The *Helvella lacunosa* species complex in western North America: cryptic species, misapplied names and parasites

Nhu H. Nguyen

Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, California 94720

Fidel Landeros

Departamento de Botánica y Zoología, Universidad de Guadalajara, Mexico

Roberto Garibay-Orijel

Instituto de Biología, Universidad Nacional Autónoma de México, Mexico

Karen Hansen

Swedish Museum of Natural History, Department of Cryptogamic Botany, P.O. Box 50007, SE-104 05 Stockholm, Sweden

Else C. Vellinga

Department of Plant and Microbial Biology, University of California at Berkeley, Berkeley, California 94720

Abstract: Based on morphology, fungal species have been considered widespread and as a result names of species from Europe or eastern North America were applied to species in western North America. However, DNA sequences have shown that many western taxa are different from their European counterparts; one such case is presented here. Comparisons of ITS and LSU rDNA sequences from ectomycorrhizal root tips and ascomata of specimens identified as *Helvella lacunosa* from North America, Europe and Asia revealed that the taxa from western North America and Mexico formed a well supported clade different from the eastern North American, European and Asian taxa. Within this western North American clade there are at least four taxa. Here we describe two of these western taxa as new species: *Helvella vespertina* and *Helvella dryophila*. *Helvella vespertina* is a bigger version of *H. lacunosa*, is variable in hymenial color and shape and forms ectomycorrhizae with conifers; it fruits typically Oct–Jan. *Helvella dryophila* is characterized by a dark almost black, squat pileus, a light stipe when young, medium size and forms ectomycorrhizae with *Quercus* species; it fruits Jan–Jun. Due to insufficient material, the two other *Helvella* taxa are discussed but not formally described here. We also examined the *Hypomyces* and other mycoparasites associated with the ascomata of *Helvella* species and discuss misleadingly labeled sequences in public databases.

Key words: biodiversity, Helvellaceae, host plants, *Hypomyces*, North American mycoflora

INTRODUCTION

Macrofungal taxa in western North America have been known under European or eastern North American names for many years. It recently has become clear in the light of sequence data that these names often have been misapplied. Through sequence data and phylogenetic analyses, we here infer the situation of the supposedly common and globally distributed species *H. lacunosa*, with emphasis on western North America and Mexico.

*Helvella lacunosa* Afzel. : Fr described from Sweden (Afzelius 1783) is a common and widespread species in Europe and North America that is easily recognized by a tan to dark gray to black convoluted cap with a stipe that either has deep grooves (sulcate) or deep holes (lacunose). This species is commonly seen in western North America and parts of Europe, and because it was deemed common, specimens are not commonly collected. Authors of monographs in both Europe (Dissing 1966) and North America (Weber 1972; Abbott and Currah 1988, 1997) used a broad morphological species concept for *H. lacunosa* and included within it was an entity called *Helvella sulcata* Afzel. with a grooved stipe. This has led to the use of the name “*H. lacunosa*” throughout both continents for different looking mushrooms (FIG. 1A–K).

Fungal collections made during a foray to Santa Cruz Island (California, USA) by Grubisha et al. (2005) and several mycoblitzes at Point Reyes National Seashore (California, USA; http://nature.berkeley.edu/brunslab/tour/mycoblitz2.html) were subjected to a rigorous sequencing project with two goals: to understand the limits of local species and to compare the coastal California mycoflora with those from other parts of California, eastern North America and Europe. Many of the Californian taxa were morphologically similar to but differed in ITS sequences from eastern North American and European species of the same names. *Helvella lacunosa* was one of these taxa. Comparisons of specimens and root-tip data available from GenBank showed that perhaps three different taxa were hiding under that one name. This finding also corroborates with the
results of Matthew Smith (pers comm) who sequenced a number of oak root tips associated with Helvella from California.

