SYSTEMATICS AND PHYLOGENY

Molecular phylogeny and taxonomy of the endolithic lichen genus *Bagliettoa* (Ascomycota: Verrucariaceae)

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Abstract Despite their importance in bio-deterioration, the taxonomy of the calcicolous endolithic lichen genus *Bagliettoa* is still problematic, mostly because of difficult identifications and unsettled species concepts. In this study, the traditional morphology-based species and genus delimitations were tested using the phylogenetic markers ITS, *mcm7* and mtSSU. Bayesian and maximum likelihood approaches were used on a dataset of 82 specimens to reconstruct phylogenetic relationships between the eight most common species of *Bagliettoa*. Molecular data confirmed the placement of *B. limborioides*, the type of the generic name, within the clade including species usually considered as part of *Bagliettoa*. Five other species were monophyletic and well supported (*B. baldensis*, *B. calciseda*, *B. cazzae*, *B. marmorea*, *B. parmigerella*). Relationships within a clade including *B. parmigera*, *B. steineri* and specimens with intermediate characters (here referred to as *B.* cf. *suzaeana*) were not resolved and weakly supported. This species complex (also called *B. parmigera* s.l.) is in need of a further revision. After revision of the type material, a lectotype is chosen for *Bagliettoa parmigerella*. Additionally, the new combinations *Bagliettoa rubrocincta* and *Bagliettoa suzaeana* are proposed based on their morphological similarity to other species of *Bagliettoa*. A key to the 12 currently recognized species is provided, which will hopefully help ecologists and biogeologists to identify species from this difficult genus of endolithic lichens.

Keywords biopitting; calcicolous lichens; *mcm7*; molecular taxonomy; Verrucariales

■ INTRODUCTION

In karstic systems, microorganisms that live on or within the rock interface belong to ecological communities important to erosion, soil formation and carbon cycling (Burford & al., 2003; Lian & al., 2011). Together with bacteria and other fungi, lichens are an important part of these communities (Nash, 2010; Tang & Lian, 2012), where they occur either as epilithic or endolithic colonizers. In endolithic lichens, the fungal hyphae penetrate calcareous substrates along preexisting passageways (cracks or pores) or passageways created by active dissolution processes (Favero-Longo & al., 2009). One example of common endolithic lichens living on calcareous rocks is *Bagliettoa* A.Massal., a genus of lichenized ascomycetes of the family Verrucariaceae. Its involvement in rock weathering on both natural geological formations and stone monuments of cultural and heritage importance has been previously studied (Pinna & al., 1998; Pinna & Salvadori, 2000; Carter, 2002; Bungartz & al., 2004; Favero-Longo & al., 2009; McIlroy de la Rosa & al., 2012a). The thallus of *Bagliettoa* is entirely immersed in the rock with which it is intermingled. Its fruiting bodies (perithecia) are immersed in the thallus and the rock. They create small pits on the surface of the substrate after they die and decompose, a process known as biopitting. These microscale biopits are then further colonized and degraded by other endolithic microorganisms (Viles & Cutler, 2012) and can contribute to the creation of large geological alterations such as the formation of mesoscale basins (McIlroy de la Rosa & al., 2012b) or the destruction of calcareous monuments (e.g., Blazquez & al., 1995; Gazzano & al., 2009).

Although lichens have often been associated with rock weathering, some studies suggest that they can also contribute to rock surface protection, as a lichen cover can act as a buffer from wind, sand and rain (e.g., Ariño & al., 1995; Mottershead & Lucas, 2000; Carter & Viles, 2003, 2004, 2005). Through the formation of a mineralized upper cortex, species of *Bagliettoa* could possibly also slow the weathering process down. In fact, the biogeochemical interactions of endolithic lichens within karstic systems and their ecological importance in these ecosystems are not yet fully understood, and it is still debated

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whether they participate in biodegradation or bioprotection (Concha-Lozano & al., 2012; McIlroy de la Rosa, 2012). The limited knowledge currently available on their contribution to ecological and biogeochemical processes is partly due to the poorly known taxonomy of these endolithic groups (Tretiach & Geletti, 1997). It is therefore crucial to clarify the taxonomy of endolithic lichen genera, including *Bagliettoa*, a genus for which conflicting species concepts caused common misidentification.

