

## ***Psilocybe allenii* – a new bluing species from the Pacific Coast, USA**

JAN BOROVIČKA<sup>1,2</sup>, ALAN ROCKEFELLER<sup>3</sup>, PETER G. WERNER<sup>4</sup>

<sup>1</sup>Institute of Geology, v.v.i., Academy of Sciences of the Czech Republic, Rozvojová 269, CZ-16500 Prague 6, Czech Republic

<sup>2</sup>Nuclear Physics Institute, v.v.i., Academy of Sciences of the Czech Republic, Řež 130, CZ-250 68 Řež near Prague, Czech Republic  
bore.bor@gmail.com

<sup>3</sup>alanrockefeller@gmail.com

<sup>4</sup>Bioscience Microscopy Facility, Merritt College, 12500 Campus Dr., Oakland, CA, USA  
pgwerner@pobox.com

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*Psilocybe allenii* is a new bluing wood-rotting species from the Pacific Coast, USA. Both morphological and molecular features (ITS rDNA phylogeny) indicate its close relationship to *Psilocybe cyanescens* Wakef. Despite the shape and size of spores and cystidia of this new species falling within the variability of *P. cyanescens*, *P. allenii* can be distinguished by its convex to hemispheric pileus, not wavy at maturity, and ITS rDNA sequence. The description of *P. allenii* is accompanied by sequences obtained from the holotype and paratype collections (ITS rDNA, LSU, EF-1 $\alpha$  and RPB2). Furthermore, similar species of this relationship are discussed and an epitype of *P. cyanescens* is designated.

**Key words:** Basidiomycota, Agaricales, *Strophariaceae*, taxonomy, molecular phylogeny.

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Lysohlávka Allenova – *Psilocybe allenii* je nový druh modrající dřevní lysohlávky z tichomořského pobřeží USA. Jak morfologické, tak molekulární znaky (fylogeneze ITS rDNA) ukazují na blízkou příbuznost s lysohlávkou modrající – *P. cyanescens* Wakef. Přestože tvar a velikost spor a cystid tohoto nového druhu spadají do rámce variability lysohlávky modrající, lysohlávka Allenova se liší tvarem klobouku, který je vypouklý až polokulovitý, v dospělosti bez zvlněného okraje, a odlišnou sekvencí ITS rDNA. Popis nového druhu je doplněn souborem molekulárních dat získaných z holotypu a paratypů (ITS rDNA, LSU, EF-1 $\alpha$  a RPB2). Dále jsou diskutovány příbuzné druhy z tohoto okruhu a je stanoven epityp lysohlávky modrající.

### INTRODUCTION

Northern California and the Pacific Northwest, including British Columbia, are regions with many psychoactive wood-rotting *Psilocybe* species, namely *P. cyane-*

*scens* Wakef., *P. azurescens* Stamets & Gartz, *P. cyanofibrillosa* Stamets & Guzmán, *P. stuntzii* Guzmán & Ott, *P. pelliculosa* (A.H. Sm.) Singer & A.H. Sm., *P. sylvatica* (Peck) Singer & A.H. Sm., and *P. baeocystis* Singer & A.H. Sm. (Stamets & Gartz 1995, Stamets 1996, Guzmán et al. 2008). Despite not being reported in the literature, *P. ovoideocystidiata* Guzmán & Gaines, which is known from the eastern USA (Guzmán et al. 2007), also has been found in this area (Borovička et al., unpublished).

In last few years, J.B. received several collections of an unusual *Psilocybe* species collected by John W. Allen in Seattle (WA, USA). Judging from photographs, they were rather similar to *P. cyanescens* but the pileus margins were not wavy. However, their microscopic characteristics fell within the range of variability of *P. cyanescens*. As has been recently demonstrated in the group of the European *P. serbica* M.M. Moser et E. Horak, the macro- and microcharacters can be highly variable in this species (Borovička 2008, Borovička et al. 2011); therefore, one would not place emphasis on the macroscopic differences observed in the collections from Seattle and would attribute them to intraspecific variability of *P. cyanescens*.

However, DNA sequencing of the collections from Seattle and also from the San Francisco Bay Area (Northern California) has revealed that there is a stable 5 base-pair difference in the DNA sequence of the ITS rDNA region (containing ITS1, 5.8S, ITS2 sequences and flanking SSU and LSU regions) which is commonly used for separation of agaric species (Miller & Buyck 2002, Antonín et al. 2009a), including *Strophariaceae* (Antonín et al. 2009b, Borovička et al. 2011). We therefore consider the collections to represent a new species.