Based on these preliminary results, we decided to test the hypothesis that H. lacunosa in western North America is a species complex and that these species differ from H. lacunosa from other parts of the world. We methodically collected many samples from different regions and habitats in California. We also borrowed specimens from established institutions and collectors from eastern North America, Mexico, Europe and Asia. Then we compared the LSU and ITS sequences along with morphology of Californian material with those from other localities and especially the type locality for H. lacunosa in northern Europe. Here we focus on Helvella taxa from western North America, their hosts and their associated parasites. We describe two Helvella taxa as new species and compare these with other Helvella species.

**MATERIALS AND METHODS**

*Collections and morphology.*—We sampled collections of “Helvella lacunosa” from western North America (most intensively in California), Mexico, eastern North America (the Midwest and Northeast), both southern (Montenegro) and northern Europe (Norway, Sweden, Denmark, the Netherlands) and Asia (southwestern China). Because no holotype exists for this species, we tried to obtain samples from locations near where it was described in Sweden.

Fresh collections of “Helvella lacunosa” and other Helvella species were made throughout California in the 2011–2012 mushroom seasons, photographed and vouchers at the University Herbarium at the University of California, Berkeley (UC). Specimens were borrowed from the University of Minnesota Herbarium (MIN), the NAMA Environmental Herbarium (F), and the general fungal collection at F, the University of Florida Herbarium (FLAS) and various collectors. Herbarium abbreviations are according to Thiers (continuously updated). (See Supplementary Table I for details.)

Spores were measured from mature ascomata in Melzer’s reagent; the notation (65/4/4) means that 65 spores were measured from four ascomata and four collections with a minimum of 15 per collection.

*DNA extraction and sequencing.*—We extracted DNA with several protocols depending on the age of the specimens as follows: for recently collected Helvella specimens (after 1990) we used a modified Sigma Extract-N-Amp kit (Sigma Aldrich, St Louis, Missouri); for specimens that had been stored in a herbarium before 1990, we used a standard 2% CTAB (Cetyltrimethylammonium bromide) buffer lysis followed by chloroform extraction and ethanol precipitation. We PCR-amplified the ITS region for recently collected specimens with the standard fungal specific primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). For herbarium specimens with degraded DNA, we amplified the ITS gene in two segments with ITS1F-ITS2 and ITS3-ITS4 (White et al. 1990) when necessary. We amplified the D1/D2 loop of the LSU gene with the primers LSI (5’ AGTACCCGCTGAACCTTAG 3’) (Hausner et al. 1993) and LR5 (5’ TCCTGAGGGAAAACCTTGC 3’) (White et al. 1990). We also produced ITS sequences for seven Hypomyces collections on Helvella and three on other mushrooms using ITS1F and ITS4. Sequences were produced with the standard BigDye Terminator 3.1 Kit and were run on an ABI Prism 3700 Genetic Analyzer (Life Technologies) with the above PCR primers. We examined each automated sequence and manually interpreted and corrected ambiguous bases with Sequencher 4.7 (Gene Codes Corp.). All sequences were deposited in GenBank (Supplementary Table I).

*Phylogenetic methods.*—We analyzed the Helvella sequences in the following ways. Using BLAST from GenBank, we gathered all ITS and LSU sequences similar to our sequenced specimens (in the Helvellaceae), including environmental sequences and all sequences labeled Helvella. We excluded from our datasets sequences labeled “Helvella” that were determined to belong outside the Helvellaceae. The GenBank LSU numbers for these erroneously labeled sequences are: AJ544211 (H. lacunosa) and EU669264 (H. maculata). The GenBank accession numbers of the ITS sequences are: AF046219 (H. or Pindara terrestris), AJ544211 (H. lacunosa), AJ879645 (uncultured root isolate), EU669212 (H. maculata), EU834201 (H. maculata), FJ235143 (Helvella sp.), FJ235145 (Helvella sp. SOC902), JF908044 (H. phlebophora) and JF908045 (H. epithidium representing Cortinarius sp.). Gyromitra esculenta (Pers.: Fr.) Fr. was chosen as outgroup in the LSU analyses and Helvella latisspora Boud., and H. lactea Boud. were chosen as the outgroup for the ITS analyses. Sequences were aligned with MAFFT (Katoh et al. 2002) and optimized manually. For the ITS matrix, we restricted the dataset to close relatives of our taxa of interest because the region is variable among species in the genus, as is the case for various other Pezizales, such as Morchella (Du et al. 2012) and Peziza s. str. (Hansen et al. 2002). The alignments were deposited in TreeBASE under 13596. Phylogenetic analyses of LSU, ITS and combined LSU+ITS alignments were performed under maximum likelihood and maximum parsimony criteria. The maximum likelihood method was performed with the GTRGAMMA model of nucleotide substitution in RAxML (Stamatakis 2006). Maximum likelihood bootstrapping was performed locally in RAxML using stringent bootstrapping criteria with 1000 replications (Stamatakis 2006). Maximum parsimony bootstrap analysis was performed in PAUP 4.0b6 with 1000 replications (Swofford 2001).