Bagliettoa was first described by Massalongo (1853) for *Bagliettoa limborioides* A.Massal., a species of Verrucariaceae with a distinctive endolithic thallus and a shield-like involucrellum with a star-shaped aperture. Several other endolithic species sharing the same diagnostic characters were however still subsequently referred to as *Verrucaria* Schrad. by many authors among whom Zschacke (1933) and Servít (1939) both attempted to revise these endolithic taxa in the 1930s to 1950s. Although Zschacke limited the number of endolithic *Verrucaria* with a shield-like involucrellum to a realistic seven species (Zschacke, 1933), Servít (1929, 1931, 1934, 1936, 1939, 1952, 1953, 1954, 1955) described a plethora of new species, varieties and forms, and proposed the new genus *Protobagliettoa* Servít for species lacking spores at maturity, unnecessarily complicating the taxonomy of this group. Later on, the genus *Bagliettoa* was accepted by several authors (Poelt & Vězda, 1981; Santesson, 1993; Santesson & al., 2004; Eriksson, 2006) and other species of *Verrucaria* with a similar combination of characters (*V. baldensis* A.Massal., *V. cazzae* Zahlbr., *V. parmigera* J.Steiner, *V. parmigerella* Zahlbr., *V. steineri* Kušan) were transferred to this genus (Poelt & Vězda, 1981). More recently, Halda (2003) revised this group of endolithic *Verrucaria* and, as a result of a morphological study of about 3000 specimens (including most of the varieties and forms described by Servít), reduced the number of taxa to four: *V. baldensis*, *V. limborioides* (A.Massal.) Clauzade & Cl.Roux, *V. marmorea* (Scop.) Arnold and *V. parmigerella*. He also chose lectotypes and neotypes for several names in order to settle species concepts otherwise variable depending on the regional Flora used (e.g., Clauzade & Roux, 1985; Smith & al., 2009). However, Halda (2003) did not accept *Bagliettoa* as a separate genus and synonymized many additional names (e.g., the synonymy between *V. cazzae* and *V. marmorea*), leading to even more confusion.

Recently, molecular data have confirmed that *Bagliettoa* is a distinct genus, and it was therefore reinstated within the classification of the Verrucariaceae (Gueidan & al., 2007, 2009). Moreover, these data showed that this genus could not be restricted to species with an involucrellum with a star-shaped aperture as two endolithic *Verrucaria* (*V. marmorea*, *V. calciseda* DC.) lacking this diagnostic feature were shown to belong to the same lineage (Gueidan & al., 2007, 2009). In these studies, *Bagliettoa* was defined by (1) a crustose endolithic thallus, often with a visible margin, (2) an upper cortex differentiated into a lithocortex, (3) immersed perithecia and, when present, shield-shaped involucrellum with a star-shaped aperture, (4) oil cells often present in the lower part of the medulla, (5) ascospores often absent or deformed, simple and hyaline, (6) pseudoparaphyses numerous and short, with detachable apical cells in most species (Gueidan & al., 2007, 2009). However, only a limited sampling of *Bagliettoa* was included in these molecular-based studies and the type of the genus name, *B. limborioides*, was missing.

The goal of our study was to use a broader sampling in order to test the morphology-based species boundaries within *Bagliettoa* using molecular data. For this purpose, we first investigated the variability of three different markers (ITS, mtSSU, *mcm7*) within *Bagliettoa*. Then, with a sampling of the eight most common species of *Bagliettoa*, including the generic type *B. limborioides*, we used a three-gene phylogenetic analysis to investigate species relationships within *Bagliettoa* and revise the current species concept within this genus. A key to the currently accepted species is also provided.

