ECHINOSPHAERIA MEDUSA, A NEW SPECIES FROM NEW ZEALAND, WITH NOTES ON RELATED SPECIES.

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Abstract

This paper describes a new species of the ascomycete genus *Echinosphaeria*, which was found in New Zealand on dead wet wood. It is compared with existing species of the genus and with closely related species of the genus *Lasiosphaeria*.

Key words: Helminthosphaeriaceae, Lasiosphaeriaceae, systematics.

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Introduction

An undescribed species of *Echinosphaeria* was collected on unidentified dead wood in the Woodside Glen reserve during the course of the 22nd New Zealand Fungal foray held at Dunedin, Otago, New Zealand.

Materials and Methods

Morphological and microscopic details were studied in water mounts after which a number of semi-permanent slides were made using Shear's mounting fluid (Bell 2005). Due to the morphological similarities with *Echinosphaeria canescens*, a species with which we are familiar, both line drawings and photographs were undertaken of this species too in order that the reader might more readily discriminate the differences between them. The ascospore size ranges for each species were determined by measuring at least 50 ascospores. Colour references below follow the notation of Kornerup & Wanscher (1989).

Echinosphaeria medusa A. Bell & D.P. Mahoney *sp. nov*. (Figures 1A–F, 2A–C, 3A–B).

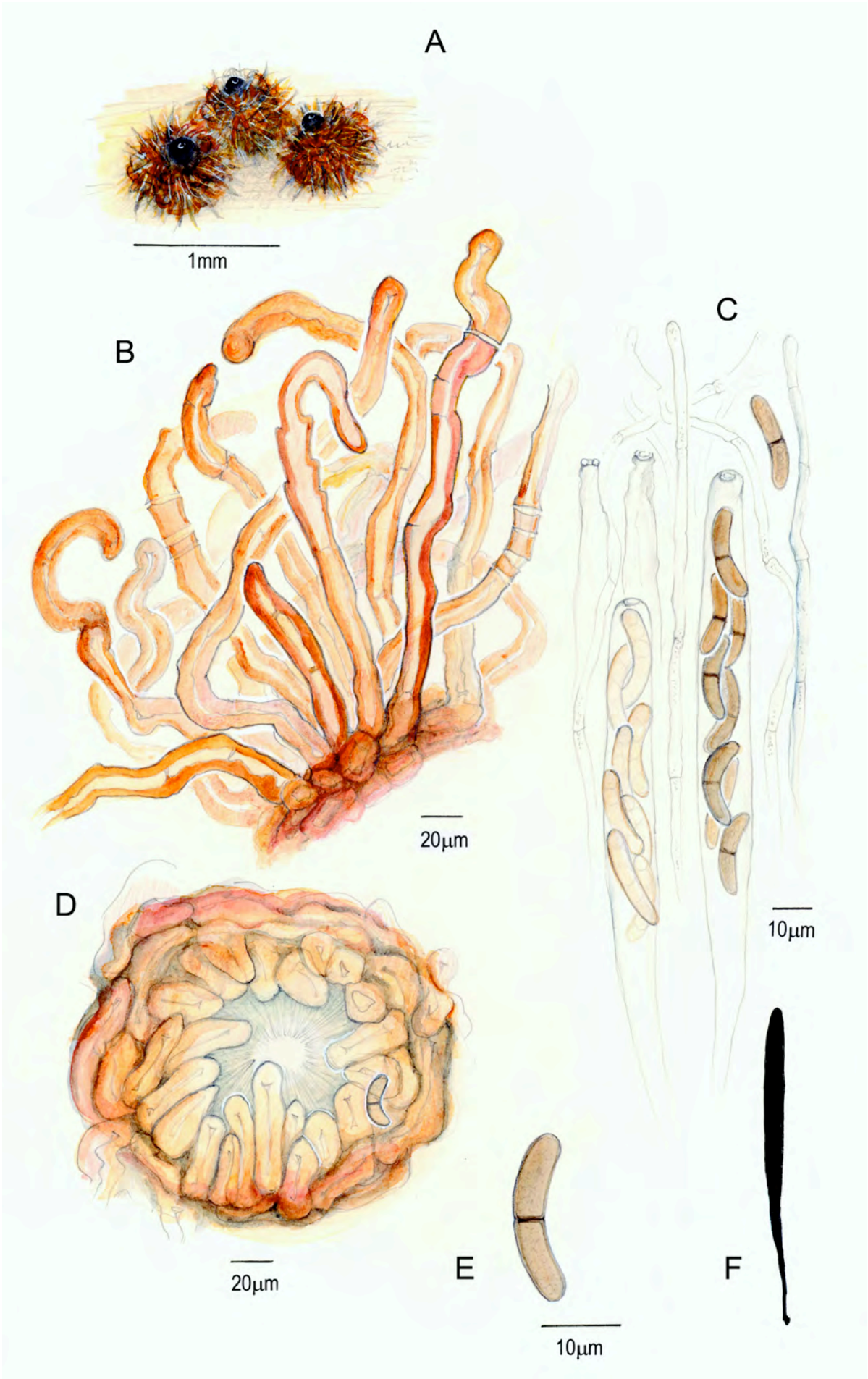
Etymology: *medusa* = resembling the entangled snake hair of the mythical Greek goddess Medusa.