#### MATERIALS AND METHODS

**Morphological study.** The macroscopic description is based on observations of fresh and dried specimens, colour photographs and personal communication with John W. Allen. Microscopic features are described from dried material mounted in a 5% KOH aqueous solution or 1% aqueous Congo Red; observations were made using Zeiss Primo Star LED microscope (full-Köhler) and Zeiss Plan-Achromat 100×/1.25 oil-immersion objective. Basidiospores were measured from mature fruit bodies (lamellae); statistical analysis is based on the measurement of 200 spores in 5 collections. Minimum and maximum length/width values of spore size are given in brackets and represent the 5th and 95th percentiles, respectively. Spore length/width quotients (Q-values) are presented as 5th percentile, median and 95th percentile, respectively. Spore dimensions were measured in the hymenium from pictures taken with a Canon PowerShot A650 IS digital camera

connected to a Zeiss Primo Star microscope; measurements on screen and estimations were carried out using the AxioVision 4.8.1 software.

Voucher specimens are deposited in the herbarium of the Mycological Department, National Museum, Prague, Czech Republic (PRM), University of Washington, Seattle, WA, USA (WTU), University of British Columbia, Vancouver, BC, Canada (UBC), and San Francisco State University, San Francisco, CA, USA (SFSU). Collections of *Psilocybe subaeruginosa* agg. were studied from New Zealand (Landcare Research, Auckland, PDD), continental Australia (kept at SFSU) and Tasmania (Tasmanian Museum and Art Gallery, Hobart, HO). Herbarium acronyms are used according to Thiers (2012).

**DNA study.** To get insight into the taxonomic position of the new species, nuclear DNA was extracted from six collections and from additional selected species (Tab. 1) identified/revised by Jan Borovička or Alan Rockefeller. A small piece of a dried basidiocarp was extracted using the NucleoSpinR Plant II extraction kit (Macherey-Nagel) according to the manufacturer's instructions. The ITS rDNA region was amplified by polymerase chain reaction (PCR) using primer pairs and the PCR regime as described in Borovička et al. (2011). In order to characterize the holotype of *P. allenii*, additional molecular markers were amplified and sequenced: LSU and EF-1 $\alpha$  partial sequences according to Borovička et al. (2011) and RPB2 (RNA polymerase II second largest subunit) partial sequence using the primer pair bRPB2-5F/bRPB2-7.1R according to Liu et al. (1999) and Matheny (2005). The obtained amplicons were purified with isopropanol and both strands were sequenced at Macrogen Europe (Netherlands). The DNA sequences were edited using the biological sequence alignment editor BioEdit (Hall 1999) and the ITS rDNA sequences were aligned using the ClustalW Multiple Alignment tool. Edited nucleotide sequences were submitted to EMBL-Bank (EMBL Nucleotide Sequence Database).

The evolutionary history was inferred by using the Maximum Likelihood method based on the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985). The tree with the highest log likelihood (-1935.8300) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches; the branch supports were estimated using 1,000 bootstrap replicates. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise the BIONJ method with MCL distance matrix was used. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.4154)]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 nucleotide sequences. There were a total of 589 positions in the final dataset with 178 variable and 42 singleton sites. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

**Tab. 1.** Species under molecular study.

Identifier	Species	Herbarium specimen	Origin	EMBL-Bank
				ITS
P26	<i>Psilocybe allenii</i> , holotype	PRM 899876	USA	HE994440
P40	<i>Psilocybe allenii</i> , paratype	PRM 899877	USA	HE994441
P41	<i>Psilocybe allenii</i> , paratype	PRM 899878	USA	HE994442
P42	<i>Psilocybe allenii</i> , paratype	WTU (Stamets, 16.XII.2002)	USA	HE994443
TW0880	<i>Psilocybe allenii</i>	SFSU (Rockefeller, 14.XI.2010)	USA	HE994450
AR25	<i>Psilocybe allenii</i>	SFSU (Klein, 30.XI.2011)	USA	HE994448
P39	<i>Psilocybe cyanescens</i>	PRM 899875	USA	HE994444
TW0881	<i>Psilocybe ovoideocystidiata</i>	SFSU (Auweia, 11.XI.2010)	USA	HE994451
TW0882	<i>Psilocybe ovoideocystidiata</i>	SFSU (Cuthbert, 5.XI.2010)	USA	HE994452
TW0883	<i>Psilocybe ovoideocystidiata</i>	SFSU (Auweia, 1.X.2006)	USA	HE994453
TW0884	<i>Psilocybe ovoideocystidiata</i>	SFSU (Auweia, 6.II.2009)	USA	HE994454
P44	<i>Psilocybe subaeruginosa</i> agg.	PDD 783551	New Zealand	HE994446
P43	<i>Psilocybe subaeruginosa</i> agg.	PDD 91967	New Zealand	HE994447
AR22	<i>Psilocybe subaeruginosa</i> agg.	SFSU (Feelers, 26.IV.2011)	Australia	HE994449
P33	<i>Hypholoma marginatum</i>	PRM 921867	Czech Rep.	HE994445