■ MATERIALS AND METHODS

Specimen sampling. — According to Index Fungorum, 16 species names are available in *Bagliettoa* (www.indexfungorum.org, 20/02/2013). However, several names have been placed in synonymy in the past (e.g., in Servít, 1939, 1955; Poelt & Vězda, 1981; Clauzade & Roux, 1985; Nimis, 1993; Halda, 2003), and the current number of species is estimated to be between 4 and 16 depending on the authors (Table 1). In the present work, the generic concept follows the one published in recent molecular-based studies (Gueidan & al., 2007, 2009) and the species concepts follow Clauzade & Roux (1985), except for *B. parmigera* (J.Steiner) Vězda & Poelt and *B. calciseda* (DC.) Gueidan & Cl.Roux, which are recognized as two different species as in Gueidan & Roux (2007). The genus *Bagliettoa* is mostly known from Europe and is more species diverse in the Mediterranean area. It is also present in other parts of the world (e.g., Australia, China, North America), although it has generally been overlooked in these regions. In our study, we were able to sample specimens from the Mediterranean area (southern France, Italy), northern Europe (northern France, United Kingdom) and from North America and, therefore, to cover several different climatic and geographic zones within their distribution range. A total of 60 specimens of *Bagliettoa* were collected from limestone rocks in France and 2 in the United Kingdom. Material was also obtained from specimens borrowed from E (2), NMW (1) and NY (8). In total, 73 specimens representing the eight most common species of *Bagliettoa* (*B. baldensis* (A.Massal.) Vězda, *B. calciseda*, *B. cazzae* (Zahlbr.) Vězda & Poelt, *B. limborioides*, *B. marmorea* (Scop.) Gueidan & Cl.Roux, *B. parmigera*, *B. parmigerella* (Zahlbr.) Vězda & Poelt, *B. steineri* (Kušan) Vězda; Fig. 1, Table 2) were studied. Seven specimens of *Parabagliettoa* Gueidan & Cl.Roux (*Parabagliettoa cyanea* (A.Massal.) Gueidan & Cl.Roux and *Parabagliettoa dufourii* (DC.) Gueidan & Cl.Roux), the sister genus of *Bagliettoa*, were also collected or borrowed on loan from E to be used as an outgroup in our study. Finally, type specimens or potential type material were borrowed from various herbaria for study: *B. cazzae* (W 1916-4447, W 1916-4495, W 1912-4574), *B. marmorea* (PRM 900619), *B. parmigera* (PRM 758127, WU 072982, WU 072983), *B. parmigerella*

Table 1. List of currently available names in *Baglietto* and species accepted in six recent taxonomic treatments of the genus.

