

## Two species of *Inocybe* (fungi) introduced into Western Australia

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### Abstract

Bougher, N.L. and Matheny, P.B. Two species of *Inocybe* (fungi) introduced into Western Australia. *Nuytsia* 21(3): 139–148 (2011). This paper reports for the first time the introduction into Western Australia from the northern hemisphere of *Inocybe curvipes* P.Karst. and *Inocybe rufuloides* Bon. The fungi are associated with planted non-native ectomycorrhizal trees – *Quercus*, *Pinus*, and possibly *Salix*. Previously, *I. curvipes* was confirmed from South Australia where it has been present for at least almost a century, but it was not known from Western Australia. *Inocybe rufuloides* had not been recorded anywhere in Australia. In this paper *I. curvipes* is also reported for the first time in Papua New Guinea, in association with planted *Quercus* and *Pinus*. *Populus* and *Quercus* are confirmed as ectomycorrhizal partners of *I. curvipes* based on ITS BLASTn analysis of environmental sequences.

### Introduction

Numerous ectomycorrhizal macrofungi have been introduced from the Northern Hemisphere into Western Australia with their exotic plant associates. The most recent conspicuous introduction is the long anticipated first record of *Amanita muscaria* in Western Australia, in this case associated with *Betula pendula* (Robinson 2010). Eleven species of ectomycorrhizal fungi have been recognized in association with *Pinus* in Western Australia (Dunstan *et al.*, 1998). Some species of *Inocybe* are known to occur with planted *Pinus* in other regions of the Southern Hemisphere, but to date none have been reported in Australia (Dunstan *et al.* 1998; Walbert *et al.*, 2010). This paper reports and describes two introduced north temperate species of *Inocybe* previously unrecorded in Western Australia – *Inocybe curvipes* P. Karst., and *Inocybe rufuloides* Bon. *Inocybe curvipes* has been present in Australia for at least almost a century. This is indicated by some of the specimens in a mixed collection from South Australia in 1917 (associated plants not recorded) that recently were recognized for the first time as *Inocybe curvipes* by Matheny & Bougher (2010). *Inocybe rufuloides* had not been reported anywhere in Australia.

### Methods

Fresh fruit bodies collected from the field were described following colour notations of Munsell Soil Color Charts (1954), Ridgway (1912), or Kornerup and Wanscher (1967) and then air-dried for

later examination. Hand sections for microscopic observations were mounted in 3% KOH and in 1% Congo Red. Microscopic characters were drawn with a Nikon drawing tube system. Spore measurements include the hilar appendix and nodules.

DNA sequences of the internal transcribed spacer regions (ITS) and the 5' end of the nuclear large subunit ribosomal RNA region (nLSU) were produced following protocols outlined in Judge *et al.* (2009) and Matheny (2005). Seven new sequences (JN035289–JN035295) have been deposited at GenBank. The new sequences were used as queries of the NCBI nucleotide sequence database using the BLASTn search algorithm.

### Taxonomic description

**Inocybe curvipes** P.Karst., *Hedwigia* 29: 176 (1890). *Type*: Tammela, Mustiala, Myllyperä, Finland, 22 August 1890, *P.A. Karsten s.n. (holo: H 6025661)*.

*Inocybe radiata* Peck, *Bull. Torrey Bot. Club* 22: 488 (1895).

*Inocybe lepidoccephala* Speg., *Anales Mus. Nac. Buenos Aires* 6: 126 (1898).

*Inocybe variabilissima* Speg., *An. Mus. Nac. Bs. As.* 6: 125 (1899).

*Inocybe decipientoides* Peck, *Bull. Torrey Bot. Club* 34: 100 (1907).

*Inocybe astoriana* Murrill, *Mycologia* 3: 104 (1911).

*Inocybe jamaicensis* Murrill, *Mycologia* 4: 82 (1912).

*Inocybe ochraceoscabra* Atk., *Am. J. Bot.* 5: 214 (1918).

*Inocybe globocystis* Velen., *Ceské Houby*: 368 (1920).

*Inocybe languinella* (J.Schröt.) Konrad & Maubl., *Icon. Select. Fung.* 6: 137 (1937) *sensu auct. div., non* Konrad & Maubl.

*Astrosporina decipientoides* (Peck) Pearson, *Trans. Brit. Mycol. Soc.* 22: 28 (1938).

*Inocybe cinerascentipes* Huijsman, *Fungus* 25: 26 (1955).