Ascomata aggregata, superficialia. Ventre globosus, 0.5–0.75 mm diam, capillatura. Pili copiosus, rigide crispatus, brunneo-aurantiacus vel brunneo-bubalinus, crassitunicatus, ad 550 µm longi, 20 µm latae. Paraphyses hyalinae, septatae, circa 3 µm latae, longitudine indeterminata. Asci qua cylindracei qua clavati, circa 165 x 12 µm, longulus stipitati, octospori. Ascosporae biseriatae, transeuns uniseptatae, brunneolus, (19–) 20–24 (–27) x 4–5 µm. Fungus lignicolous.

Holotypus: On dead decorticated wet wood, Woodside Glen, near Outram, Otago, New Zealand, collected 15/5/08 by D.P. Mahoney, PDD 94222 (= Bell & Mahoney 1043).

Ascomata superficial and densely crowded on decorticated very wet wood, approx. 0.5–0.75 mm diam, enveloped in a radiating entanglement of thick-walled, brownish-orange to brownish-buff (7C7/D7) stiff hairs of variable thickness, but many as wide as 20 µm (Figs 1A, 2A). Hairs difficult to measure accurately due to their curly nature, but can reach at least 550 µm. Central canal in the hairs may be trabeculate or completely occluded. Some hairs also exhibit annular cracking (Figs 1B, 2C). The underlying perithecial wall is composed of thick-walled