Species previously treated as *Baglietto* or *Protobaglietto**	Servít (1939)	Servít (1955)	Poelt & Vězda (1981)	Clauzade & Roux (1985)	Nimis (1993)	Halda (2003)
B. bagliettoiformis (Hazsl.) Gams***	as ***V. bagliettoiformis*** Servít	as ***P. bagliettoiformis*** (Hazsl.) Servít***	as a synonym of *B. parmigerella*	—	as a synonym of *B. parmigerella*	as a synonym of *V. parmigerella*
B. baldensis (A.Massal.) Vězda	as ***V. baldensis*** A.Massal.	as ***P. baldensis*** (A.Massal.) Servít***	***B. baldensis*** [synonyms: *Protobaglietto exesa*, *P. kutakiana*, *Verrucaria parmigera* f. *subconcentrica* J.Steiner]	as ***V. baldensis*** [synonyms: *Protobaglietto exesa*, *P. kutakiana*, *Verrucaria subconcentrica*]	***B. baldensis*** [synonyms: *Amphoridium baldense* (A.Massal.) A.Massal., *A. uberrimum* (A.Massal.)?, *Protobaglietto exesa*, *Verrucaria subconcentrica*]	as ***V. baldensis*** [synonyms: *Limboria sphinctrina* “Duf.”, *Protobaglietto lactea*, *Verrucaria bosniaca*, *V. calciseda* f. *alocyza* Arnold, *V. dalmatica* Servít, *V. grummannii*, *V. gyelnikii*, *V. parmigera*, *V. serbica* Servít, *V. steineri*, *V. subconcentrica*, *V. suzaeana*]
B. calciseda (DC.) Gueidan & Cl.Roux	—	—	—	as ***V. calciseda*** DC. [synonyms: *Protobaglietto inaequata*, *Verrucaria calciseda* var. *lactea*, *V. gyelnikii*, *V. parmigera*, *V. subrosea*]	as a synonym of *B. parmigera*	—
B. cazzae (Zahlbr.) Vězda & Poelt	as ***V. cazzae*** Zahlbr.	as ***P. cazzae*** (Zahlbr.) Servít	***B. cazzae*** [synonyms: *Protobaglietto alocyza*, *Verrucaria subrosea* Servít]	as ***V. cazzae***	***B. cazzae*** [synonyms: *Protobaglietto alocyza*, *Verrucaria subrosea*]	as a synonym of *V. marmorea*
B. ceracea (J.Steiner) Jatta	as a synonym of *V. sphinctrina* var. *limborioides* (A.Massal.) Servít	—	as a synonym of *B. sphinctrina* “(Ach.) Körb.”**	as a synonym of *V. limborioides* (A.Massal.) Clauzade & Cl.Roux	as a synonym of *B. limborioides*	as a synonym of *V. limborioides*
B. inaequata (Servít) Gams***	as ***V. inaequata*** (Servít) Servít	—	as a synonym of *B. parmigera*	as a synonym of *V. calciseda*	as a synonym of *B. parmigera*	as a synonym of *V. parmigerella*
B. limborioides A.Massal.	as a synonym of *V. sphinctrina* var. *limborioides*	—	as a synonym of *B. sphinctrina* “(Ach.) Körb.”**	as ***V. limborioides*** [synonyms: *Verrucaria bosniaca*, *V. ceracea*, *V. grummannii*, *V. sphinctrina* auct. non Ach., *V. suzaeana*]	***B. limborioides*** [synonyms: *Baglietto sphinctrina* auct. non (Ach.) Körb., *Verrucaria bosniaca*, *V. ceracea*, *V. grummannii*, *V. sphinctrina* auct. non Ach., *V. suzaeana*]	as ***V. limborioides*** [synonyms: *Verrucaria ceracea*, *V. quarnerica*]

Table 1. Continued.

Species previously treated as *Bagliettoa* or *Protobagliettoa**	Servít (1939)	Servít (1955)	Poelt & Vězda (1981)	Clauzade & Roux (1985)	Nimis (1993)	Halda (2003)
B. marmorea (Scop.) Gueidan & Cl.Roux	—	—	—	as ***V. marmorea*** (Scop.) Arnold [synonyms: *Verrucaria purpurascens* Hoffm., *Urceolaria wulfenii* Ach.]	as ***V. marmorea*** [synonyms: *Verrucaria calciseda* var. *decipiens* Trevisan, *V. purpurascens*]	as ***V. marmorea*** [synonym: *V. cazzae*]
B. operculata (P.M.McCarthy) P.M.McCarthy	—	—	—	—	—	—
B. parmigera (J.Steiner) Vězda & Poelt	as ***V. parmigera*** J.Steiner	as ***P. parmigera*** (J.Steiner) Servít	***B. parmigera*** [synonyms: *Protobagliettoa inaequata* (Servít) Servít***, *P. lactea*, *P. obscurata*, *Verrucaria gyelnikii*]	as a synonym of *V. calciseda*	***B. parmigera*** [synonyms: *Protobagliettoa inaequata*, *P. obscurata*, *Verrucaria caesiella* Servít, *V. calciseda*, *V. calciseda* f. *caesia* Anzi, *V. calciseda* var. *calcivora* A.Massal., *V. calciseda* var. *lactea*, *V. crassa* var. *lactea* f. *croatica* Servít, *V. crassa* var. *lactea* f. *geographica* Servít, *V. lactea* (Arnold) Servít, *V. saxivora* Servít]	as a synonym of *V. baldensis*
B. parmigerella (Zahlbr.) Vězda & Poelt	as a synonym of *V. sphinctrinella* var. *parmigerella* (Zahlbr.) Servít	as ***P. parmigerella*** (Zahlbr.) Servít	***B. parmigerella*** [synonyms: *Protobagliettoa erumpens*, *Verrucaria calciseda* f. *bagliettoiformis* Hazsl., *V. sphinctrinella*]	as ***V. parmigerella*** Zahlbr. [synonyms: *Verrucaria pinguis* J.Steiner, *V. sphinctrinella*]	***B. parmigerella*** [synonyms: *Protobagliettoa erumpens*, *P. bagliettoiformis*, *Verrucaria harrimannii* sensu Anzi, *V. pinguis*, *V. sphinctrinella*]	as ***V. parmigerella*** [synonyms: *Protobagliettoa kutakiana*, *Verrucaria bagliettoiformis* var. *erumpens*, *V. calciseda* f. *bagliettoiformis*, *V. inaequata*, *V. sphinctrinella*]
B. quarnerica (Zahlbr.) Vězda	as ***V. quarnerica*** Zahlbr.	—	***B. quarnerica***	as ***V. quarnerica***	—	as a synonym of *V. limborioides*
B. sphinctrina auct. non (Ach.) Körb.**	as ***V. sphinctrina*** auct.	***B. sphinctrina*** auct.	***B. sphrinctrina*** auct. [synonyms: *Bagliettoa limborioides*, *Verrucaria bosniaca* Servít, *V. ceracea* J.Steiner, *V. grummannii*, *V. suzaeana* Servít]	*V. sphinctrina* auct. non Ach. as a synonym of *V. limborioides*	*B. sphinctrina* auct. non (Ach.) Körb. as a synonym of *B. limborioides*	*B. sphinctrina* "(Duf.) Körb." as a synonym of *V. baldensis*

