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Two new species of *Agaricus* from the Subantarctic

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Abstract—Molecular and morphological data are presented for three *Agaricus* species on Campbell Island, New Zealand. Two of these are new species, *A. campbellensis* and *A. subantarcticus*. The third may be conspecific with *A. subrutilescens*, although the molecular data suggest a lack of gene flow between subantarctic and North American populations. Alternatively, it could be a closely related, but undescribed species. This is the first report of the *Subrutilescens* group from the Southern Hemisphere.

Key words— *Basidiomycota,* fungi, ITS, LSU, phylogenetics

Introduction

Agaricus L. is a diverse, cosmopolitan genus with an estimated 300 species worldwide (Cappelli 1984, Bas 1991). Recently, efforts have been made to reconstruct the evolutionary history of the genus from molecular data (Bunyard et al. 1996, Mitchell & Bresinsky 1999, Robison et al. 2001, Challen et al. 2003, Geml 2004, Geml et al. 2004) and to detect and describe new species using molecular and morphological data (Callac & Guinberteau 2005, Kerrigan 2005). Studies to date mostly have included European and North American taxa while those in the subantarctic regions remain largely unknown. Mitchell & Walter (1999) provided a taxonomic key to all known *Agaricus* species in New Zealand. Here we provide molecular phylogenetic analyses to reveal the evolutionary history of three *Agaricus* species from Campbell Island in the Subantarctic and use molecular and morphological data to describe two of these as new species.

Campbell Island (S 52° 33', E 169° 10') is located c. 300 km from the nearest land (Auckland Islands) and c. 650 km south of the main islands of New Zealand. Basement rocks of the island are composed of schist (dating from

640 Ma), Cretaceous sandstone, Tertiary limestone, and more recent volcanic flows. Deep peat is found in flat and gently sloping areas, while mineral content of the soil is more pronounced on steeper slopes (Campbell 1981, Foggo & Meurk 1983). Vegetation is predominantly tussock grassland, shrubland and herbfield divided into upper alpine, lower alpine, and subalpine zones (Meurk 1977). *Agaricus* collections were made in the shrubland to upper alpine zones characterized by dwarf forests of *Dracophyllum scoparium* and *D. longifolium*, with *Coprosma* and *Myrsine* species (Figs. 1, 2).

Methods and materials

Thirteen specimens were collected from the subantarctic Campbell Island (Table 1). Voucher specimens are deposited in the University of Alaska Fairbanks Herbarium (ALA) and in the New Zealand Fungal Herbarium (PDD). DNA was extracted from small samples of dried specimens using the E-Z 96® Fungal DNA Kit (Omega Bio-tek, Inc., Doraville, GA). ITS and LSU sequences of an additional thirty *Agaricus* species, representing the known diversity of the genus, were downloaded from GenBank (Table 1.). Homologous sequences of *Chlorophyllum molybdites* (U85309, U85303) were used to root all trees.

Table 1. Specimen code and GenBank accession numbers of *Agaricus* spp. included in this study.

Species	Collection number	GenBank accession number		Origin, reference
		ITS	LSU	
A. campbellensis	GAL9379	DQ232638	DQ232651	Campbell Island, New Zealand
	GAL9420	DQ232644	DQ232657	Campbell Island, New Zealand
	GAL9573	DQ232640	DQ232653	Campbell Island, New Zealand
	GAL9603	DQ232639	DQ232652	Campbell Island, New Zealand
	GAL9633	DQ232641	DQ232654	Campbell Island, New Zealand
	GAL9605	DQ232645	DQ232658	Campbell Island, New Zealand
	GAL9649	DQ232646	DQ232659	Campbell Island, New Zealand
A. subantarcticus	GAL9604	DQ232637	DQ232650	Campbell Island, New Zealand
	GAL9419	DQ232642	DQ232655	Campbell Island, New Zealand
	GAL9425	DQ232647	DQ232660	Campbell Island, New Zealand
	GAL9418	DQ232648	DQ232661	Campbell Island, New Zealand
	GAL9572	DQ232643	DQ232656	Campbell Island, New Zealand
A. cf. *subrutilescens*	GAL9422	DQ232649	DQ232662	Campbell Island, New Zealand
A. abruptibulbus Peck	-	AY484673	AY484673	Geml et al. (2004)
A. albolutescens Zeller	-	AY484675	AY484675	Geml et al. (2004)
A. arvensis Schaeff.	-	AY484691	AY484691	Geml et al. (2004)
A. augustus Fr.	-	AY484672	AY484672	Geml et al. (2004)

Figs. 1-2. Characteristic vegetation of the subalpine zone of Campbell Island: *Dracophyllum scoparium* and *D. longifolium* (shrub), *Poa litorosa* (tussock grass), *Polystichum vestitum* and *Histiopteris incisa* (ferns).