Figure 1 (page 142). *Echinosphaeria medusa*. (A) Fresh ascomata as they appear on dead wood. (B) Detail of ascomal appendages. (C) Asci, ascospores & paraphyses. (D) Ostiolar region. (E) Mature ascospore. (F) Silhouette of ascus.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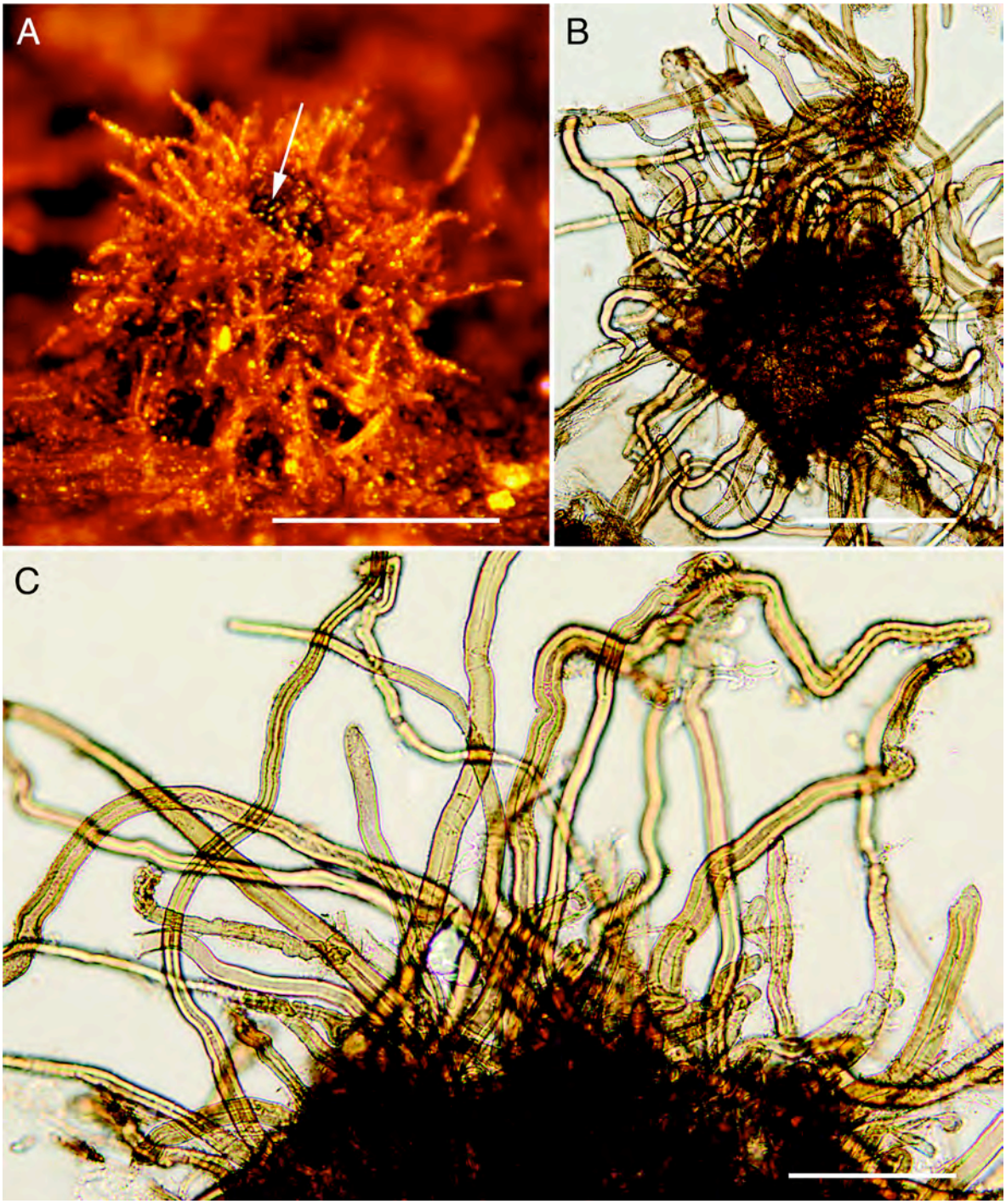


Figure 2. *Echinosphaeria medusa*. (A) In situ view of a fresh ascoma on dead wood. Arrow on ostiole. Bar = 400 µm. (B–C) Peridial fragments with medusoid appendages mounted in Shear's mounting fluid. Bars = 140 and 80 µm, respectively.

cells (Figs 1B, 2B). Due to the density of the vestiture, it is unclear if each of the perithecial cells produces a hair. Young ostioles are surrounded by shorter thick-walled hairs and the periphyses can be seen beneath (Fig. 1D). In mature perithecia the neck appears as a small black dome emerging from the hairs (Figs 1A, 2A). *Paraphyses* hyaline, septate, unbranched, approx. 3 µm diam, free-ended, longer than the asci. *Asci* cylindrical to slightly clavate with elongated stalk, difficult to separate from the centrum material, approx. 165 µm long, 12 µm at their widest point with a distinct non-amyloid apical ring, but no subapical globulus (Figs 1C, 3A). *Ascospores* biseriately arranged within ascus, allantoid, initially hyaline but becoming brown with maturity with a single thickened central septum, (19–) 20–24 (–27) x 4–5 µm (Figs 1C, 1E, 3A–B).