B. sphinctrinella (Zsch.) Gams***	as ***V. sphinctrinella*** Zsch.	—	as a synonym of *B. parmigerella*	as a synonym of *V. parmigerella*	as a synonym of *B. parmigerella*	as a synonym of *V. parmigerella*
B. steineri (Kušan) Vězda	as ***V. steineri*** Kušan	as ***P. steineri*** (Kušan) Servít***	***B. steineri***	as ***V. steineri***	***B. steineri***	as a synonym of *V. baldensis*
B. subconcentrica (J.Steiner) Gams***	as ***V. subconcentrica*** J.Steiner (Servít)	as ***P. subconcentrica*** (Servít) Servít	as a synonym of *B. baldensis*	as a synonym of *V. baldensis*	as a synonym of *B. baldensis*	as a synonym of *V. baldensis*
P. alocyza (Arnold) Servít	as *V. parmigera* var. *alocyza* (Arnold) J.Steiner	***P. alocyza*** (Arnold) Servít	as a synonym of *B. cazzae*	—	as a synonym of *B. cazzae*	as a synonym of *V. baldensis*
P. erumpens (Servít) Servít	as *V. baglietoiformis* var. *erumpens* Servít	***P. erumpens***	as a synonym of *B. parmigerella*	—	as a synonym of *B. parmigerella*	as a synonym of *V. parmigerella* Zahlbr.
P. exesa (Servít) Servít	as *V. subconcentrica* var. *subconcentrica* f. *exesa* Servít	***P. exesa***	as a synonym of *B. baldensis*	as a synonym of *V. baldensis*	as a synonym of *B. baldensis*	as a synonym of *V. baldensis*
P. graeca (Servít) Servít	as *V. bagliettoiformis* var. *graeca* Servít [synonym: *V. cazzae* var. *graeca* Servít]	***P. graeca***	as a synonym of *B. cazzae*	—	—	as a synonym of *V. baldensis*
P. grummannii (Servít) Servít	***V. grummannii*** Servít	—	as a synonym of *B. sphinctrina* “(Ach.) Körb.”**	as a synonym of *V. limborioides*	as a synonym of *B. limborioides*	as a synonym of *V. baldensis*
P. gyelnikii (Servít) Servít ex J.Nowak & Tobol.	as ***V. gyelnikii*** Servít	***P. gyelnikii*** (Servít) Servít ***	as a synonym of *B. parmigera*	as a synonym of *V. calciseda*	—	as a synonym of *V. baldensis*
P. kutakiana Servít	—	***P. kutakiana***	as a synonym of *B. baldensis*	as a synonym of *V. baldensis*	—	as a synonym of *V. parmigerella*
P. lactea (Arnold) Servít	as *V. steineri* var. *lactea* (Arnold) Servít [synonym: *V. calciseda* var. *lactea* Arnold]	***P. lactea***	as a synonym of *B. parmigera*	as a synonym of *V. calciseda*	as a synonym of *B. parmigera*	as a synonym of *V. baldensis*
P. obscurata (Servít) Servít	as *V. gyelnikii* var. *gyelnikii* f. *obscurata* Servít	***P. obscurata***	as a synonym of *B. parmigera*	—	as a synonym of *B. parmigera*	as a synonym of *V. baldensis*