Table 1, concluded

Species	Collection number	GenBank accession number		Origin, reference
		ITS	LSU	
A. bernardii (Quél.) Sacc.	-	AY484678	AY484678	Geml et al. (2004)
A. bisporus (J.E. Lange) Imbach	-	AY484692	AY484692	Geml et al. (2004)
A. bitorquis (Quél.) Sacc.	-	AY484695	AY484695	Geml et al. (2004)
A. blazei Murrill	-	AY484697	AY484697	Geml et al. (2004)
A. californicus Peck	-	AY484679	AY484679	Geml et al. (2004)
A. campestris L.	-	U85307	U85273	Johnson & Vilgalys (1999)
A. cupreobrunneus (Jul. Schaeff. & Steer) Pilát	-	AY484680	AY484680	Geml et al. (2004)
A. devoniensis P.D. Orton	-	AJ418755	AF059225	Challen et al. (2003), Mitchell & Bresinsky (1999)
A. diminutivus Peck	-	AY484681	AY484681	Geml et al. (2004)
A. excellens (F.H. Møller) F.H. Møller	-	AY484682	AY484682	Geml et al. (2004)
A. fissuratus (F.H. Møller) F.H. Møller	-	AY484683	AY484683	Geml et al. (2004)
A. fuscofibrillosus (F.H. Møller) Pilát	-	AY484684	AY484684	Geml et al. (2004)
A. fuscovelatus Kerrigan	-	AY484677	AY484677	Geml et al. (2004)
A. hondensis Murrill	-	AY484685	AY484685	Geml et al. (2004)
A. inapertus Vellinga	-	AF482834	AF482878	Vellinga et al. (2003)
A. langei (F.H. Møller) F.H. Møller	-	AY484699	AY484699	Geml et al. (2004)
A. lilaceps Zeller	-	AY484676	AY484676	Geml et al. (2004)
A. macrocarpus (F.H. Møller) F.H. Møller	-	AY484686	AY484686	Geml et al. (2004)
A. macrosporus (F.H. Møller & Jul. Schäff.) Pilát	-	AY484687	AY484687	Geml et al. (2004)
A. nivescens (F.H. Møller) F.H. Møller	-	AY484670	AY484670	Geml et al. (2004)
A. pocillator Murrill	-	AF041542	U85308	Hopple & Vilgalys (1999, Johnson & Vilgalys (1999)
A. semotus Fr.	-	AJ133390	AF059224	Challen et al. (2003), Mitchell & Bresinsky (1999)
A. subfloccosus (J.E. Lange) Hlaváček	-	AY484698	AY484698	Geml et al. (2004)
A. subperonatus (J.E. Lange) Singer	-	AF432889	AF059216	Challen et al. (2003), Mitchell & Bresinsky (1999)
A. subrutilescens (Kauffman) Hotson & D.E. Stuntz	-	AY484688	AY484688	Geml et al. (2004)
A. xanthoderma Genev.	-	AY484689	AY484689	Geml et al. (2004)

Table 2. Selected characteristics of subantarctic *Agaricus* spp. included in this study.

Species	UAF/ALA Collection	Spore dimensions (µm)		Spore shape	Schaeffer reaction	KOH reaction	Color change	Pileus	Stipe length × diameter (cm)	
	number	length	width	Q-value			context	(cm)		
A. campbellensis	GAL9379	8.74 ±0.79	4.26 ±0.25	1.93 ±0.14	+	yellow	yellow, tan	3.5-4.0	4.0-5.5 × 0.8	almond
	GAL9420	7.06 ±0.49	4.29 ±0.34	1.69 ±0.22	+	yellow	yellow	3.0-5.0	4.0-6.0 × 0.8-1.1	almond
	GAL9573	7.04 ±0.66	4.48 ±0.41	1.56 ±0.17	+	yellow	N/A	N/A	N/A	N/A
A. subantarcticus	GAL9604	8.16 ±0.64	4.86 ±0.45	1.75 ±0.22	+	yellow	N/A	N/A	N/A	N/A
	GAL9419	7.78 ±1.31	4.83 ±0.28	1.68 ±0.15	+	yellow	yellow	4.0-13.0	4.0-9.0 × 0.8-3.5	almond
	GAL9425	7.72 ±1.25	4.80 ±0.41	1.53 ±0.32	+	yellow	yellow	7.0-8.0	4.0 × 1.5-2.0	almond
A. cf. *subrutilescens*	GAL9422	4.98 ±0.69	3.21 ±0.25	1.51 ±0.19	-	olive green	none	6.0	6.0 × 0.9-1.5	sweet, nutty

Symbols: + = present or positive, - = absent or negative, N/A = no data.
Spore dimensions and shape are given as mean ± standard deviation, based on twenty-five measurements per specimen.