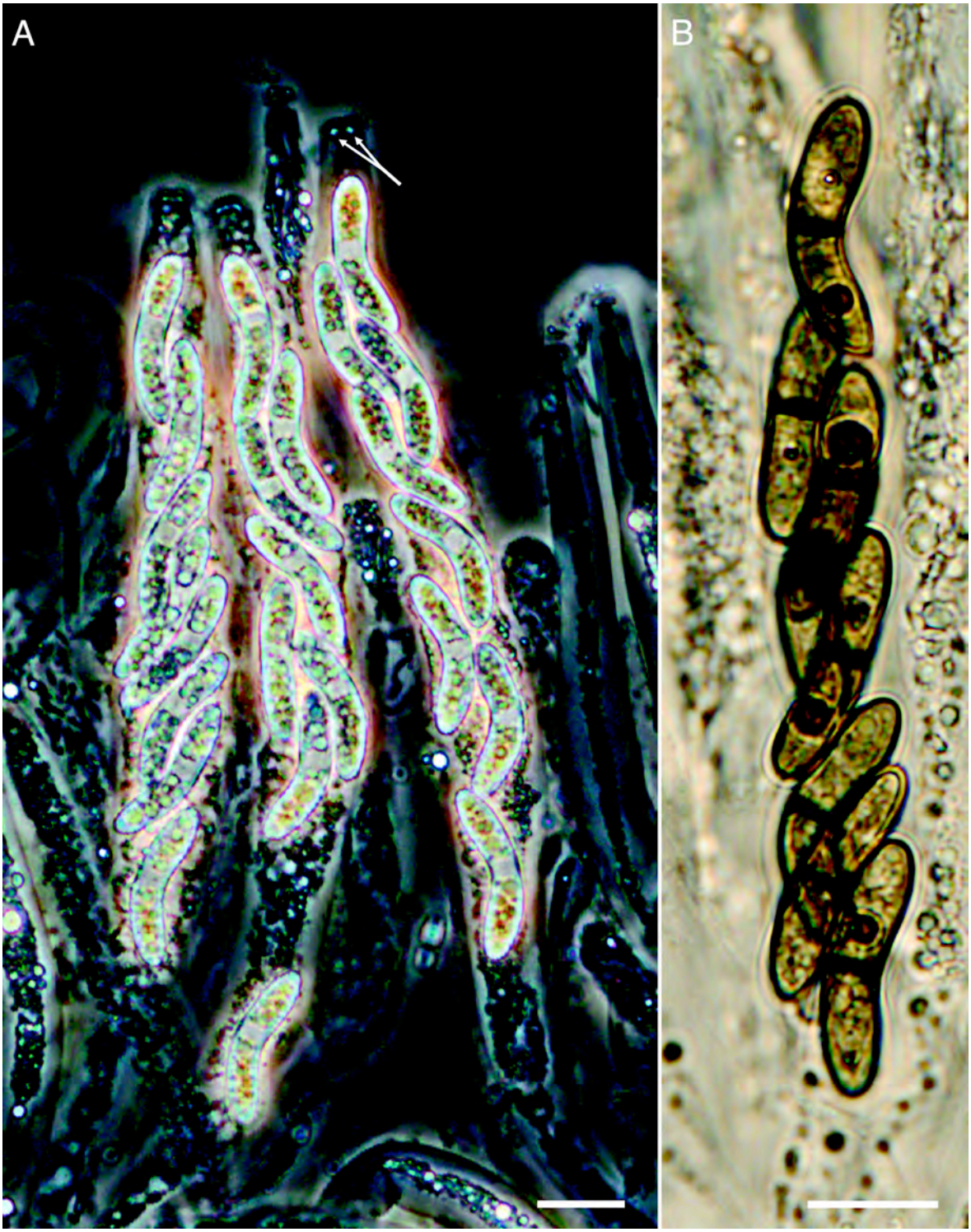