*Inocybe sericeopoda* Furrer–Ziogas, *Beih. Sydowia* 10: 62 (1995).

*Inocybe curvipes* var. *globocystis* (Velen.) Bon, *Docs. Mycol.* 28 (109–110): 10 (1998).

[*Inocybe cicaetricata* auct. non Ellis & Everh.: R.Heim, *Encylop. Mycol.*, 1 Le Genre *Inocybe* (Paris) 1: 351 (1931)].

[*Inocybe carpta* auct. non (Scop.:Fr.) P.Kumm.: Ricken, *Die Blätterpilze*: 101 (1915)].

[*Inocybe lanuginella* auct. non (J.Schröt.) Konrad & Maubl.: W.A.Dunstan, B.Dell & N. Malajczuk, *Mycorrhiza* 8: 73 (1998)].

*Pileus* to 27 mm diam., conical, hemispherical, or obtusely conical in youth, becoming subcampanulate, obtusely conical or more or less convex upon expansion, margin incurved when young, later decurved; velipellis absent, surface dry, appressed fibrillose with an unbroken center when young, later finely appressed scaly on and around the center and fibrillose towards the margin; margin torn and at times radially rimulose; dark brown (10YR 3/3) throughout or brown (10YR 4/3) or shading brown towards the margin, at times the edge of the margin is yellowish brown (10YR 5/4) – ‘Mummy Brown’ to ‘Bister’ to ‘Snuff Brown’, shading to ‘Snuff Brown’, ‘Saccardo’s Umber’ to ‘Tawny-Olive’ towards the margin; context pallid or white, not changing color where bruised. *Lamellae* adnate to adnexed, moderately close, 34–48 L with several tiers of lamellulae, ventricose in age, up to 4 mm broad; light gray when immature to light yellowish brown or brown, eventually dark yellowish brown in age; edges pallid and fimbriate. *Stipe* 20–40 mm × 3–7 mm at apex, even or with a slightly swollen base; cortina fugacious, surface nowhere pruinose, finely-fibrillose to fibrillose or twisted fibrillose, generally pallid above shading to light brown or ‘Avellaneous’ below, but brown to dark brown or ‘Bister’ at the base and upwards in age; context solid, mostly pallid, at times with subtle pinkish brown cortex. Odour spermatic when first cut, later mixed with an acidulous component. Taste not recorded. Spore deposit not recorded.

*Basidiospores* (8.5) 9–12 × 5–6.5 μm, mean 10.3 × 5.6 μm, mean profile 10.3 × 5.6 μm (n= 30), mean face view 9.9 × 5.7 μm (n= 30), mean L/B ratio profile 1.82, mean L/B ratio face view 1.75, gibbous about a trapeziform outline with 6–10 moderate to small-sized nodules to 2 μm tall, at times these merely corners, usually with a distinct apical nodule, yellowish brown, apiculus distinct. *Basidia* 26–34 × 8–11 μm, predominantly quadrisporic, but also sometimes bisporic and monosporic then with long sterigmata to 15 μm, clavate, cylindric or ventricose (particularly when mono- and bi-sporic). *Lamellae trama* of parallel hyphae – 12 μm wide, with similar but narrower hyphae in the subhymenium. *Pleurocystidia* 47–73 × 19–27 μm, broadly fusiform or saccate and noticeably tapered towards the apex, this usually acute (not obtuse or well-rounded) and crystalliferous, with a slender, long basal pedicel (e.g. 12 × 5 μm); thick-walled, walls to 2.5 μm thick, hyaline. *Cheilocystidia* 48–66 × 15–24 μm similar to pleurocystidia but more variable in shape with abundant small clavate and vesiculose paracystidia among the larger cystidia. *Stipitipellis* with no caulocystidia; sparse vesture hyphae observed on lower part of the stipe. *Pileipellis* a cutis of cylindric hyphae 4–15 μm wide, many with brown encrusting pigment, some emerging in scattered bundles and with cystidioid terminal elements e.g. lageniform 51 × 4 μm. *Clamp connections* present in all tissues. (Figures 1, 2)