The entire ITS and partial LSU regions were PCR amplified in reaction mixtures containing 1.75µl Ultrapure Water (Invitrogen), 1µl 10x Herculase PCR buffer (Stratagene), 0.05µl 100mM dNTP mixture, 25mM of each dNTP (Applied Biosystems), 0.2µl Herculase DNA polymerase (Stratagene), 2 µl of 1µM forward primer, ITS1F (Gardes & Bruns 1993) and reverse primer, TW13 (White et al. 1990), and 3µl of template DNA at a concentration of 0.1ng/ µl. PCR reactions were performed using the following temperature program: 95 °C/2 min, 34 cycles of 95 °C/0.5 min, 54 °C/1 min, 72 °C/2 min; and 72 °C/10 min. The concentration of the amplification products was determined using Picogreen (Molecular Probes). The amplification products were normalized to a concentration of 4ng/µl and sequenced using the Applied Biosystems (ABI) BigDye v. 3.1 terminator kit and an ABI 3730xl automated capillary DNA sequencer (Applied Biosystems, Foster City, CA). We used two internal primers for cycle sequencing, ITS4 and CTB6 (White et al. 1990), in addition to the primers used in the PCR reactions.

Sequence data obtained for both strands of each locus were edited and assembled for each isolate using Aligner v. 1.3.4 (CodonCode Corporation, Dedham, MA). Sequence alignments were initiated using Clustal W (Higgins et al. 1991) and subsequently corrected manually. Ambiguously aligned positions were recoded using INAASE 2.3b (Lutzoni et al. 2000) to retain the phylogenetic information present in the region without violating positional homology. The code matrix was attached to the alignment and was included in MP analyses. Phylogenetic analyses were conducted using the maximum-parsimony (MP) method in PAUP* 4b10 (Swofford 2002). MP analyses were carried out with the heuristic search option using the "tree bisection and reconnection" (TBR) algorithm with 100 random sequence additions to find the global optimum without limiting the maximum number of trees. Gaps were scored as "new state". The stability of clades was tested using the bootstrap test (Felsenstein 1985) with "full heuristic search" and 500 replications.

Fresh collections were described, photographed, and dried for later microscopic study. Specimens from three collections of each species were chosen for study. The ISCC-NBS Dictionary of Color Names was used to describe all combinations of basidiome color. Twenty-five basidiospores were measured from each specimen. Basidiospores, cheilocystidia and basidia were measured in 5% KOH using a 100× objective. Numerical data were analyzed by one-way analysis of variance (ANOVA) using JMP 3.2.6 (SAS Institute Inc.). Where the null-hypothesis was rejected, that all means were the same, Student's t-test (Ott 1993) was used to detect significant differences by testing each pair of means. Two chemical tests were performed on the pileus: the Schaeffer-reaction (using aniline and nitric acid) and the KOH-reaction. The Schaeffer-reaction was considered positive when the intersection of the two reagents became bright

