller than *A. bovei*. The KOH extractable pigment is released slowly, initially pale green, fading to blackish.

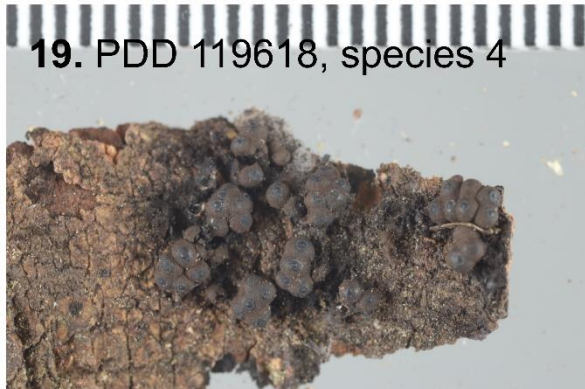
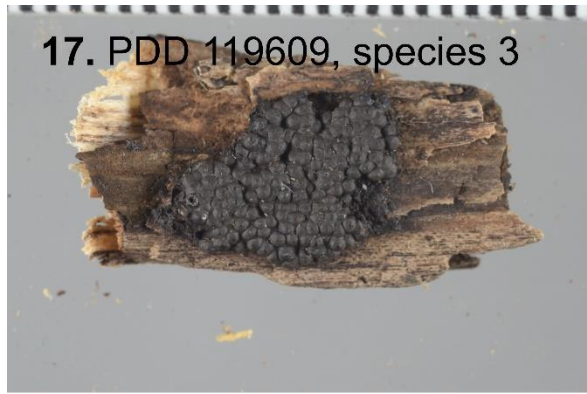
More than 20 PDD specimens have been identified as *A. bovei* var. *microspora*, they have not been compared critically with the sequenced specimens.

Fig. 1. Phylogeny based on ITS sequences. New Zealand species compared with close BLAST matches and other taxa deposited in GenBank under names of relevance to New Zealand. *Hypoxylon perforatum* used as outgroup.









References

Ju YM, Rogers JG 1996. A revision of the genus *Hypoxylon*. The Mycological Society of America, Mycological Memoir 20: 1–363.

Kuhnert, E; Sir, EB; Lambert, C; Hyde, KD; Hladki, AI; Romero, AI; Rohde, M; Stadler, M. 2016. Phylogenetic and chemotaxonomic resolution of the genus *Annulohypoxylon* (Xylariaceae) including four new species. *Fungal diversity*. 85:1–43.