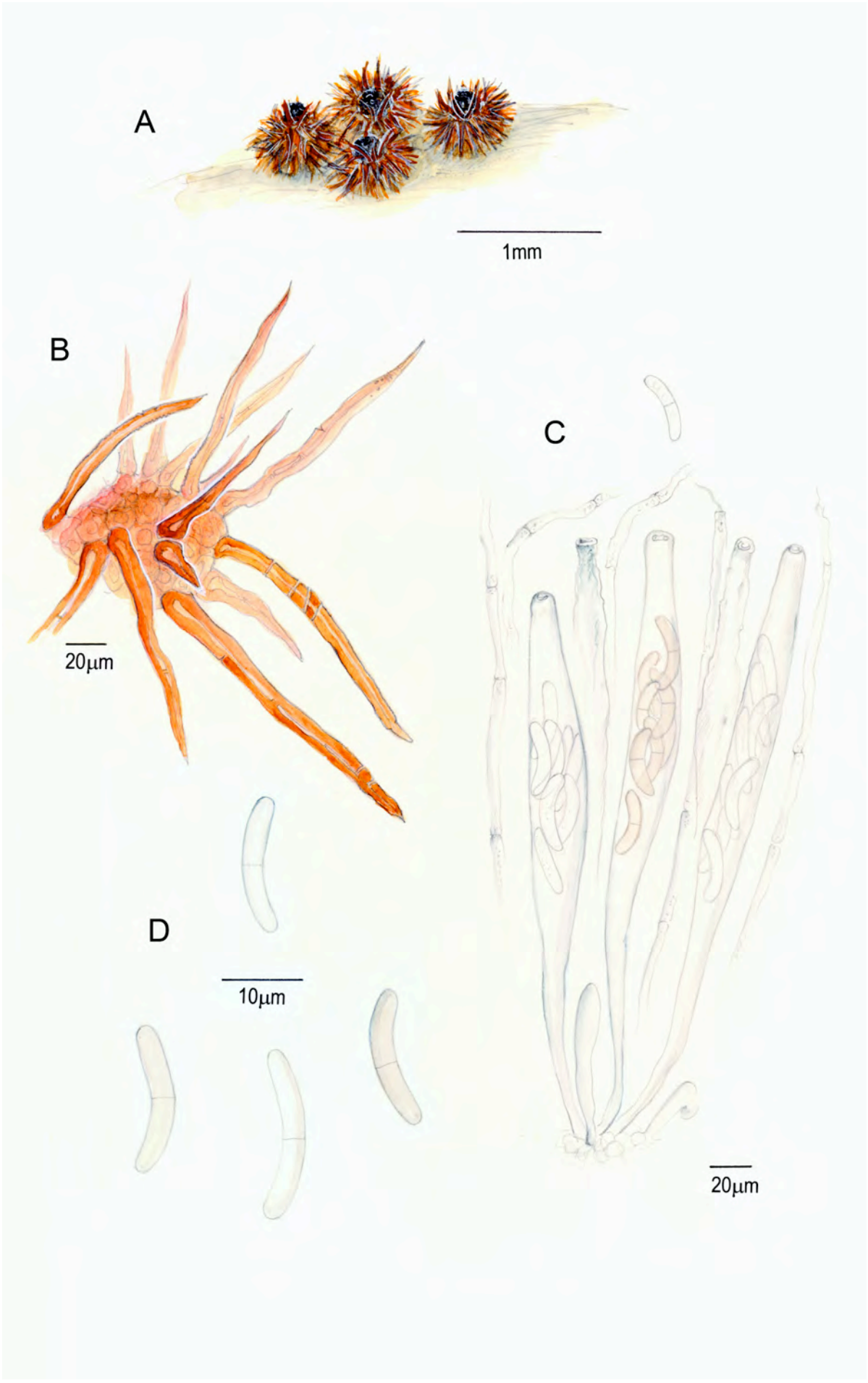