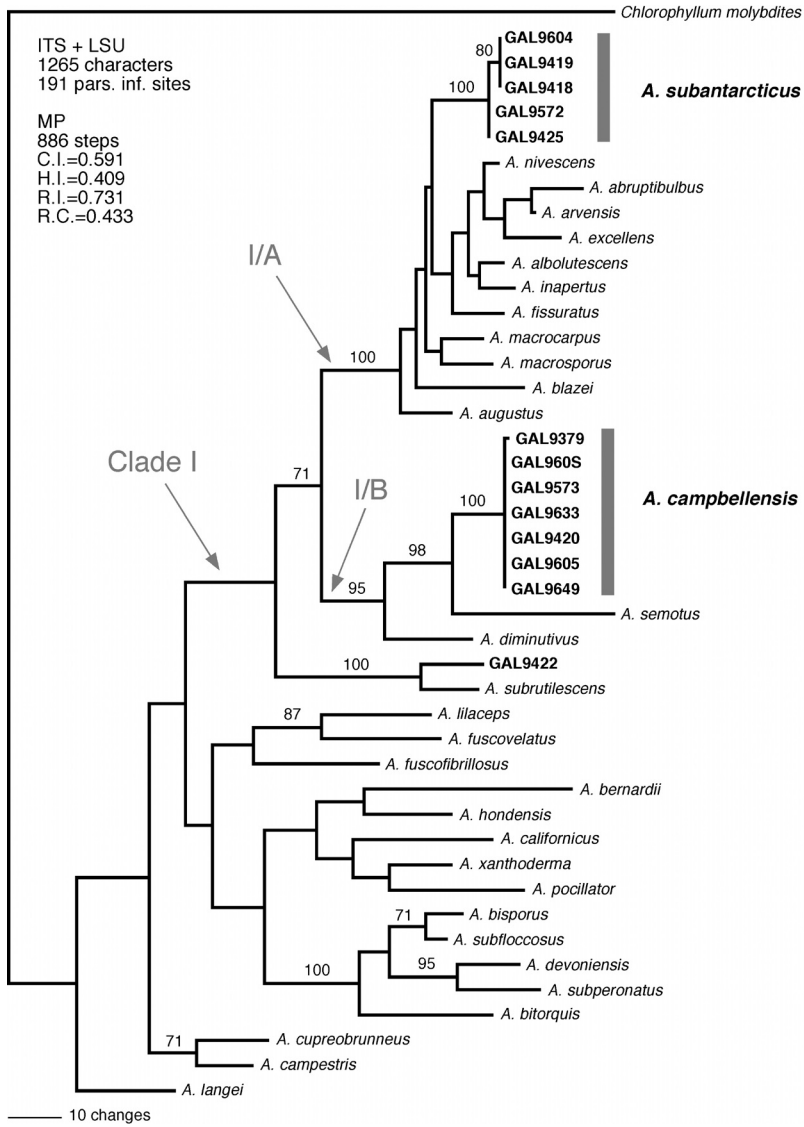

Fig. 3. Phylogram of *Agaricus* species based on maximum parsimony analyses of the combined (ITS, LSU) dataset. Bootstrap values are shown above the branches. Subantarctic isolates are in bold.

orange or red, while the KOH reaction was positive when yellow or green color was observed in the area of contact on the pileus surface.

Results

The combined ITS+LSU datasets of the 33 taxa consisted of 1414 characters, including gaps. Of these, 155 characters were excluded, containing ambiguous positions (235-239, 326-330, 558-568, 585-601, 719-731, 735-739) and incomplete data on both ends (1-44, 1360-1414). After including the character matrices of the ambiguous regions recoded by INAASE, the final alignment consisted of 1265 characters. The number of parsimony-informative sites was 191. One of the 3 most parsimonious trees is shown with bootstrap values in Fig. 3. The subantartic specimens grouped in three species clades, all within Clade I (Geml et al. 2004) that corresponds to section *Arvenses* (Heinemann 1977). One specimen (GAL9422) formed a well-supported (100%) monophyletic clade with an *A. subrutilescens* specimen from North America. Seven specimens (GAL9379, GAL9420, GAL9573, GAL9603, GAL9605, GAL9633, GAL9649) had virtually identical ITS and LSU sequences and formed a new species clade, *A. campbellensis* with 100% bootstrap support. This species clade formed a sister clade to *A. semotus* in clade I/B (Geml et al. 2004) corresponding to subsection *Minores* (Heinemann 1977). The third species clade, *A. subantarcticus*, included five collections (GAL9418, GAL9419, GAL9425, GAL9572, GAL9604) and also received 100% support. Although confidently placed in Clade I/A with species of subsection *Arvenses* (Heinemann 1977), the closest relatives of this species could not be specified with statistical support.

Selected morphological characteristics of the three species of *Agaricus* found on Campbell Island are summarized in Table 2. Macro- and microscopic characteristics of the subantarctic *A.* cf. *subrutilescens* are in agreement with those traits specified by Kerrigan (1986) for *A. subrutilescens* in California. The only difference was in habitat: in California, *A. subrutilescens* is found in *Sequoia* and *Pseudotsuga* forests, while on Campbell Island *A.* cf. *subrutilescens* was found under *Dracophyllum* shrub in the subalpine zone. *Agaricus campbellensis*, represented by GAL9379, GAL9420, and GAL9573, showed relatively low variation in spore dimensions within specimens, similar to *A.* cf. *subrutilescens*. However, variation between specimens was sometimes great, for example, GAL9379 had much more elongated basidiospores ($P<0.05$) than the other two collections. On the other hand, intraspecimen variation was substantial in *A. subantarcticus*, although statistically significant differences were not detected among collections. At the species level, basidiospore length values of *A. subantarcticus* and *A. campbellensis* were not significantly different, while the basidiospore width in *A. campbellensis* was significantly less than in *A. subantarcticus* ($P<0.05$). Both newly described species shared characteristics with species in the section *Arvenses*, notably the presence of cheilocystidia, positive Schaeffer's reaction, yellow KOH reaction, yellowish change of context, and almond-like fragrance. *Agaricus subantarcticus* produces mid-

Fig. 4. *Agaricus* cf. *subrutilescens* (GAL9422). Scale bar equals 5 cm.

sized basidiomes with well-developed fibrillose patches on the pileus, while *A. campbellensis* has small basidiomes with tiny oppressed fibrils on the pileus.