*Specimens examined.* WESTERN AUSTRALIA: Carine, Perth, gregarious on soil under *Quercus* in suburban backyard garden, 31 Aug. 2001, *B. Dunstan* PBM 2210 (PERTH 007676859, formerly CSIRO E7058); Botany Department, campus of University of Western Australia, Crawley, Perth, in rose garden, 6 June 1973, *D. Waldie* s.n. (PERTH 00763578). AUSTRALIAN CAPITAL TERRITORY: Black Mountain CSIRO, Canberra, in grass at base of *Pinus radiata* near the herbarium, 14 Feb. 1990, *E. Cooper* EC 4 (CANB 404918, ITS and LSU sequences respectively: JN035289, JN035293). SOUTH AUSTRALIA: Belair National Park, unrecorded habitat and plant association, 12 May 1917, *J. B. Cleland* s.n. (AD12143). VICTORIA: Templestowe, Melbourne, growing in lawn in rings under

weeping willow (presumably *Salix*), 29 May 1963, *P. Jenkins s.n.* (MEL 1054882). PAPUA NEW GUINEA: near Massy, along road under *Pinus*, 27 July 2003, *R. Treu* TR37–03 (M–0138268, ITS and LSU sequences respectively: JN035290 and JN035294). JAMAICA: Cinchona, wet mountains area, 4500–5200 ft elev, in a clay road, collected between 25 Dec. 1908 and 8 Jan. 1909, *W.A. Murrill & E.L. Murrill* WAM & ELM 595 (WTU, isotype of *Inocybe jamaicensis* Murrill). UNITED STATES OF AMERICA: Massachusetts, in open grassy ground, Aug. and Sep., *W.D. Jackson* (WTU, isotype of *Inocybe radiata* Peck); Washington, next to Burke–Gilman Trail near the Wall of Death, University District, Seattle, on disturbed soil along side of road near *Corylus*, *Prunus*, and *Populus*, 18 June 1998, *P.B. Matheny* PBM1007 (WTU); on grassy lawn under introduced *Arbutus unedo* with *Pinus* in the vicinity, 28 Oct. 1998, *P.B. Matheny* PBM 1328 (WTU); Green Lake, Seattle, King County, on ground under *Betula*, 15 Sep. 2002, *J.F. Ammirati & M.T. Seidl* PBM 2401 (WTU).

*Distribution and habitat.* On soil under introduced *Quercus* or *Pinus radiata*, and possibly *Salix*, introduced into Western Australia, Australian Capital Territory, South Australia, Victoria, and Papua New Guinea. Fruiting February, May to August (in Australia).

*Notes.* *Inocybe curvipes* is distinctive microscopically by its broadly fusiform hymenial cystidia that are tapered at the acute apex (Figures 2C, 2D), and its long spores with 6–10 moderate to small-sized nodules or merely corners (Figure 2A). The presence of monosporic and bisporic basidia (Figure 2B) is not a consistent feature of this species but they are present in some collections from Australia and elsewhere. They occur in only one of the two Western Australian collections examined (PERTH 07676859). *Inocybe curvipes* has a restricted habitat in Australia as it occurs in gardens and suburban plantings of *Quercus* or *Pinus* or possibly *Salix*. It has not been found in natural vegetation or in exotic plantations of *Pinus* in Australia. We previously recognized this species for the first time in Australia – from South Australia (Matheny & Bougher 2010), but it had not been reported in other parts of Australia or in Papua New Guinea.

BLASTn results of our *I. curvipes* ITS sequences reveal 16 matches (97–99% similarity) with unidentified environmental sequences from ectomycorrhizas of *Populus* in plantations (Stefani *et al.* 2009), two matches (99% similarity) with *Quercus* ectomycorrhizas from a nursery setting (Leski *et al.* 2010), two Swedish isolates labeled *I. curvipes* (99% similarity), and one Estonian isolate labeled *I. cf. rennyi* (99% similarity) (Ryberg *et al.* 2008). These data unequivocally confirm *I. curvipes* as an ectomycorrhizal associate of *Populus* and *Quercus* in plantation and nursery settings outside Australia. The high similarity with the isolate labeled *I. cf. rennyi* is noteworthy because presumably the collection from which the sequence was obtained must feature the unusually elongated spores typical of *I. rennyi* (Kropp & Matheny 2004). Evidence is mounting that either *I. rennyi* represents a polyphyletic taxon, or is at least merely an aberrant spored version of *I. curvipes*. ITS sequences of collections from the ACT and Papua New Guinea differ at three sites, two of which are polymorphic in the ACT collection.