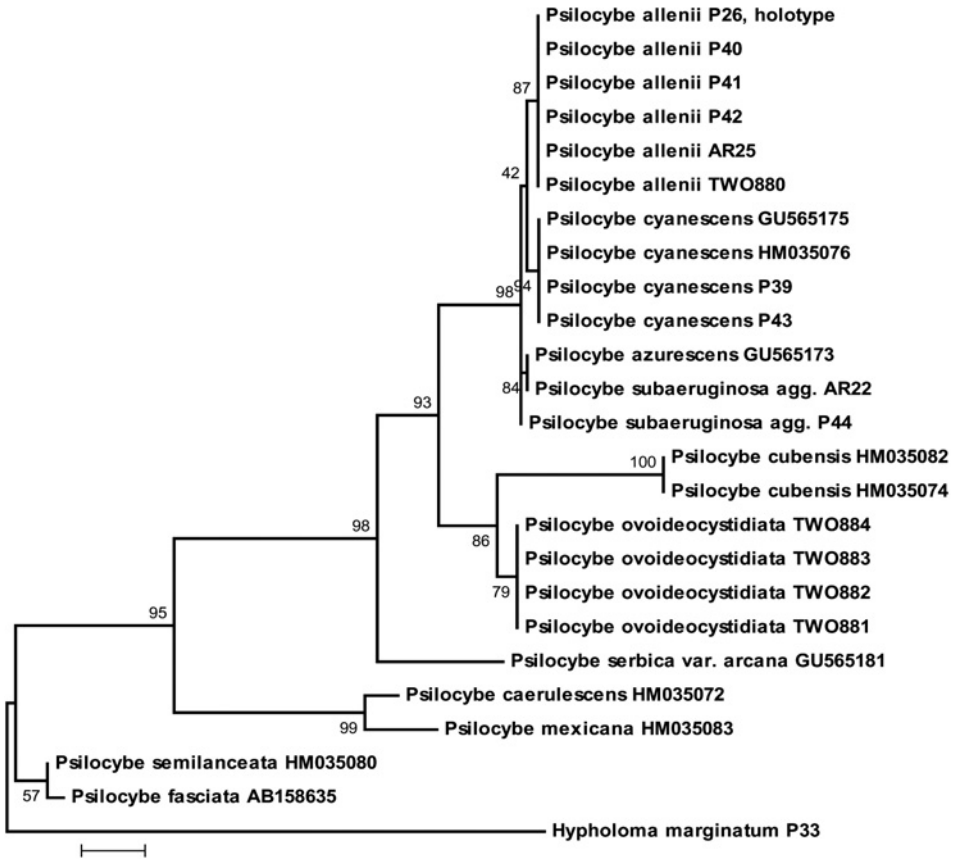
## RESULTS

As has been demonstrated by phylogenetic analysis (Fig. 1), *P. cyanescens*, *P. azurescens* and *P. allenii* are very closely related species. However, in all sequenced collections of *P. allenii* from both California and Washington, the same sequence of the ITS rDNA region was observed, differing by 5 base pairs from collections of *P. cyanescens* both from Europe and the USA. Furthermore, another DNA marker system was used (in collaboration with colleagues at Florida International University, Miami, FL) supporting the finding within this paper. The microsatellite profiles show *P. allenii* as being different from *P. cyanescens* (manuscript in prep., B. Kallifatidis et al., personal communication). On the other hand, no significant differences were observed in the obtained sequences of LSU, EF-1 $\alpha$ , and RPB2. Since the macroscopic differences between *P. cyanescens* and *P. allenii* are obvious and even easily recognized by people lacking formal mycological training, we have decided to describe *P. allenii* at the species level.