Taxonomy

Agaricus* cf. *subrutilescens (Kauffman) Hotson & D.E. Stuntz **Figs. 4, 13**

Pileus to 60 mm diam, broadly convex-umbonate; ground color orange to tan, radiating surface fibrils bronze to snuff brown (5E5-5F6); partial veil remnants on margin; context whitish, unchanging, to 5 mm thick. **Lamellae** to 5mm broad, unequal in length, crowded, edges entire, free, thin; pinkish clay (5B2). **Stipe** 60 mm long, 9-15 mm thick at bulbous base; apex smooth; white (5A1), surface with floccose patches to base; context white, unchanging, stuffed to solid. Smell: sweet. Taste: nutty. **Partial veil** white, forming an inferior, membranous annulus. **Chemical reactions**: pileus cuticle olive green with 5% KOH, pileus context negative; Schaeffer reaction negative. **Microscopical characteristics**: Basidiospores: 4-6.2 × 3-3.7 µm, short to medium-elliptical, wall yellow to reddish-brown (5% KOH), smooth, thick, entire, apiculate; Q=1.51±0.19. Basidia: 18.5-23.3 × 7-7.5 µm, 4-spored. Clamps: lacking throughout. Subhymenium undifferentiated. Pileipellis of interwoven, inamyloid, cylindrical hyphae 2.6-12.3 µm in diam. Pileus context of interwoven, inamyloid, cylindrical hyphae 2.2-11µm in diam. Lamellar trama of parallel to interwoven, inamyloid hyphae 4.4-22.9 µm in diam.

Habitat – On litter, under *Dracophyllum scoparium* in the subalpine belt.

Collection examined: NEW ZEALAND. Campbell Island: perseverence harbour, tucker cove (S52° 33', E169° 10') *PDD92092, GAL9422*, 9 March 2000, coll. G. A. Laursen, Specimen deposited in ALA.

Comments –– Although this taxon has a somewhat unique ITS sequence, it is likely conspecific with or very closely related to *A. subrutilescens* described from North America, this is supported by the phylogenetic analyses and the shared characteristics (green KOH reaction, negative Schaeffer reaction, small spore size, unchanging context, and brown, fibrillose pileus surface etc.). It is worth noting, however, that the ITS sequences of the two isolates are relatively distinct, suggesting some divergence between populations in North America and the subantarctic region. Future investigations involving a substantially larger sample size are needed to learn more about the population structure of the species. It appears that this is the first report of *Subrutilescens* group (Kerrigan 1986) from New Zealand as well as the Southern Hemisphere.

Agaricus campbellensis Geml, Laursen & D. Lee Taylor, **sp. nov.** **Figs. 5-8, 14**
MycoBank # mb510665

Pileus 30-50 mm, convexus, forte convexo-planus, siccus, alutaceus, fibrillulae appressae, rufobrunneae, in margine fragmentis veli albis ornatus. Lamellae liberae, confertae, latae, e pallidis griseo- ad roseobrunneae, denique fuscae, inaequalis, exilis, acie sterili. Stipes 40-60 mm longus, 8-11 mm crassus, albidus, fibrillosus, basi clavata, attenuatus ad pileum, anguste fistulosus, superficies sicca, caro alba, mox griseolutescens. Annulus superus, albus, membranaceus, simplex. Odore amygdalino. Sporae ovatae, aliquando productae, mono- vel biguttulatae, fuscae, (6-) 7-8.8 (-10) × 4-4.5 (-4.8) µm. Basidia bi- et tetrasporigera, (20.2-) 21.1-24.6 × (6.6-) 7.0 µm. Typus: New Zealand, Campbell I.: Perseverence Harbour, Tucker Cove (S52° 33.1', E169° 9.3'), infra Dracophyllum scoparium, D. longifolium, et Polystichum vestitum in zona alpina et subalpina, 9 Mar 2000, coll. Gary A. Laursen, holotypus PDD92093, isotypus GAL9420 in herbario ALA depositur.

Etymology –– The name refers to the type locality.