*Inocybe curvipes* has been present in Australia for at least almost a century. A collection of it from South Australia (AD12143) was made by J. B. Cleland in 1917. That collection was originally unidentified by Cleland, and later incorporated together with *Asterosporina asterospora* sensu Cleland and merged into the protologue of *Inocybe dewrangia* Grgur. (Grgurinovic 1997, Matheny & Bougher 2010). *Inocybe curvipes* was likely introduced into Australia and Papua New Guinea together with imported north temperate ectomycorrhizal plants such as *Quercus* and *Pinus* species. It has been similarly introduced into other southern hemisphere regions, e.g. *I. curvipes* (as *I. lanuginella*) is recorded as an ectomycorrhizal fungus associated with *Pinus* in South Africa (Dunstan *et al.*, 1998). It is also widespread in South America under *Pinus* (Cortez & Coelho 2005).



Figure 1. Basidiomes of *Inocybe curvipes* (PERTH 007676859).

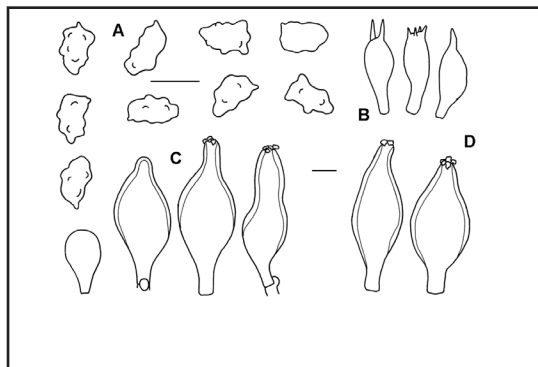


Figure 2. Micromorphology of *Inocybe curvipes* (PERTH 007676859). A – spores; B – basidia; C – cheilocystidia; D – pleurocystidia. Scale bars = 10  $\mu$ m (longer bar for spores only).

*Inocybe curvipes* is a toxic species that can be expected to occur in urban or suburban habitats of non-native, north temperate ectomycorrhizal trees. While revising collections of *Inocybe* at MEL in Melbourne, we identified one from the 1960s as *I. curvipes* (originally identified as *Inocybe* sp. – MEL1054882), which was annotated with the words “cause of muscarin poisoning in woman who gathered it to eat it in mistake for mushroom”.

*Inocybe curvipes* is widespread in the northern hemisphere temperate zone having been described numerous times in Europe, eastern and western North America, and Central America. As is the case with many other Inocybaceae, *I. curvipes* has been misidentified numerous times since it was originally published in 1890 by Karsten; for example, two eminent American mycologists, C. H. Peck and W. A. Murrill, both subsequently published this species under two different newly proposed names (see synonymy above). Kauffman (1924) reported *I. ochraceoscabrosa* Atk. as a synonym of *I. decipentoides*, both of which are later synonyms of *I. curvipes* (Stangl 1989). Our list of synonyms follows Stangl (1989), to which we add the following: *I. radiata* Peck and *I. jamaicensis* Murrill based on studies of

the types. Stangl's list of synonyms of *I. curvipes* follows much of Singer (1949), who reported the following species as synonyms of *I. decipientoides*: *I. astoriana* Murrill, *I. ochraceoscabrosa* Atk., *I. globocystis* Velen. as reconfirmed by Kuyper (1985), *I. carpta* (Scop.: Fr.) Sacc sensu Ricken, and later in the same publication *I. variabilissima* Speg. We also list *I. sericeopoda* Furrer–Ziogas as a synonym of *I. curvipes* based on the figures depicted in Furrer–Ziogas (1995) and in line with the synonymy presented in Esteve–Raventós & Caballero (2009); however, we have not examined the type, which is indicated in a private herbarium.

*Inocybe cicatricata* sensu R. Heim (1931; Fig. 202) is *I. curvipes*, no doubt, given the morphology of the cystidia depicted in Heim's drawings. Stuntz (1947) separated *I. cicatricata* from *I. decipientoides* and his line drawings bear out their separation; however, Stuntz states that his material of *I. cicatricata* '... agrees quite well with Heim's description of *Inocybe cicatricata* Ellis & Ev.' *Inocybe cicatricata* sensu Stuntz (1947), however, in our opinion is not *I. curvipes*.

***Inocybe rufuloides*** Bon, *Docums. Mycol.* 14 (53): 28 (1984). *Type*: Cayeux-sur-Mer (Somme), France, 19 May 1983, Bon, Vast & Claus s.n. (*holo*: MB 83038).