Figure 3. *Echinosphaeria medusa*. (A) Asci with apical rings and 8 nonseptate hyaline ascospores. Arrows on apical ring in optical section. Water mount. (B) Eight brown 2-celled ascospores. Shear's mounting fluid preparation. (A–B) Bars = 12 µm.

Echinosphaeria canescens (Pers.) A.N. Mill. & Huhndorf (Figs 4A–D, 5A–D).

Ascomata superficial and densely crowded on decorticated very wet wood, approx. 0.5–0.75 mm diam, enveloped in radiating thick-walled brownish-orange to brownish-buff (7C7/D7), stiff, straight to slightly curved spines averaging 8–12 µm wide at base and up to 350 µm long (Figs 4A, 5A). Central canal in the hairs may be trabeculate or completely occluded. A number of hairs also exhibit annular cracking (Fig. 4B). The underlying perithecial wall is composed of thick-walled cells (Fig. 4B). A black slightly roughened glabrous domed neck is particularly visible in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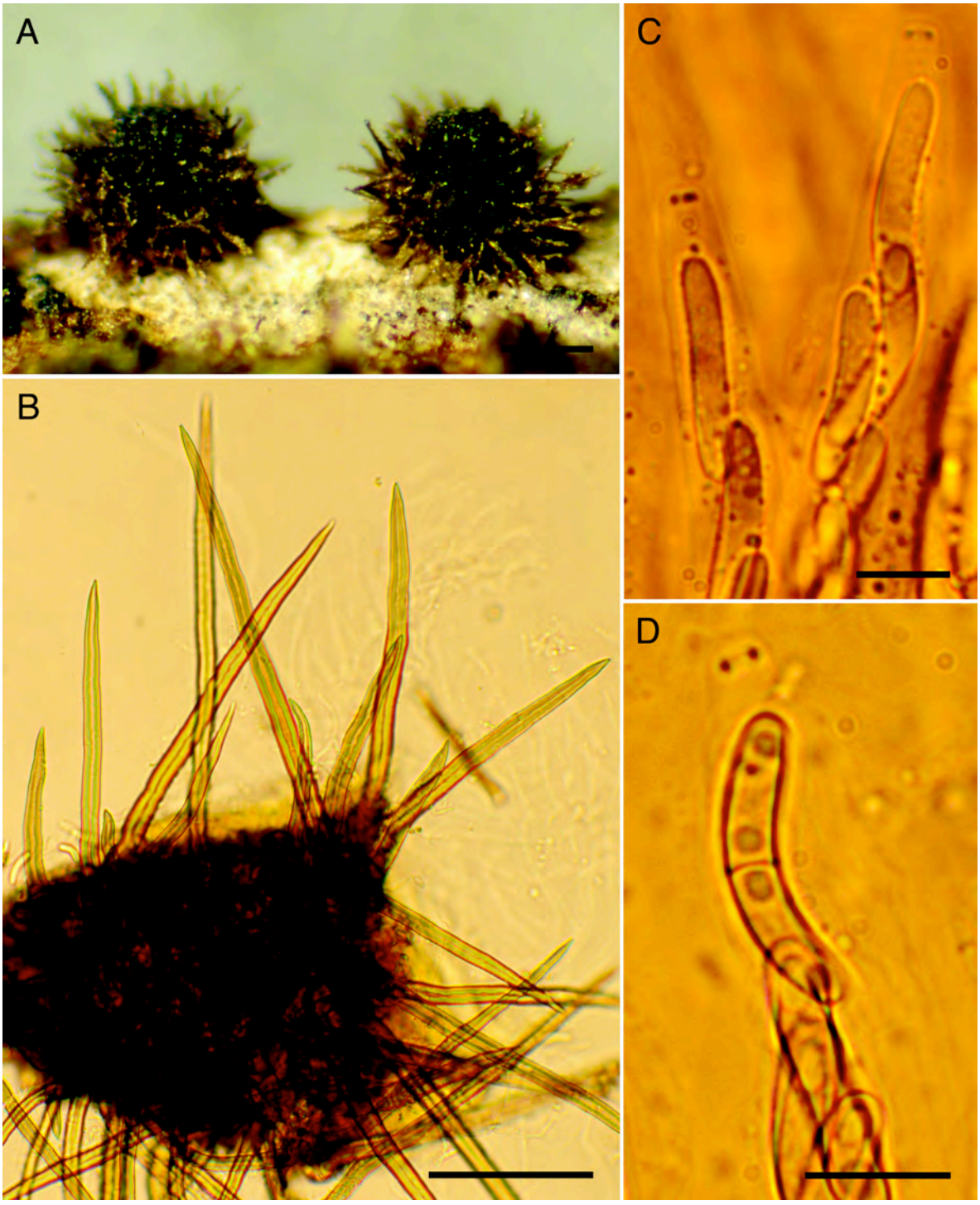

Figure 5. *Echinosphaeria canescens*. (A) In situ view of fresh ascomata on dead wood. Bar = 250 µm. (B) Peridial fragment with spines. Bar = 80 µm. (C) Asci with apical rings and nonseptate ascospores. (D) Ascus with apical ring and 2-celled ascospore. (C–D) Bars = 10 µm. (B–D) water mounts irrigated with Melzer's reagent.