Pileus 30-50 mm diam, convex to plano-convex, surface dry, tan (5A2, orange white) with appressed, clay or mustard brown (5D5, 5E6) fibrils, margin with veil remnants. **Lamellae** 4.5 mm broad, pinkish to pale grayish brown (5C3, 5D4, 5D5) at first, later dark brown (5E4, 5E5), free, crowded, unequal in length, thin. **Stipe** 40-60 mm long, 8-11 mm thick, whitish with yellow-brown fibrils (3A6, 3A7, 4A6, 4A7), base swollen, tapering toward the pileus, dry, context hollow, white, turning a marbled yellow, tan or grey. Odor and taste: almond. **Partial veil** white forming an inferior, membrane-like annulus, evanescent in age. **Chemical reactions**: pileus context and surface yellowing in 5% KOH; Schaeffer reaction positive. **Microscopical characteristics**: Basidiospores short to medium-elliptical, sometimes elongated, yellow to reddish-brown, wall smooth, thick, entire, apiculate, one- or two-guttulate, dark brown at maturity, (6-) 7-8.8 (-10) × 4-4.5 (-4.8) µm, mean and standard deviation are: L 7.61±1.03,

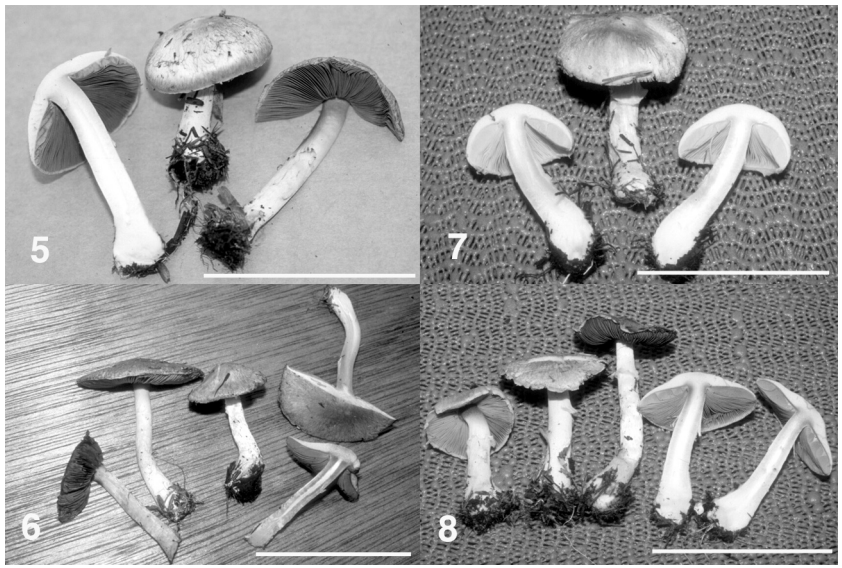

Fig. 5-8. *Agaricus campbellensis*. 5. GAL9379. 6. GAL9420. 7. GAL9573. 8. 9605. Scale bar equals 5 cm.

W 4.34±0.34; Q=1.69 ± 0.22. Basidia: 2-4-spored, (20.2-) 21.1-24.6 × (6.6-) 7.0 µm, clavate. Subhymenium of irregular, swollen, inamyloid cells. Pileipellis of interwoven, inamyloid, cylindrical hyphae, terminal cells cylindrical to slightly swollen tipped. Pileus context of interwoven, inamyloid, cylindrical hyphae. Lamellar trama of parallel to interwoven, inamyloid hyphae.

Characteristic fixed ITS rDNA polymorphisms. Characteristic fixed DNA polymorphisms were determined based on the sequence alignments used in the phylogenetic analyses. Only those characters present in all *A. campbellensis* isolates and not present in *A. semotus*, the most closely related known *Agaricus* species, were included. These fixed DNA polymorphisms are indicated with capital and italicized letters (nucleotides) or italicized numbers (gaps) with the alignment position given: tctT[-1-]tag @ 81-82; gggTat[-2-]Cgag @ 154, 157-159; aggTggtcAGcct @ 167, 172-173; ttTgct @ 189; tgtGagg @ 199; gctTtgc @ 222; tgaCccc @ 242; gttTA[-1-]ctTGcCaga @ 255-256, 260-261, 263; gtCGaat @ 296-297; cttTgaa @ 306; tctTtac @ 315; catGggc @ 321; tt[-1-]T[-1-]catgCcta @ 328, 334; aatCata[-2-]ata @ 346, 350-351; aacGcag @ 400; aaaGgca @ 617.

Habitat –– On litter, under *Dracophyllum scoparium*, *D. longifolium*, and *Polystichum vestitum* in the subalpine and alpine zones.