***Psilocybe allenii* Borov., Rockefeller & P.G. Werner, sp. nov.**

(MycoBank: MB 564115)

= *Psilocybe cyanofriscosa*, nom. prov. (see Etymology)? = *Psilocybe cyanescens* s. Arora p.p. (1986, Fig. 88)



**Fig. 1.** Phylogenetic placement of *Psilocybe allenii* and related species inferred from ITS rDNA molecular data. The best tree resulting from the Maximum Likelihood analysis in MEGA5 is presented. Numbers beside the internal nodes are Maximum Likelihood bootstraps. Sequences without database accession numbers were obtained during this study (Tab. 2). The tree was rooted using *Hypholoma marginatum* as outgroup.

**Holotype.** USA, WA, King County, Seattle, University of Washington Campus, 3 November 2009, leg. John W. Allen, PRM 899876. **Isotypes.** WTU (2 complete fruit bodies), UBC (1 complete fruit body).

**Etymology.** *Psilocybe allenii* has been known for many years in the San Francisco Bay Area and despite being hypothesized new (see Stamets 2005: 286), to our knowledge it has neither been published nor discussed in the scientific literature. The online mycological community has been using the provisional name "*Psilocybe cyanofriscosa*" for this taxon, coined by "Quankus" on www.shroomery.org (1 November 2006); this name has also been mentioned in Wikipedia (5 January 2012). However, this provisional name is not grammatically correct Latin and we have decided to name this species in honour of John W. Allen who deeply believed in this new species and repeatedly insisted on a detailed study and DNA sequencing; without his persistence and enthusiasm, this study would likely not have occurred in a timely manner.

**Tab. 2.** Species under molecular study – comparison with reference (epitype) collection of *P. cyanescens*; numbers of base pair (bp) changes in particular DNA regions are indicated.

Species	Origin	Sequence	18S	ITS1	5.8S	ITS2	28S
			8 bp	217 bp	159 bp	215 bp	52 bp
<i>Psilocybe cyanescens</i>	Germany	GU565175	ref.	ref.	ref.	ref.	ref.
<i>Psilocybe cyanescens</i>	not reported	HM035076	0	0	0	0	0
<i>Psilocybe cyanescens</i>	USA, Washington	HE994444	0	0	0	0	0
<i>Psilocybe allenii</i>	USA, Washington	HE994440	0	2	0	3	0
<i>Psilocybe allenii</i>	USA, Washington	HE994441	0	2	0	3	0
<i>Psilocybe allenii</i>	USA, Washington	HE994442	0	2	0	3	0
<i>Psilocybe allenii</i>	USA, California	HE994443	0	2	0	3	0
<i>Psilocybe allenii</i>	USA, California	HE994450	0	2	0	3	0
<i>Psilocybe allenii</i>	USA, California	HE994448	0	2	0	3	0
<i>Psilocybe subaeruginosa</i> agg.	Australia	HE994449	0	4*	0	2	0
<i>Psilocybe subaeruginosa</i> agg.	New Zealand	HE994446	0	0	0	1	0
<i>Psilocybe subaeruginosa</i> agg.	New Zealand	HE994447	0	0	0	0	0
<i>Psilocybe azurescens</i>	USA, Oregon	GU565173	0	2*	0	2	0

Heterozygous positions were not considered.