**RESULTS**

We produced three alignments, one for LSU (71 sequences, of which 36 are newly generated), one for ITS (89 sequences, of which 57 are newly generated) and one for ITS+LSU (85 sequences). The LSU

**NGUYEN ET AL.: HELVELLA LACUNOSA SPECIES COMPLEX**

1277
region was uniform, with only 170/789 (= 22%) informative characters. The ITS region provided twice as many informative characters (413/927 = 45%), but the ITS1 and ITS2 sequences of distant Helvella species could not be aligned. To avoid the ITS alignment problems that accompany the divergent sequences, in addition to missing LSU or ITS data for certain specimens, we chose to present the results as separate LSU and ITS trees (Figs. 2, 3). In both trees the taxa of interest, particularly those that we are describing here, are well represented.

Analyses of the LSU and ITS alignments (Figs. 2, 3) showed that the collections from eastern North America, Europe and Asia originally identified as “Helvella lacunosa” fell within a gradient of small clades with low statistical support. However, despite the scant sampling and poor phylogenetic resolution of the deeper branches, we noticed some interesting patterns in several clades/species. It is apparent that some taxa are widespread. For instance a collection of H. ephippium (KC147669) from Minnesota is a close match to environmental sequences isolated from Epipactis helleborine roots in Japan (ITS 99%, LSU 99%); a specimen of “H. lacunosa” from Minnesota (KC122821) closely matches a sequence from the roots of Cephalanthera longifolia in France (ITS 96%) and Alnus roots in Estonia (ITS 97%) and a sequence from Quercus roots in China (ITS 96%); and a specimen from New Hampshire was almost identical (ITS 99%) to specimens from Austria and the Netherlands (Figs. 2, 3). With broader sampling the distribution and identity of these fungi could be better understood.

In contrast to the patterns observed above, all specimens that were identified as “Helvella lacunosa” from western North America and Mexico formed a well supported monophyletic clade, separate from the eastern North American and Eurasian taxa (Figs. 2, 3). Within this clade there are well supported branches with good sampling to support species delimitations.

One clade (CA1, Figs. 2, 3) had 100/97% bootstrap support in the ITS gene but only 66/72% in the LSU gene. The LSU+ITS combined analysis slightly improved support for this clade at 87/60% (data not shown). This clade consists of specimens that were collected from coastal areas of California north of San Francisco, northward to Oregon and British Columbia, Canada (~1300 km), and east to Idaho. The members of this clade also could be found in the foothills and lower elevations of the Sierra Nevada and Cascade Range. The association of this clade appears to be predominantly with conifers, in particular Pinus and Pseudotsuga. We propose the name H. vespertina for this clade.

A second clade (CA2, Figs. 2, 3) received 100/100% bootstrap support based on the analyses of the ITS alignment and 98/97% bootstrap support of the LSU alignment. It consisted of specimens that were collected from Riverside County and Santa Cruz Island in southern California and north to southern Oregon (~1000 km). Members of this clade also can be found at lower elevations in the Sierra Nevada, including lower valleys in the high Sierra. Ascomata have been found only where Quercus species occur, and thus we propose the name H. dryophila for this clade.

The sequences in clade CA3 (Fig. 3) are represented by several ectomycorrhizal root tips and a single ascoma collection from California. It is well supported with 95/94% bootstrap of the ITS alignment analyses. This clade probably contains two species, which occur in the northern Sierra Nevada of California and Cascades of Oregon. A much more rigorous sampling of ascomata is necessary to further taxonomic studies in this clade.