Collections examined: NEW ZEALAND. Campbell Island: perseverence harbour, tucker cove (S52° 33.1', E169° 9.3') *PDD92093* (Holotype), *GAL9420* (Isotype), 9 March 2000, coll. G. A. Laursen; same location *GAL9379*, 6 March 2000, coll. G. A.

Laursen. **NEW ZEALAND**. Campbell Island: perseverence harbour, mount honey (S52° 33.5', E169° 8.9') *GAL9573, GAL9603,* 14 March 2000, coll. G. A. Laursen. **NEW ZEALAND**. Campbell Island: perseverence harbour, mount beeman (S52° 33.7', E169° 9.2') *GAL9605, GAL9633, GAL9649*, 15 March 2000, coll. G. A. Laursen. Specimens deposited in ALA.

Comments –– Based on both molecular and morphological data, *Agaricus campbellensis* is placed in section *Arvenses*, subsection *Minores*. It differs from its closest known relative, *A. semotus* for which the name *A. dulcidulus* Schulzer was proposed (Nauta 2001), in particular by multiple ITS rDNA polymorphisms (see species description), much larger basidiospore size, the presence of two-guttulate basidiospores, and the color of the pileus. Although there are several other described species in this subsection with no available genetic data (e.g. *A. comtulus* Fr., *A. lutosus* (F.H. Møller) F.H. Møller, *A. purpurellus* (F.H. Møller) F.H. Møller, *A. luteomaculatus* (F.H. Møller) F.H. Møller, *A. xantholepis* (F.H. Møller) F.H. Møller), *A. campbellensis* clearly differs from these in morphology (i.e. spores size and pileus color). Similarly, the three *Minores* species specified by Mitchell & Walter (1999) to occur in New Zealand (i.e. *A. bambusae var. australis* Heinem., *A. semotus,* and *A. viridopurpurascens* Heinem) differ from *A. campbellensis* in the characters specified above.

Agaricus subantarcticus Geml, Laursen & D. Lee Taylor, **sp. nov.** **Figs. 9-12, 15**
MycoBank # mb510667

Pileus 40-130 mm, convexus, forte convexo-planus, albolutescens vel cremeus, denique melleus. Disco et squamuli fibrillosi cremei vel griseobrunnei, denique rufobrunnei, in margine fragmentis veli albis ornatus. Lamellae liberae, confertae, latae, e pallidis griseo-ad roseobrunneae vel aurantiobrunneae, denique fuscae, inaequalis, fragilis, acie sterili. Stipes 40-90 mm × 8-20 mm, albus, tactu lutescens, basi clavata (25-35 mm), anguste fistulosus, glabrus, caro alba, mox lutescens. Annulus inferior, albus, membranaceus, reflexus. Odore amygdalino. Sporae ovatae, fuscae, mono- vel biguttulatae, (4-) 7-9 (-9.5) × (4-) 4.5-5.5 µm. Basidia tetrasporigera, 8.8 × 17.6 µm. Typus: New Zealand, Campbell I.: Perseverence Harbour, Tucker Cove (S52° 33.1', E169° 9.3'), infra Dracophyllum scoparium et D. longifolium in zona subalpina, 9 Mar 2000, coll. Gary A. Laursen et H. H. Burdsall, holotypus PDD92094, isotypus GAL9419 in herbario ALA depositur.

Etymology –– The name refers to the geographic region, in which the species is found.

Pileus 40-130 mm diam, convex to plano-convex, surface yellow white (4A2) to cream or chamois (4A3, 4B4, 4C5) at first, later topaz or honey yellow (5C5, 5D6, 5E7). Disc and fibrillose squamules cream to grayish tan at first, later reddish brown (5D5, 5E6), margin with veil remnants. **Lamellae** 4-15 mm broad, pinkish to orange brown and pale grayish brown (5B2, 5C3, 5D4) at first, later dark brown (5E4, 5E5, 5F6), free, crowded, unequal in length, brittle. **Stipe** 40-90 mm long, 8-20 mm thick, white or dull yellow, base bulbous (25-35 mm thick), smooth, context hollow, white, turning yellow. Odor and taste: almond. **Partial veil** white forming an inferior, membrane-like, annulus, evanescent in

Fig. 9-12. *Agaricus subantarcticus*. 9. GAL9604. 10. GAL9419. 11-12. GAL9425. Scale bar equals 5 cm.