In each treatment, accepted taxa are shown in bold and synonyms are listed. A dash indicates taxa not included in the treatment.

* *Bagliettoa ocellata* C.Knight corresponds to *Pertusaria erumpescens* Nyl. (Zahlbruckner, 1928).

** The type specimen of *V. sphinctrina* Ach. (H-ACH-709) is in fact a tropical epiphytic pyrenocarpous fungus most likely from the Dothideales (Halda, 2003).

*** Non validly published new combinations.

(PRM 757645, PRM 757738, W 1913-4273, W 1913-14275) and *Verrucaria rubrocincta* Breuss (LI 364320). Type specimens from VER (*B. baldensis*, *B. limborioides*) were not available for study and the type specimen of *B. steineri* could not be located.

Species identification. — Morphological and anatomical characters were studied using a stereomicroscope and a Zeiss Axioskop light microscope. For routine identification, the star-shaped aperture of the involucrellum was observed under a stereomicroscope with oblique incident light. The presence of a shield-like involucrellum was also confirmed by exerting pressure on its side with the corner of a razor blade until it detaches. The width of the involucrellum and the color of the excipulum were assessed under the stereomicroscope by transversally cutting a perithecium in half on the substrate and pushing one half to one side to reveal the other half. Ascospores were observed using a squash mount of a perithecium extracted from the substrate with the corner of a razor blade. For more detailed observations of the perithecial characters (e.g., ostiole, excipulum), cross-sections were prepared by hand from a perithecium embedded in or extracted from the substrate and mounted in water. Photographs of specimens were taken in the Sackler Biodiversity Imaging Laboratory at the Natural History Museum using a Zeiss Stemi SV11 stereomicroscope coupled with a Canon EOS imaging system. For a better depth of field, images were stacked using the software Helicon Focus (Helicon Soft, Kharkov, Ukraine).

Molecular data. — Lichen material including thallus and perithecia was scraped from the substrate using a clean single-edged razor blade under a stereomicroscope. The resulting powder, which was a mix of fungal and algal material and calcareous substrate was then recovered on a clean piece of weighing paper and transferred to a sterile 0.5 ml Eppendorf tube. After addition of 150 µl of extraction buffer (2% sodium dodecyl sulfate), a sterile pestle was used to further homogenize the suspension. Genomic DNA was obtained using phenol-chloroform extraction (Zolan & Pukkila, 1986) as modified by Gueidan & al. (2007). DNA concentration and quality were estimated using gel electrophoresis. Based on band intensity, the appropriate