Both the LSU and ITS analyses (Figs. 2, 3) revealed a number of potentially new Mexican species, although with low support in the deepest branch of the ITS tree. It must be pointed out that the sequences in the ITS and LSU alignments did not come from the same specimens, and thus there is a disconnect between these two datasets. Nevertheless, there currently appears to be three new species in Mexico that belong to the western North American Clade and a more complete sampling and detailed study are required to delimit and describe these species. Outside the western North American Clade, there appears to be at least four species under the name “H. lacunosa”.

Based on these overall results, we hypothesize that there may be at least two additional new species in California and at least three species in Mexico. However, the sampling for the additional Californian and two (out of three) Mexican taxa is scant and a much more complete sampling is necessary before species boundaries could be delimited within this group. The exceptions are the two widespread clades that we focus on in this paper and describe here.

**TAXONOMY**

**Helvella dryophila Vellinga & N.H. Nguyen, sp. nov.**

**Figs. 1A–C, 5**

*MycoBank MB802331*

*Etymology: dryophila*, from the Greek for “oak-loving”.

*Type collection: United States of America, California, Alameda County, Berkeley, University of California, Observatory Hill opposite McCon Hall, 4 Apr 2012, N.H. Nguyen (UC 1999233), GenBank (KC122861).*
Fig. 2. LSU maximum likelihood tree of a broad sampling within the Helvellaceae. Numbers on branches are maximum likelihood/maximum parsimony bootstrap values. Each terminal leaf is annotated as: Taxon name GenBank accession – Location. Location abbreviations: CA = California, OR = Oregon, CO = Colorado, MN = Minnesota, MI = Michigan; BC = British Columbia. CA 1–3 indicate clades that occur in California. Note that the name ‘'Helvella lacunosa'' appears throughout the tree. This is due to the misapplication of this name to these terminal taxa.
FIG. 3. ITS maximum likelihood tree of the *Helvella lacunosa* species complex. Numbers on branches are maximum likelihood/maximum parsimony bootstrap values. Each terminal leaf is annotated as: Taxon name GenBank accession – Location; host when available. Location abbreviations: within California PtR = Point Reyes National Scashore, YNP = Yosemite National Park, JDSF = Jackson Demonstration State Forest, SPSP = Salt Point State Park, UCB = UC Berkeley.
Ascoma 35–85 mm high. Pileus 15–45 mm high, 21–55 mm diam, when young rounded with well defined grooves (Fig. 1C), ages to irregularly lobed and wrinkled, pinched in at apex, slightly ribbed in some places, dark gray-black, rarely pale gray, sometimes with some hints of brown, fused with stipe in several places; apothecial surface light gray, smooth. Stipe 35–75 × 10–20 mm, cylindrical to distinctly widened in basal part, regularly to irregularly grooved and lacunose, with anastomosing ribs, off-white when young and often gray with age, becoming yellowish ocher along the whole stipe, with white mycelium at base (Fig. 1A, B).

Ascospores [65/4/4] l × w = 15.7–19.6 × 9.8–12.7 μm, avl × aw = 16.4–17.6 × 10.8–11.7 μm, Q = 1.4–1.7, avQ = 1.5–1.55, smooth, with one big oil guttule. Asci pleurorhynchous, not amyloid, with eight ascospores. Paraphyses cylindrical, 3–5 μm wide, with slightly widened apex 4–6 μm wide, with brown contents. Ectal excipulum trichodermal; terminal elements 16–40 × 8–19 μm, narrowly clavate, not colored (Fig. 5).

Habitat and distribution: Mainly in small groups, rarely solitary, terrestrial and ectomycorrhizal with Quercus species (Q. agrifolia, Q. douglasii, Q. kelloggii, Q. wislizeni) in closed and open oak or mixed forests with oaks, also as a root tip on Arbutus menziesii in a pure Arbutus stand; host trees could be positively identified (Smith et al. 2007, Morris et al. 2008, Wolfe et al. 2009, Kennedy et al. 2012); widespread and common in the western parts of North America, currently known from southern Oregon south to southern California (Santa Cruz Island and Riverside County) in a range of approximately 1000 km, from low to mid-elevation of the Sierra Nevada (up to 1700 m). Fruiting from mid-Dec through end of May at higher elevations.