age. **Chemical reactions**: pileus context and surface yellowing in 5% KOH; Schaeffer reaction positive. **Microscopical characteristics**: Basidiospores short to medium-elliptical, yellow to reddish-brown; wall smooth, thick, entire, apiculate, chocolate brown to purple brown in deposit, one- or two-guttulate, exhibit great variation in size within specimen, (4-) 7-9 (-9.5) × (4-)4.5-5.5 μm, mean and standard deviation are: 7.89±1.11 × 4.83±0.38; Q=1.69 ± 0.15. Basidia: mostly 4-spored, 8.8 × 17.6 μm, clavate. Subhymenium of irregular, swollen, inamyloid cells. Pileipellis of interwoven, inamyloid, cylindrical hyphae, terminal cells cylindrical to slightly swollen tipped. Pileus context of interwoven, inamyloid, cylindrical hyphae. Lamellar trama of parallel to interwoven, inamyloid hyphae.

Characteristic fixed ITS rDNA polymorphisms. Characteristic fixed DNA polymorphisms were determined based on the sequence alignments used in the phylogenetic analyses. Only those characters present in all *A. subantarcticus* isolates and not present in any other species in Clade I/A sensu Geml et al. (2004) were included. These fixed DNA polymorphisms are indicated with capital and italicized letters (nucleotides) or italicized numbers (gaps) with the alignment position given: tagGgag @ 159; aagCggt @ 167; catActa @ 174; ctgCcct @ 228; ctcGcca @ 261; tgtTatt @ 275; tacCct @ 289.

Habitat –– On litter, under *Dracophyllum scoparium* and *D. longifolium* in the subalpine zone of subantarctic islands.

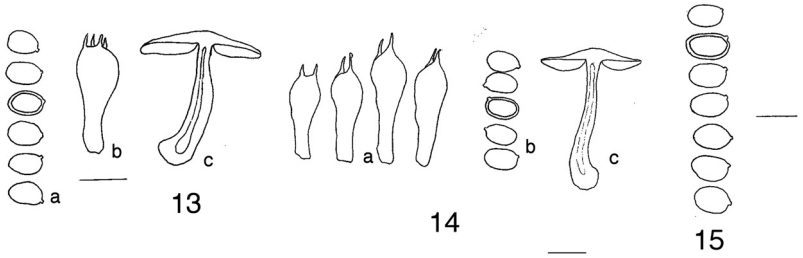

Fig. 13-15. 13. Basidiospores (a), basidium (b), and cross section of mature basidiocarp (c) of *Agaricus* cf. *subrutilescens* (GAL9422). 14. Basidia (a), basidiospores (b), and cross section (c) of mature basidiocarp of *Agaricus campbellensis* (GAL9420). 15. Basidiospores of *Agaricus subantarcticus* (GAL9419). Scale bar equals 10 μm.

Collections examined: NEW ZEALAND. Campbell Island: perseverence harbour, tucker cove (S52° 33.1', E169° 9.3') *PDD92094* (Holotype), *GAL9419* (Isotype), 9 March 2000, coll. G. A. Laursen and H. H. Burdsall; same location and date *GAL9418*, coll. G. A. Laursen. **NEW ZEALAND**. Campbell Island: perseverence harbour (S52° 33.2', E169° 10.1') *GAL9425*, 10 March 2000, coll. G. A. Laursen. **NEW ZEALAND**. Campbell Island: perseverence harbour, mount honey (S52° 33.5', E169° 8.9') *GAL9572,* 14 March 2000, coll. G. A. Laursen. **NEW ZEALAND**. Campbell Island: perseverence harbour, mount beeman (S52° 33.7', E169° 9.2') *GAL9604*, 15 March 2000, coll. G. A. Laursen. Specimens deposited in ALA.

Comments –– Based on molecular and morphological data, *A. subantarcticus* is placed in section *Arvenses*, subsection *Arvenses*. The cream then honey-brown colored pileus, the relatively large fibrillose squamules, and the characteristic inferior position of the annulus are unique among known species in the section *Arvenses*. Although species with brown scales are found in the section (e.g. *A. augustus, A. subrufescens* Peck), the squamules are much larger in *A. subantarcticus* and sometimes less pronounced, particularly in older specimens. Also these species are very different phylogenetically as well. Superficially similar pilei can be observed in *A. romagnesii* Wasser; however, this latter species is obviously different in many other aspects (ITS sequence, negative Schaeffer reaction, phenolic odor, etc.). There are three species in subsection *Arvenses* sensu Mitchell & Walter (1999) in New Zealand (i.e. *A. arvensis, augustus,* and *A. lanipes* (F.H. Møller & Jul. Schäff.) Hlaváček) and they clearly differ from *A. campbellensis* in both molecular and morphological characters.

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