*Pileus* 5–35 mm diam.; convex to hemispherical in button, then conico-campanulate, maintaining a broad central umbo and not or very reluctantly expanding to appanate in old age; margin plane at first with evanescent whitish appendiculate (suggestive of a partial veil); surface dry radially fibrillose with appressed pale silvery appressed fibrils some fusing into narrow appressed squamules, centre of pileus more matted-fibrillose, overlying the mousey brown (near 6E4) to dull bronze-brown (near 6E5) then later darker brown (near 7F8) pileal surface; context dull cream, paler in cap, up to 5 mm thick above stipe apex. Partial veil: sparse, rapidly evanescent whitish fibrils. *Lamellae* broadly adnexed, slightly ventricose, to 7 mm deep, crowded then closely spaced with abundant lamellulae in three tiers; edge minutely cystidiate (but not conspicuous to the eye), edge paler or concolorous with face, pale cream in button, then pale fawn (near 5B4), finally dull brown (near 5D6–6D6) or dull greyish brown (near 6D4) never dark or rusty brown. *Stipe* to 50 × 8 mm; cylindrical with base slightly swollen or clavate; solid; surface longitudinally silky-fibrillose with whitish appressed fibrils over entire length (easy to remove on handling), fibrils breaking up into minute floccules near apex, but no pruinosity present; dull whitish with slight pinkish tinge especially towards the apex in some specimens, darker and dull when old. *Basal mycelium* white but not copious or conspicuous. *Odour* grassy, spermatic. *Taste* not recorded. Spore deposit dark brown (near 6F5–6F6 to 7F6).

*Basidiospores* 11–12.5 (13) × 5.5–6.5 µm, mean 11.3 × 6.1 µm, mean profile 11.3 × 6.1 µm (n= 30), mean face view 11.4 × 6.1 µm (n= 30), mean L/B ratio profile 1.85, mean L/B ratio face view 1.87, smooth, ellipsoid to ovoid in face view, ovoid to subamygdaliform in profile sometimes with slight adaxial depression, with slightly thick wall, yellowish brown, apiculus small but prominent. *Basidia* 25–37 × 8.5–11.5 µm, quadrisporic, clavate to narrowly ventricose, hyaline. *Lamellae trama* of parallel clamped hyphae to 16 µm wide, subhyenium of narrower hyphae 2–4 µm wide. *Pleurocystidia* 53–75 × 14–17 µm, lageniform, subfusiform or utriform, crystalliferous; thick-walled, walls to 2 µm thick, hyaline. *Cheilocystidia* 20–56 × 11–15.5 µm, similar to pleurocystidia, crowded forming sterile lamella edge, thin-walled paracystidia sometimes in short chains. *Stipitipellis* with few caulocystidia similar to the hymenial cystidia at the stipe apex, end cells otherwise undifferentiated. *Pileipellis* a cutis of cylindrical hyphae to 10 µm wide, many with brown encrusting pigment, subpellis not well differentiated but with more tightly packed hyphae. *Clamp connections* present in all tissues. (Figures 3, 4)

*Specimens examined*. WESTERN AUSTRALIA: Modong Nature Reserve, Jandakot Regional Park, Thomas Rd, Oakford, Perth, in gregarious troops in disturbed gravelly roadside edge near *Pinus*

plantation with *Melaleuca preissiana* and *Adenanthos* sp. also present, 9 July 2006, *N. Bougher*; *N. Goldsbrough* & *J. Keeble* E8353 (PERTH07700598 (ITS and LSU sequences respectively: JN035292, JN035295); Bold Park, Perth, abundant in litter under *Pinus radiata* trees, adjacent to Ecology Centre, just South of Oceanic Drive, 10 July 2008, *N. Bougher* 00479 (PERTH 08073147); Bold Park, Perth, same location, 1 June 2010, *N. Bougher* 00618 (PERTH 08305978)(ITS sequence: JN035291); Bold Park, Perth, abundant along edge of limestone track in banksia woodland with *Pinus radiata* plantation nearby (30 m to nearest pine tree), Bold Park's central pine plantation, 29 July 2009, *N. Bougher* 00541 (PERTH 08305951).

*Distribution and habitat.* On soil, often in abundance, under planted *Pinus radiata*, introduced to Western Australia. Fruiting June to July.