\* including 1 indel position

ref. – reference sample

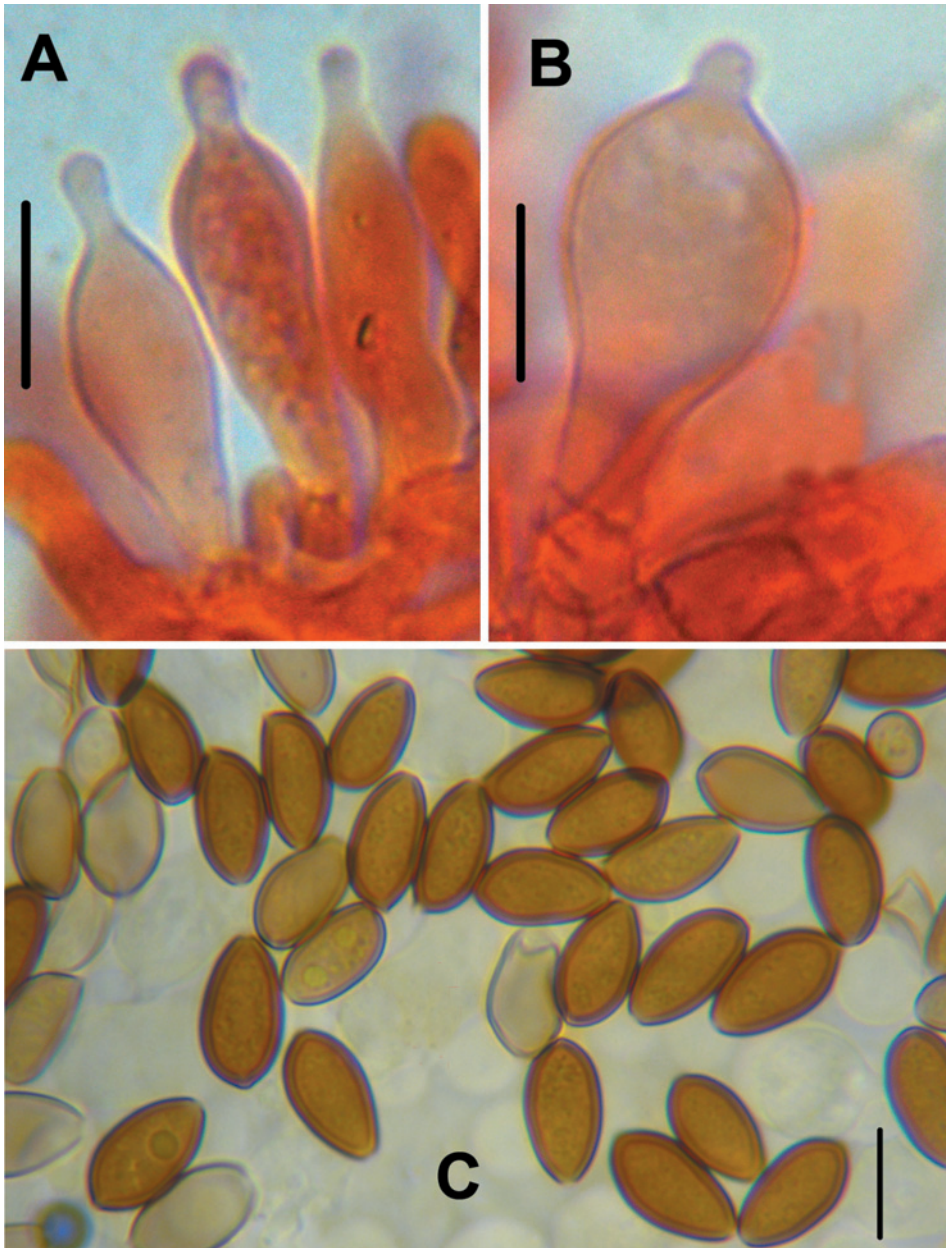
**Description.** Habit collybioid; fruit body size rather variable, depending on substrate quality and environmental conditions (Figs. 2 and 3). Pileus 1.5–7(9) cm diam., rarely larger, broadly convex to plane when mature, often also almost hemispheric and not umbonate, sometimes slightly depressed in the centre, with a straight margin, sometimes slightly incurved, only rarely somewhat wavy, sometimes with a striate margin in mature specimens when moist (striations continue one fifth to half of the way to the pileus centre); surface smooth, viscid when moist, with a separable gelatinous pellicle, hygrophanous, pale orange brown or caramel brown when moist, fading to a light yellowish buff as it dries; staining blue when damaged or sometimes in response to environmental conditions. Lamellae adnate to sinuate, cream to pale gray brown when young, dark purple brown mature, margin pale to whitish. Spore print dark violet brown to dark tobacco brown. Stipe 4–7(9) × 0.2–0.7 cm, cylindrical, hollow, rather firm, apex pruinose, slightly enlarged at base, with thick white rhizomorphs; surface smooth to silky fibrillose, whitish when young and strongly bluing when bruised, later off-white and/or with yellowish shades. Mycelium white, rhizomorphic, sometimes staining sky blue, odour and taste farinaceous. Veil present in young specimens, cortinate, snow-white, later disappearing. Like *P. azurescens* or *P. serbica* var. *moravica*, a cortinate zone can be present and coloured purplish brown by spores. Flesh tan, staining blue when damaged, odour and taste strongly farina-



**Fig. 2.** *Psilocybe allenii*. USA, CA, San Francisco Bay Area, 16 December 2002 leg. Paul Stamets (WTU, paratype). EMBL-Bank: HE994443, HE994464. Photo courtesy of Paul Stamets.