dried specimens (Fig. 4A). Ostioles of young perithecia are surrounded by short thick-walled hairs very similar to those of *E. medusa* in Fig. 1D. *Paraphyses* hyaline, septate, unbranched, approx. 3 µm diam, free-ended, longer than the asci. *Asci* cylindric to clavate, crowded and not detaching therefore difficult to measure lengths accurately, width 8–10 µm at the widest point with distinct non-amyloid apical ring, subapical globulus absent (Figs 4C, 5C–D). *Ascospores* biseriately arranged within

Figure 4 (page 145)**.** *Echinosphaeria canescens*. (A) Fresh ascomata as they appear on dead wood. (B) Detail of ascomal appendages. (C) Asci, ascospores & paraphyses. (D) Mature ascospores.

ascus, allantoid, initially hyaline becoming a very dilute brown with maturity with a single thin central septum, 17–24 x 3–4 µm (Figs 4C–D, 5C–D).

Specimen examined: On dead decorticated wood, Snowbank Lake, near Ely, Lake County, Minnesota, USA, collected 15/9/04 by D.P. Mahoney & A. E. Bell, PDD 82112 (= Bell & Mahoney 890). A duplicate of this material is also held at the Bell Museum, University of Minnesota (= MIN 882059).

Discussion

Viewed under low magnification (X10), *Echinosphaeria medusa* and *E. canescens* are more or less indistinguishable when both species are turgid and water soaked. The domed neck region of *E. canescens* is more obvious especially in dried material. The differences are only manifest upon greater magnification and in subsequent slide mounts. The perithecial hairs are much larger and form an unruly entanglement in *E. medusa* and the individual hairs are much more variable in both length and width as compared with the stiffer, pointed, more slender hairs of *E. canescens* (compare Figs 1B & 2C with Figs 4B & 5B). The ascospores of *E. medusa* are slightly larger and a darker brown and the central septum is much more prominent, appearing as twice the thickness of the lateral walls (compare Figs 1C, 1E & 3B with Figs 4D & 5D).

Miller & Huhndorf (2004) erected the genus *Echinosphaeria* (Pers.) A.N. Mill. & Huhndorf to accommodate a species formerly known as *Lasiosphaeria canescens* (Pers.) Karst. based upon their sequencing data which placed it in a different clade to their new and restricted definition of *Lasiosphaeria*. Thus, *Echinosphaeria* was further defined as possessing perithecia clothed in brown setae having a black glabrous papillate neck, unitunicate cylindrical asci bearing an apical ring (but no subapical globulus) and allantoid ascospores without appendages that are initially hyaline but become pale brown with age. Furthermore *Echinosphaeria canescens* was removed from the *Lasiosphaeriaceae* and placed in the *Helminthosphaeriaceae*. Miller & Huhndorf corroborate that *Echinosphaeria* exhibits ascospores that conform to the Group A morphology as defined by Candoussau *et al*. (2001). Group A ascospores are shared by a number of other species including *Lasiosphaeria strigosa* (Alb. & Schw.) Sacc. and *L. stuppea* Ellis & Everh., both of which have perithecia which superficially appear identical to the perithecia of *E. canescens* and *E. medusa*. It is only by close examination of the asci and ascospores that differences are readily seen. *L. stuppea* has much broader uniseptate ascospores (23–34 x 6.8–11 µ m). Although we have not seen *L. stuppea*, there are excellent photographs of both perithecia and ascospores of this species available on the Internet site www.ascofrance.fr. by Alain Gardiennet. We have examined material of *L. strigosa* (PDD 14975, originally collected in England) and confirm that in this collection at least, the ascospore measurements fall within the described range for that species (34–40 x 6–7 µm). However, Candoussau *et al.* (2001) stated that although they temporarily keep the usual distinction between *L. strigosa* and *L. canescens* "*intermediate forms may be encountered*". Much earlier, Berlese (1893) illustrated *L. strigosa* and *L. strigosa* var. *canescens*, indicating that he considered them to belong to the same species. Seaver (1912) also considered that *L. canescens* Karst. may be synonymous with *L. strigosa* (Alb. & Schw.) Sacc. However, these two specific names remain in the literature until more conclusive evidence should suggest otherwise.