*Notes.* *Inocybe rufuloides* can be recognized in Western Australia by its occurrence in pine plantations together with a combination of features including its dull bronze-brown, radially fibrillose pileus with some whitish overhanging fibrils when young, pale lamellae, pinkish upper stipe, and grassy/spermatocoid odour (Figure 3). It has smooth, ellipsoid to subamygdaliform spores, and metuloid cheilocystidia and pleurocystidia (Figure 4). *Inocybe rufuloides* is an ectomycorrhizal associate of *Pinus* species in Europe, e.g. *Pinus pinea* (Iotti *et al.* 2005). Collections of *I. rufuloides* from France listed by Bon (1984) in his original description indicate a possible association with *Pinus* and other plants such as *Picea*. In Western Australia *I. rufuloides* is an exotic species apparently exclusively associated with *Pinus*, and it is not yet known to associate there with other plants.

We identify the Western Australian collections cited above as *Inocybe rufuloides* in view of their conformity with the range of attributes presented in the literature for that species, and sequence similarity with material identified by J. Vauras as *I. rufuloides* (Ryberg 2009). Vesper and Richter (2010) present a comparison of their morphological data for *I. rufuloides* against data published by eight other authors. The Western Australian material conforms within the range of attributes presented, but the attributes presented show considerable variation. For example, caulocystidia are variably considered absent or present by the authors listed by Vesper and Richter (2010). The Western Australian collections have very few differentiated terminal cells on the stipe surface, with only some cauloparacystidia (broad shape, not or barely thick-walled) and rare metuloid caulocystidia (we observed only one metuloid in samples from 3 fruit bodies in each of 3 different collections). Further evidence pointing to morphological variability in *I. rufuloides* is that the Western Australian collections are much less reddish-brown overall than for specimens shown in the image of *I. aff. rufuloides* by Vesper and Richter (2010).

Iotti *et al.* (2005) reported that *I. rufuloides* is one of two *Inocybe* species found contaminating *Tuber* infected plants in experimental truffières in Italy. However, the identity of their material is called into question given that a BLASTn search of DQ067579 (sequence generated from a sporocarp) shows it to be highly similar (98% similarity) to *I. pseudodestructa* Stangl & J. Veselský, FN550908, based on material identified by J. Vauras. BLASTn searches of Western Australian *I. rufuloides* match a sequence produced by Ryberg (2009), using material from Italy identified by J. Vauras as *I. rufuloides*.

The gross morphology of *Inocybe pseudodestructa* as described by Stangl and Veselský (1973) is indeed similar to that of the Western Australian collections, including having a pale brownish stipe that is reddish along the upper third. However, the protologue for *I. pseudodestructa* by Stangl and Veselský (1973) shows at least two significant microscopical attributes that differ from the Western Australian collections: (1) more strongly amygdaliform spores lacking the adaxial flattening or depression present on the spores of Western Australian collections; (2) presence of long, narrow caulocystidia.



Figure 3. Basidiomes of *Inocybe rufuloides* (PERTH 08073147).

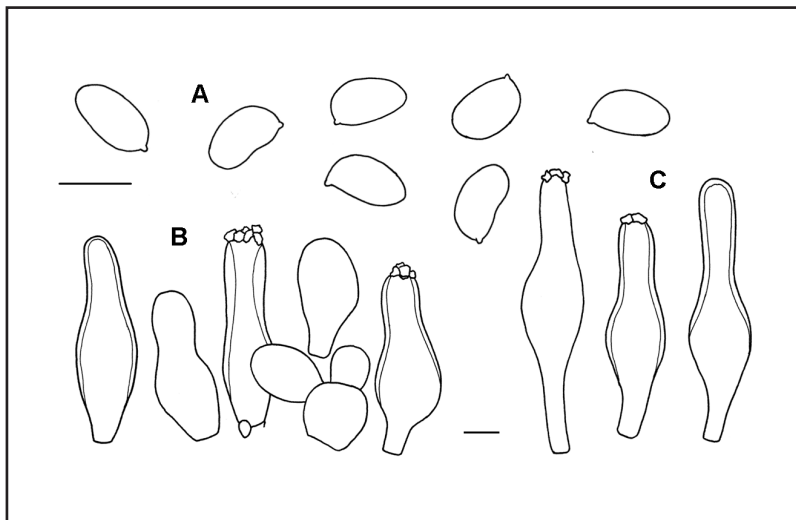


Figure 4. Micromorphology of *Inocybe rufuloides* (PERTH 08073147). A – spores; B – cheilocystidia; C – pleurocystidia. Scale bars = 10  $\mu$ m (longer bar for spores only).



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