**Fig. 3.** *Psilocybe allenii*. USA, CA, Oakland, 5 January 2006 leg. Peter G. Werner. Photo by Peter G. Werner.



**Fig. 4.** *Psilocybe allenii*, holotype (PRM 899876). **A** – cheilocystidia. **B** – pleurocystidium. **C** – spores. Scale bar = 10  $\mu$ m. Photo by Jan Borovička.



ceous. Basidia cylindrical, mostly  $27\text{--}37 \times 9\text{--}11 \mu\text{m}$ , 4-spored, sterigmata usually  $4\text{--}5.5 \mu\text{m}$  long. Clamp connections abundant. Spores  $(11.1)12.0\text{--}12.6\text{--}13.1(14.2) \times (6.5)6.8\text{--}7.1\text{--}7.4(7.9) \mu\text{m}$ ,  $Q = 1.6\text{--}1.8\text{--}1.9$ ; slightly narrower in side view (median  $\sim 6.8 \mu\text{m}$ ), elongate-ellipsoid, equilateral in face view, somewhat inequilateral in side view, with an apical pore, relatively thick-walled ( $0.8\text{--}1 \mu\text{m}$ ), brownish with a yellow tinge in 5% KOH (Fig. 4C). Cheilocystidia abundant, variable in shape, narrowly clavate-mucronate, narrowly lageniform (neck no longer than  $8 \mu\text{m}$ ), rarely with a forked neck, infrequently narrowly fusiform to fusiform, hyaline, thin-walled, mostly  $20\text{--}30 \times 6\text{--}8 \mu\text{m}$  (Fig. 4A). Pleurocystidia common, narrowly to broadly clavate-mucronate (rarely with subcapitate apex), hyaline, thin-walled, mostly  $25\text{--}35 \times 9\text{--}14 \mu\text{m}$  (Fig. 4B). Caulocystidia present, variable in shape but generally similar to cheilo- and pleurocystidia. All types of cystidia sometimes finely encrusted at apex.

**Habitat and phenology.** Scattered to gregarious, sometimes caespitose, growing on woody debris, usually on wood chips (*Pinus radiata*, *Cupressus macrocarpa*, *Eucalyptus*, *Pseudotsuga menziesii*, *Alnus* and others). Synanthropic, most common in urban wood chip landscaping and also found in wood chipped gardens, parks and similar urban locations. This species is easy to cultivate on agar, grain spawn, and sawdust or wood chips. Fructifies in cold weather, from late September to January.

**Distribution.** Known from Los Angeles (CA, USA) to Seattle (WA, USA). This species is not as common in coastal dune grasses as its close relative *P. cyanescens*, though it does occur there in Northern California. While most collections have been found in the San Francisco Bay Area and Humboldt County (CA) within 10 miles of the ocean or bay, it has been found at least 100 miles inland in California.

Chemical analyses are not available. However, *P. allenii* is consumed for its hallucinogenic properties, and is commonly sought out by some mushroom hunters; it is roughly equivalent in potency to *P. cyanescens*.

#### Collections studied

**Holotype.** USA, WA, King County, Seattle, University of Washington Campus, 3 November 2009, leg. John W. Allen, PRM 899876 (EMBL-Bank: HE994440, HE994455, HE994459, HE994463). **Isotypes.** WTU (2 complete fruit bodies), UBC (1 complete fruit body). **Paratypes.** 1. USA, WA, King County, Seattle, University of Washington Campus, 30 October 2010, leg. John W. Allen, PRM 899877 (EMBL-Bank: HE994441, HE994456, HE994460). – 2. USA, WA, King County, Seattle, University of Washington Campus, 3 November 2010, leg. John W. Allen, PRM 899878 (EMBL-Bank: HE994442, HE994457, HE994461). – 3. USA, WA, King County, Seattle, University of Washington Campus, 3 November 2010, leg. John W. Allen, PRM 899879. – 4. USA, CA, Mountain View, 14 November 2010, leg. A. Rockefeller, SFSU (EMBL-Bank: HE994450). – 5. USA, CA, Sunnyvale, 30 November 2011, leg. Debbie Klein, SFSU (EMBL-Bank: HE994448). – 6. USA, CA, San Francisco Bay Area, 16 December 2002, leg. P. Stamets, WTU (EMBL-Bank: HE994443, HE994464).

## DISCUSSION

**Similar taxa**

Due to its non-umbonate hemispherical pileus and fibrillose zone on the stipe, some specimens of *P. allenii* are similar in appearance to *P. serbica* var. *moravica* (Borovička 2003, 2008). However, this is a European species with different microscopic characteristics and, furthermore, its fruit bodies are more slender than those of *P. allenii*.