In summary, it is impossible to know which of these four species one has collected in the field simply armed with a hand lens. As yet we do not know if any of them are restricted geographically. Since they reside on very decayed wet wood and wood has been transported to all parts of the world over some considerable time, it seems unlikely that this question will ever be satisfactorily answered. To date *E. canescens, L. stuppea* & *L. strigosa* have been found in Europe and the USA on a variety of woody substrates, but not recorded in New Zealand and *E. medusa* has been found only in New Zealand. In our view it is far too early to pronounce *E. medusa* as endemic, since there are so few mycologists studying these fungi, and vast areas of the world remain mycologically uninvestigated. Even when one is specifically looking, a certain amount of serendipity is involved in finding them because of their small size.

Anamorphs associated with *Echinosphaeria canescens* include *Endophragmiella* and *Selenosporella* (Hughes 1979, Samuels *et al.* 1987), but there is a lack of information on cultural characteristics and anamorphs in general within the *Lasiosphaeriaceae sensu lat.* Candoussau *et al.* (2001) were of the opinion

that: "*scanty information about lasiosphaeriaceous anamorphs does not yet allow further subgeneric splitting of the genus Lasiosphaeria"*. Subsequent sequencing work by Miller & Huhndorf (2004) indicated that based principally on evidence provided by analyses of partial nuclear large subunit (LSU) rDNA sequences, the genus should be split into a number of new genera including *Echinosphaeria*, which was additionally placed in a new family *Helminthosphaeriaceae*. At present many unanswered questions surround these fungi including whether or not *L. strigosa* and *E. canescens* are separate species and whether or not future sequencing of the additional taxa *L. strigosa*, *L. stuppea* and *E. medusa* will add further support to the existence of *Echinosphaeria* as a genus. However, what is clear is that the two fungi illustrated herein with the specific epithets *canescens* and *medusa* are clearly distinguishable on morphological grounds, although the genus to which they are assigned may change with time.

An additional species *Echinosphaeria macrospora* Puja, Bhat & K.D. Hyde was described by Puja *et al*. (2006). It developed in culture during the isolation of endophytes from living stems and leaves of *Centella asiatica*. Its anamorph is reported to be the new setose sporodochial species *Vermiculariopsiella endophytica* Puja, Bhat & K.D. Hyde. Although its asci and ascospores resemble those of other *Echinosphaeria* species, its perithecia lack appendages and its anamorph is distinctly different than those attributed to *E. canescens*. Thus this species remains distinct from other known species of *Echinosphaeria* and their *Lasiosphaeria* relatives described herein.

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References

Bell, A. (2005). *An illustrated guide to the coprophilous Ascomycetes of Australia*. CBS Biodiversity Series No. **3**, 1–172. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Berlese, A.N. (1893). *Icones Fungorum omnium hucusque cognitorum ad usum Sylloges Saccardianae adcommodatae* **1**, plate 107. Avellino; E. Pergola.

Candoussau, F., Fournier, J. & Magni, J.-F. (2001). New and rare species of *Lasiosphaeria* in southwestern France. *Mycotaxon* **80**, 201–240.

Hughes, S.J. (1979). Relocation of species of *Endophragmia* auct. with notes on relevant generic names. *New Zealand Journal of Botany* **17**,139–188.

Kornerup, A. & Wanscher, J.H. (1989). *Methuen Handbook of Colour,* 3rd edition. Metheun, London.

Miller, A.N. & Huhndorf, S.M. (2004). A natural classification of *Lasiosphaeria* based on nuclear LSU rDNA sequences. *Mycological Research* **108**, 26–34.

Puja, G., Shenoy, B.D., Hyde, K.D. & Bhat, D.J. (2006). *Echinosphaeria macrospora* sp. nov., teleomorph of *Vermiculariopsiella endophytica* sp. nov. *Cryptogamie Mycologie* **27**, 11–20.

Samuels, G.J., Muller, E. & Petrini, O. (1987). Studies in the *Amphisphaeriaceae* (sensu lato) 3. New *Monographella* and *Pestalosphaeria*, and two new genera. *Mycotaxon* **28**, 473–499.

Seaver, F. (1912). The genus *Lasiosphaeria*. *Mycologia* **4**, 115–124.