Comments: Helvella dryophila looks similar to H. vespertina, but the contrast in color between pileus and stipe is striking; the pileus is very dark and squat and rounded with distinct well defined grooves when young (Fig. 1A–C), and the ascomata are up to 85 mm high. We could not find any reliable microscopic differences. The two species can actually grow near each other in a mixed oak-conifer forest. The collection from Oregon under the name H. lacunosa (U42681) in O’Donnell et al. (1997) is nested within the H. dryophila clade (CA2 in Fig. 2) and is almost certainly H. dryophila. Other western North American Helvella species are discussed below.

Helvella vespertina N.H. Nguyen & Vellinga, sp. nov.
Fig. 3D–I. MycoBank MB802330
Etymology: vespertina, Latin for ‘western, where the sun sets’.

Type collection: United States of America, California, Sonoma County, Salt Point State Park, 15 Jan 2012, T.W. Osmundson (UC 1999204), GenBank (KC122858).

Misapplied name: Helvella lacunosa sensu Morse, Mycologia 37:417. 1945 and sensu other American authors.

Ascoma 50–300 mm high. Pileus 25–55 (–150) mm high, 25–50 (–120) mm broad, with three almost smooth lobes and wavy when young developing brain-like convolutions with age, often with one lobe
descending on stipe, light gray to dark gray, evenly colored to dark-spotty; apothecial surface pale when young to pale gray, sometimes with some anastomosing ribs, smooth, fused with stipe in several places. Stipe up to 250 mm high, up to 105 mm broad, cylindrical, with rounded to sharp, lengthwise, anastomosing ribs, grooved and lacunose, smooth, white, turning to gray with age, sometimes ochraceous along the lower portions of the stipe along the ribs (FIG. 1D–I).

Ascospores [61/4/4] l 3 w 5 15.7–21.4 3 9.8–12.2(–13.7) mm, avl 3 avw 5 17.1–17.6 3 10.9–11.4 mm, Q 5 1.4–1.8, avQ 5 1.55–1.6, smooth, with one guttule. Asci pleurorhynchous, not amyloid, with eight ascospores. Paraphyses cylindrical, 3–5 mm wide, with 4–9 mm wide apex, with brown intracellular pigment. Ectal excipulum trichodermal with terminal narrowly clavate to clavate, rarely cylindrical cells, 15–53 3 10–20 mm, with brown to dark gray parietal pigments (Fig. 4).

Habitat and distribution: In groups and clusters, terrestrial in forests and grassy verges, ectomycorrhizal with *Pinus* species (*P. muricata, P. lambertiana, P. ponderosa*), *Pseudotsuga menziesii* and possibly *Abies concolor* and *Arbutus menziesii*; widespread and common in the western parts of North America, at sea level in the coastal areas to around 1800 m in the Sierra Nevada and other mountain regions, known from Vancouver Island (British Columbia, Canada), California, Oregon and Idaho (USA.), covering a distance of approximately 1300 km and probably more widespread. Oct–Mar (rarely in Apr).

Comments: *Helvella vespertina* resembles European *H. lacunosa* macroscopically but can form ascomata of gigantic proportions with stipes up to 20 cm tall and 10.5 cm across (see also Morse 1945). To our knowledge no European collections of *H. lacunosa* reach such dimensions. It is equally variable in colors, and complexity of the hymenium and also very similar in microscopic characters to *H. lacunosa*. When young it sometimes can be indistinguishable from *H. dryophila* (see description below), but the host tree and fruiting time will be the determining factors; it is also similar microscopically to *H. dryophila*. Older specimens of these species are more easily recognized and distinguished.