*Psilocybe cyanescens* Wakef. is very similar to *P. allenii* in our experience, and it cannot be distinguished from it by size or shape of the spores or the cystidia. However, *P. allenii* can be recognized macroscopically by the shape of the pileus, which is convex to hemispheric and not wavy at maturity, its thinner stipe, thinner pileus context and slightly darker pileus colour. Furthermore, *P. allenii* often appears a few weeks before *P. cyanescens* in similar habitats, and has a shorter season, being less common in the spring. In addition, there is a stable 5-bp difference in its ITS rDNA sequence (Tab. 2). The main macroscopic differences between *P. allenii* and its relatives from the Pacific Coast are summarized in Tab. 3. In *Mushrooms Demystified* (Arora 1986), the colour plate of *P. cyanescens* possibly shows several fruit bodies of *P. cyanescens* growing from wood chips, with several fruit bodies of *P. allenii* placed in front of them to show the underside.

**Tab. 3.** Characteric macrocharacters of *P. allenii* and its relatives from the Pacific Coast.

	<i>Psilocybe allenii</i>	<i>Psilocybe cyanescens</i>	<i>Psilocybe azurescens</i>
<b>Pileus shape</b>	Hemispheric when young, convex when mature. Only rarely wavy in very mature specimens.	Hemispheric when young, soon becoming more or less wavy	Hemispheric when young, convex when mature. Never wavy.
<b>Pileus umbo</b>	Never umbonate	Rarely umbonate. When present, never acute.	Broadly to acutely umbonate when mature
<b>Cortinate zone on stipe</b>	Usually present	Absent	Usually present
<b>Time of year (North America)</b>	Mid September through January	Late September through April	Late September through April
<b>Stipe length</b>	4–7(9) cm	(4)6–9(11) cm	9–20 cm

*Psilocybe azurescens* Stamets & Gartz is also very close to *P. allenii* but differs by its broadly umbonate pileus, longer stem and minor differences in the ITS rDNA sequence. Furthermore, *P. azurescens* is known to occur naturally only in a small geographic area on the coast near the outlet of the Columbia River.

*Psilocybe cyanofibrillosa* Guzmán & Stamets is a somewhat uncommon bluing wood-rotting species from the Pacific Northwest. According to the descrip-

tions (Stamets et al. 1980, Guzmán 1983, Guzmán et al. 2008) and personal observations by the US co-authors, clavate-mucronate pleurocystidia are absent. Furthermore, the cheilocystidia in *P. cyanofibrillosa* have very long necks which are often highly forked, often more than two times – this might indicate an affinity to the *P. pelliculosa* group. Furthermore, spores in *P. cyanofibrillosa* are smaller than those in *P. allenii*.

*Psilocybe ovoideocystidiata* Guzmán & Gaines was recently described from the Eastern USA (Guzmán et al. 2007). Despite being uncommon, it is widespread on the Pacific Coast; for many years, these collections were incorrectly identified as *P. subaeruginascens*, *P. septentrionalis* or *P. stuntzii*. Collections from Washington (Seattle), Oregon (Portland) and California (San Francisco, Richmond and Redwood City) have been noted by the authors. The known range in the east has also expanded: this species was described from Pennsylvania and has also been found in Ohio, Kentucky, New York, New Jersey, Maryland, Rhode Island, Virginia, Washington DC and West Virginia. On the Pacific Coast, this species has not been found in the wild and shares the same woodchip habitats as *P. allenii* and allies but can be easily distinguished by the occurrence of a membranous annulus and microscopically by its subrhomboid spores. In their description of *P. ovoideocystidiata*, Guzmán et al. (2007) describe a close relationship between this North American species and the Javanese species *P. subaeruginascens* Höhn. and the Japanese species *P. septentrionalis* Guzmán, based on spore shape and the presence of an annulus on the fruiting body. Although Guzmán (1983) initially treated the majority of Japanese populations of *P. septentrionalis* as synonymous with *P. subaeruginascens*, he later (1995) accommodated that population in its own taxon, *P. septentrionalis*. More recently, Horak & Desjardin (2006) redescribed *P. subaeruginascens* based upon a study of type material and recent collections from Java, and similarly noted clear differences between Guzmán's (1983) drawings of basidiomes of Japanese collections of *P. septentrionalis* and the Javanese collections of *P. subaeruginascens* they had studied. Based upon his study of the type material of *P. subaeruginascens* and prior descriptions, photographs, and photomicrographs of *P. septentrionalis*, P.G.W. endorses the idea of a close relationship between *P. ovoideocystidiata* and *P. septentrionalis*, but notes that both species are in fact quite different from *P. subaeruginascens*, the latter having basidiospores which are much thicker-walled and darker in color than those of the other two species.

Finally, four Australian species appear to be close to *P. allenii*: *P. subaeruginosa* Cleland (Cleland 1927) and *P. australiana* Guzmán & Watling, *P. eucalypta* Guzmán & Watling, and *P. tasmaniana* Guzmán & Watling (Guzmán & Watling 1978). This species complex was studied by Chang & Mills (1992), who proposed synonymy between all these taxa. Curiously enough, Guzmán observed chocolate brown pleurocystidia in the original collection of *P. subaeruginosa* (AD, Cleland 13251)

and classified it in a separate section *Subaeruginosae* (Guzmán 1983, 1995). However, Chang & Mills (1992) did not confirm the occurrence of chocolate brown pleurocystidia in any of the collections studied (including the lectotype); only pale yellow coloration was occasionally noted in some pleurocystidia.

We have recently revised several collections from Australia, Tasmania and New Zealand (AD, SFSU, HO and PDD) identified as *P. subaeruginosa* by various native mycologists and have not observed pigmented pleurocystidia. Phylogenetic analysis (Fig. 1) has revealed that this group of fungi is closely related to *P. cyanescens*, as already suggested by Chang & Mills (1992); interestingly, the ITS rDNA region of the collection PDD 91967 collected in Auckland was 100% similar with *P. cyanescens* (Tab. 2). In conclusion, the group of *P. subaeruginosa* requires a thorough revision supported by well-documented morphological and molecular data. However, our preliminary screening does not indicate conspecificity between *P. allenii* and the investigated collections within the complex of *P. subaeruginosa*.

### ***Psilocybe cyanescens* complex**

In recent studies of the *P. cyanescens* complex in Europe (Borovička 2008, Borovička et al. 2011), it was demonstrated that even stable macro- and micro-characters observed in various collections over the years, which would normally support a rather narrow species concept of *P. serbica*, are not supported by molecular data: neither by ITS rDNA, nor LSU, nor EF-1 $\alpha$ . The question has arisen if a similar problem would appear in the complex of species closely related to *P. cyanescens* from North America (*P. azurescens*) and south-eastern Asia & Australia (*P. subaeruginosa* agg.). Molecular data are not available in public databases and our sequencing of several collections of *P. subaeruginosa* has revealed only subtle differences in the ITS rDNA region when compared to *P. cyanescens*. Unfortunately, further confusions are to be expected in the future since bluing *Psilocybe* species are outdoor-cultivated by “mushroom fans” and might be introduced to various parts of the world.

Despite being described from Kew Gardens, UK, synanthropic habitats and the distribution of *P. cyanescens* in Western Europe suggest that this species was introduced to Europe and is indigenous to North America, where it is known from natural habitats on the Pacific Coast. This hypothesis proposed by Borovička (2005) was accepted in the recent monograph of *Strophariaceae* by Noordeloos (2011) and available molecular data (ITS rDNA, LSU and EF-1 $\alpha$  sequences) are identical in European and American collections of *P. cyanescens* (Borovička et al. 2011, this study).

The holotype collection of *P. cyanescens* (at K) is very old and in bad condition. The macrocharacters observed on the holotype and especially the macroscopic appearance of collections from the UK and continental Europe known to

the authors indicate that *P. allenii* has not been reported from Europe and that the holotype collection belongs to *P. cyanescens* s.s. (Borovička 2005, 2008; Borovička et al. 2011, Noordeloos 2011). However, to avoid further confusion, an epitype collection characterized by molecular data (ITS rDNA, LSU, EF-1 $\alpha$ ) is designated:

### ***Psilocybe cyanescens***

Holotype. United Kingdom, England, Surrey, Royal Botanic Gardens, Queen's Cottage grounds, 25 October 1911 leg. E.M. Wakefield, K(M) 63976.

Epitype (designated here). Germany, Hamburg, Altona District, Klein Flottbeck Botanical Garden, on mulch, 26 November 2003 leg. Jürgen Hechler (PRM 901481). GenBank: GU565175, GU565167, GU565158. Another collection from the same locality (PRM 901480), showing the typical appearance of *P. cyanescens*, is depicted in Borovička (2005, Fig. 8).

A thorough investigation is needed to better understand the species delimitation using both morphological and molecular data – future results from molecular and possibly other research (e.g. interbreeding studies) might lead to new taxonomic concepts in various groups of Basidiomycetes, including the complex of *P. cyanescens*. In view of the problems discussed within this paper, especially a revision of the Australasian bluing *Psilocybe* species related to *P. cyanescens* is needed.

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