Thanks to sampling of ectomycorrhizal root tips in various locations, *Pinus muricata* and *Pseudotsuga menziesii* could be positively identified as conifer hosts for this species (Peay et al. 2010, Kranabetter et al. 2012). It also was found once on root tips of *Arbutus menziesii* in a predominantly conifer forest (Kennedy et al. 2012), although ascomata have not been collected in a pure *A. menziesii* stand.

Knowledge of host plant is an important feature (sometimes essential) in recognition of *H. vespertina* and *H. dryophila*, where *H. vespertina* associates with conifers and *H. dryophila* with oaks. This is also the case in distinguishing between two very similar European species, *H. fusca* Gillet and *H. lacunosa* based on the association of *H. fusca* with *Populus* (Landeros and Korf 2012). Therefore we recommend that a combination of host and macro-morphological characters be used to distinguish between *H. dryophila* and *H. vespertina*.

**DISCUSSION**

It is surprisingly difficult to morphologically distinguish between European *H. lacunosa* and western North American *H. vespertina*. Dissing (1966) in his monograph of European *Helvella* species, applied a broad morphological species concept to *H. lacunosa*. Furthermore, he synonymized several eastern North American taxa with *H. lacunosa*. Because he studied many collections from Europe, Asia, North America and South Africa, his description is probably a mixture of a number of species. We sequenced one of his collections, a typical *H. lacunosa* from Sorø Sønderskov, Denmark, a locality where many ascomata occurred (Dissing 1966). This specimen does not belong to the western North American clade but grouped with other European and Asian collections and was pivotal to our understanding of the group (Fig. 3). More than two decades later German
mycologist Häffner (1987) in his taxonomic treatment of Helvella in Germany did not give a description of typical H. lacunosa but only of two variants that stood out, namely H. lacunosa var. sulcata f. minima and H. lacunosa f. phlebophoroidea-maxima (an invalid name). Our results suggest that European H. lacunosa also harbors several cryptic species that could be recognized by applying phylogenetic species concepts. The distribution of these taxa is insufficiently known as yet, and the identity of the specimens cited by Dissing (1966) as introduced into South Africa’s pine plantations is open for reassessment.

In North America, similar to European literature, the descriptions of H. lacunosa often are based on collections from different regions; Weber (1972) in her overview of Michigan Helvella species included western North American collections in her descriptions, which almost certainly belong to different species than the ones from Michigan. But, contrary to Dissing (1966), Weber (1972) considered H. palustris Peck separate from H. lacunosa.

The name H. lacunosa has been widely applied in North America (e.g. Anderson and Ickes 1921; Seaver 1928, 1942 (as Elvella mitra); Groves and Hoare 1953; Kempton and Wells 1970; Weber 1972; Abbott and Currah 1988, 1989, 1997), but our own preliminary findings, based on limited sampling outside California, indicate that there are at least eight species (four in western North America and four east of the Rocky Mountains) hiding under this name in USA and Canada, plus at least three in Mexico, and one of them could well be H. palustris. We therefore estimate at least 11 species in North America.

Kempton and Wells (1970) were insightful when they suggested that American specimens differed from European H. lacunosa, despite Dissing’s authority on the species. They suggested a careful reappraisal of western and northern North American H. lacunosa, with particular attention to spore characters. Although we could not find differences in spore sizes, the differences in ITS and LSU clearly distinguish western North American from the European collections named H. lacunosa.

The European species H. juniperi M. Filippa & Baiano looks like H. lacunosa but has a more simple, less convolute pileus than H. vespertina and H. lacunosa and differs in a thicker excipulum and in spores that are relatively long (18)19–23(24.5) × (9.5)10.5–13(14) μm; it was described from juniper forests on sandy soils in coastal Italy (Filippa and Baiano 1999).

White to off-white specimens with a general appearance of H. vespertina have been called H. crispa (Scop.) Fr.:Fr. in both western and eastern North America, but due to lack of specimens and sequences at this point we cannot confirm that the American specimens belong to the species described from Europe.

Helvella maculata N.S. Weber is a western North American species that bears a moderate resemblance to H. vespertina; it has a white sulcate stipe, a brown mottled hymenium and a light colored villose underside to the pileus (Weber 1975); the pileus is lobed and more simple in shape than that of H. vespertina and H. dryophila. ITS and LSU sequences of two H. maculata specimens in GenBank from Oregon (EU669212, EU669264, EU834201) are very different from any Helvella sequences and actually belong to a nonrelated ascomycetous fungus in the Helotiales, growing in or on the Helvella ascomata (see mycoparasite discussion below).

The five other species that were described from western North America all differ from H. vespertina, H. dryophila and H. lacunosa in shape of the pileus, shape of the stipe or in both. Short descriptions and comparisons are as follows:

Helvella crassitunicata N.S. Weber with a cupulate hymenium has thick-walled paraphyses and big spores, 22–27 μm long (Weber 1975); it is known from coniferous forests from Oregon into Alaska (Weber 1975, Abbott and Currah 1997, Castellano et al. 1999).

Helvella brevissima Peck was launched into the world with this short description: “Pileus irregular, convolute, with the deflexed margin free, blackish when dry, whitish or pallid beneath, 1–2 cm. broad; stem very short, even, solid, whitish or pallid, 1–1.5 cm. long, 3–4 mm. thick; asci cylindric; spores elliptic, commonly binucleate, 15–20 μm long, 10–12 μm broad; paraphyses filiform, thickened at the top and there brown. Ground. California. W.R. Dudley.” (Peck 1903). Dudley collected it around Stanford University in Palo Alto, south of San Francisco. The even solid stipe sets this apart from H. vespertina and H. dryophila.

Helvella faulknerae Copel. and H. hegani Copel. were described based on collections from Chico, California; H. faulknerae has a sulcate stipe and a cupulate pileus, whereas H. hegani has a smooth stipe and a bilobate pileus; both fruit in spring (Copeland 1904). The latter species could be synonymous with H. compressa (Snyder) N.S. Weber.

Helvella compressa was described in 1936 as Paxina compressa Snyder from Washington (Snyder 1936). It is a widespread, western, small vernal species with a white stipe that is cylindrical in cross section, with a saddle-shaped grayish brown to dark brown hymenium.

Mycoparasites.—Mycoparasites appear to be common on ascomata of Helvella species, possibly due to the
cartilaginous texture and relative longevity of these ascornata. One of our LSU sequences from *H. maculata* (KC122806) is a species in the Helotiiales that surprisingly matched (99%) a specimen sequenced from central Oregon collected in 2000 (EU669264 as *H. maculata*). Although based only on two specimens, the distance of about 600 km, 12 y, and repeated sequencing of this fungus from two independent labs suggest that it might be a common parasite of *H. maculata*.

Ascornata of *H. vespertina* are host to the agaricomyctete *Tricholoma sclerotoideum* Morse, also known as *Clitocybe sclerotoidea* (Morse) Bigelow (Morse 1943, 1945; Trappe 1972), which suppresses ascornata formation. We have found these mushrooms in late January in Point Reyes National Seashore, California, where *H. vespertina* grows in abundance with *Pinus muricata*. They are common where they occur. However, we have not found this agaric on a sclerotoid mass of *H. dryophila*. Morse (1943) noted that all collections of *T. sclerotoideum* came from conifer forests, where *H. dryophila* does not occur.

A specimen of *H. vespertina* with a pale ectal excipulum yielded sequences (GenBank KC122875, KC122807) that match a *Mortierella* species (Zygomycota), and we are reporting it here infecting a *Helvella*. An association between *Helvella* and *Mortierella* had not been reported previously.

*Helvella vespertina* ascornata are often parasitized by *Hypomyces cervinigenus* Rogerson & Simms, which was described from western American “*H. lacunosa*” growing in conifer forests in Washington and British Columbia (Rogerson and Simms 1971). The host was most likely *H. vespertina*. *Hypomyces cervinigenus* was described for the teleomorph stage of the European *Mycogone cervina* Ditmar. Although the name *Hyp. cervinigenus* is now widely applied to a *Helvella* parasite in Europe (Læssøe and Dissing 2000, Henrici 2002, Roobek 2012), apparently the teleomorph has not been recorded in Europe (Læssøe and Dissing 2000). Because it is not yet shown genetically that *Hyp. cervinigenus* and *M. cervina* are indeed different stages of the same species, we propose using the name *Hyp. cervinigenus* for the western North American taxon.

The first mycoparasite we encountered on *H. dryophila* we called *Hyp. cervinigenus* without confirming its seemingly obvious morphology under the microscope. However, after sequencing the ITS of *Hypomyces* from both *H. dryophila* and *H. vespertina*, we noticed that the parasites separated into two groups. This prompted a more careful morphological examination of all the *Hypomyces* collections, and we found obvious morphological differences in the aleuriospores (Fig. 1M, N), including spore color (brown in *Hyp. cervinigenus*, pink in *Hypomyces* sp.), spore wall thickness, spore shape and size (up to 14 × 14 µm in *Hyp. cervinigenus*, up to 24 × 24 µm in *Hypomyces* sp.), ornamentation and presence/absence of conidia between the two sequence groups (Fig. 1L, M). The *Hypomyces* that occurs on *H. dryophila* may be a new species. Because of the scant sampling of this potential new species, we cannot be certain whether it is exclusive to *H. dryophila* alone, but so far we have not found it among the 12 collections of *Hypomyces* on *H. vespertina*. Conversely we have found *Hyp. cervinigenus* on one collection of *H. dryophila*. More detailed studies are necessary to understand the relationship between *Helvella* species and their *Hypomyces* parasites.

Although we focused this study on the western North American species in the *H. lacunosa* complex, from the sequence data we present here it is clear that many specimens were misidentified from both fresh collections and older vouched herbarium collections in both Europe and North America. An epitype of *H. lacunosa* must be made before this species complex can be sorted out taxonomically. As a whole, the genus *Helvella* is in dire need of major revision and collections from the total distribution area of the genus must be assessed, with special attention to habitat, host tree identity and fruiting time.

It is discouraging to realize that despite many studies on the morphology of the *Helvella* species in North America, the introduction to Anderson’s and Ickis’ (1921) article on Massachusetts *Helvella* species is still valid today. The authors itemized the difficulties in identifying *Helvella* species in North America (e.g. absence of a list of all species, descriptions scattered throughout the literature and literature that is difficult to access).

If we had not sequenced this species considered to be widespread and common, we would not have discovered that we had been misapplying a European name to our taxa or realized that there are perhaps local species that could be discovered. We strongly encourage researchers and collectors who encounter specimens of “*Helvella lacunosa*” in their research to collect, voucher and sequence these specimens and in general to question names applied to other supposedly widespread species.

This article is part of a broader effort to catalog the fungal species of California, with broader applications to the North American Mycoflora Project (http://www.northamericanmycoflora.org/). There are several bottlenecks in this type of work: metadata and collections are not available, species perceived to be common are in general not collected and the necessary literature often is inaccessible, all of which must be addressed for a successful production of the mycoflora.
ACKNOWLEDGMENTS

We especially thank Rosanne Healy for useful commentaries to improve this manuscript. This work could not have been possible without many people’s help from collecting to sequencing. Alex Grote sequenced the nrITS of the Santa Cruz Island collections and Ibai Olariaga the Scandinavian collections of H. lacunosa; Zheng Wang sequenced a Chinese collection. We thank Matt Smith for several collections from New Hampshire, Branislav Perič sent material from Montenegro and Guy S. Garcia gave us a French collection. Henning Knudsen, curator at C, was instrumental in providing access to Dissing’s material. We also thank all California collectors, in particular Thea Chesney, Debbie Klein and Todd Osmundson. We thank the curators of herbaria C, UC, MIN, F (including the NAMA voucher program) and FLAS. Financial support was provided by the NSF Graduate Research Fellowship to NHN, the Mycological Society of America Travel Award to NHN for presentation of this work at the annual MSA meeting in New Haven, Connecticut, 2012, and by the Swedish Taxonomy Initiative to KH.

LITERATURE CITED


Peay KG, Garbelotto M, Bruns TD. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation