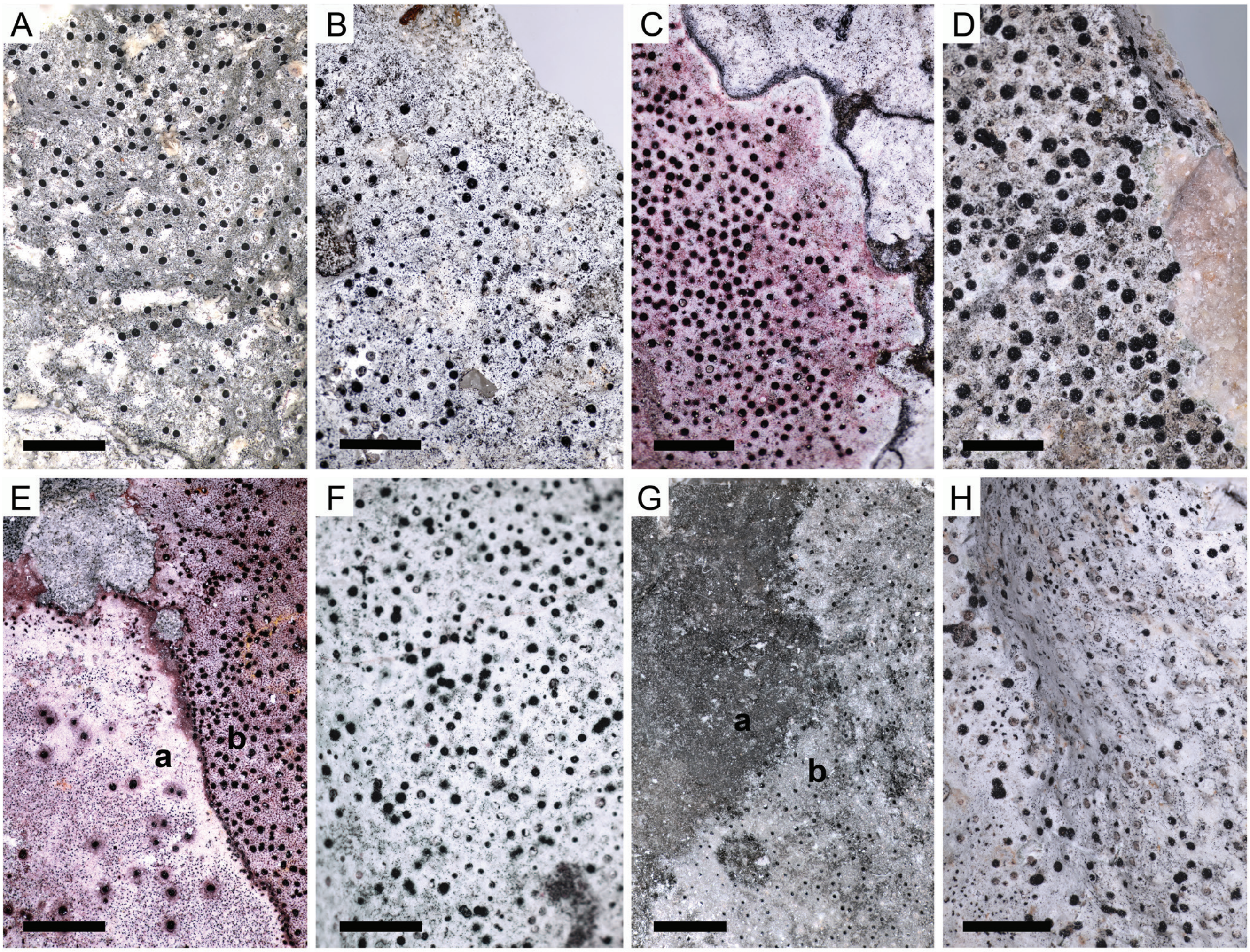


Fig. 1. Habits of the most common species of *Baglietta*. **A,** *Baglietta baldensis* (CG1952); **B,** *B. calciseda* (CG1953); **C,** *B. cazzae* (CG1531); **D,** *B. limborioides* (CG1749); **E,** *B. marmorea* (CG1526a on the bottom left corner and CG1526b on the top right corner, as indicated on the photograph); **F,** *B.* cf. *suzaeana* (CG1846); **G,** *B. parmigerella* (CG1754a on the top left corner and CG1754b on the bottom right corner, as indicated on the photograph); **H,** *B.* cf. *suzaeana* (CG2084). — Scale bars = 2 mm. Photos: J. Yuzon.

DNA dilution (1/10 or 1/100) was used for DNA amplification. One microliter of DNA dilution was added to the following PCR mix: 1.5 µl PCR buffer 10× NH_4 (Bioline, London, U.K.), 1.5 µl of $MgCl_2$ (50 mM), 0.5 µl dNTP (100 mM), 1–2 µl primers depending on the degree of degeneracy (10 µM), 0.5 µl DNA polymerase Bioline BioTaq (5 U $µl^{-1}$), and 17 µl water to a total volume of 25 µl. DNA amplification was run on a Techne TC-4000 PCR machine (Bibby Scientific, Stone, U.K.). Three markers were amplified: the intergenic transcribed spacers 1 and 2 and the 5.8S subunit of the nuclear ribosomal RNA gene (ITS), the minichromosome maintenance complex component 7 (*mcm7*) and the small subunit of the mitochondrial ribosomal RNA gene (mtSSU). All primers and PCR programs are described in Table 2. Cloning was carried out for PCR products with multiple bands and PCR products that failed sequencing using a TOPO-TA cloning kit according to the manufacturer's instructions (Invitrogen, Carlsbad, California, U.S.A.). PCR products were cleaned and sequenced at the Natural History Museum DNA Sequencing Facility in London using PCR Cleanup Filter Plates (Millipore, Billerica, Massachusetts, U.S.A.), BigDye Chemistry and an ABI 3730xl sequencing machine (Applied Biosystems, Carlsbad, California, U.S.A.).

Alignments and phylogenetic analyses. — A total of 169 new DNA sequences were obtained for this study (Appendix 1). Nine other sequences were obtained from GenBank: six published in Gueidan & al. (2008), two in Favero-Longo & al. (2009) and one unpublished sequence (FJ645261, obtained from the specimen *A. Orange 16333* kept at NMW). New sequences were edited using Sequencher v.4.8 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.) and manual alignments of all sequences were done using MacClade v.4.08 (Maddison & Maddison, 2003). Ambiguous regions were delimited as described in Lutzoni & al. (2000) and, together with the introns, were excluded from the alignments. Two datasets were assembled. The first dataset (dataset 1) aimed at comparing the variability of each gene within *Baglietto*a by comparing both branch lengths and the number of well-supported nodes obtained with each marker. These datasets included the 31 *Baglietto*a specimens for which the three gene regions were available. Each gene region was analyzed separately using maximum likelihood with RAxML VI-HPC v.7.4.4 (Stamatakis & al., 2005, 2008), as implemented on the Cipres Web Portal (http://www.phylo.org; Miller & al., 2010). The markers ITS and mtSSU had no partitions, but *mcm7* was analysed using the three codon positions as three partitions. A GTRCAT model was applied to all markers and partitions. Support values were obtained using a fast bootstrap analysis of 1000 pseudoreplicates.

The second dataset (dataset 2) combined all sequence data obtained for ITS, *mcm7* and mtSSU in order to test for species delimitation within *Baglietto*a. This dataset included a total of 82 specimens including seven outgroup taxa (Appendix 1). First, the congruence between the three markers was tested using a 70% reciprocal bootstrap criterion (Mason-Gamer & Kellogg, 1996) as following: the three matrices were analysed separately using 1000 rapid bootstrap pseudoreplicates and a GTRCAT model of molecular evolution with RAxML (partitions as previously described). After comparing the three resulting trees, detected conflicts resulting from contaminating sequences were eliminated by pruning these sequences. The three datasets were then combined. For the combined analysis, phylogenetic relationships were investigated using a Bayesian approach with MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003), as implemented on the Cipres Web Portal. Models of molecular evolution were estimated for ITS, mtSSU and each codon position of *mcm7* using the Akaike information criterion as implemented in Modeltest v.3.7 (Posada & Crandall, 1998): the GTR+I+G model was selected for ITS and mtSSU, the TIM+I model (Nst = 6, rates = equal, Pinvar > 0) for the first and the second codon positions of *mcm7*, and the TVM+G model (Nst = 6, rates = gamma, Pinvar = 0) for the third codon position of *mcm7*. Two analyses of four chains were run for 5 million generations and trees were sampled every 500 generations. All runs converged on the same average likelihood score and topology. A burn-in sample of 5000 trees was discarded for each run. The remaining 10,000 trees were used to estimate the posterior probabilities with the "compute consensus" command in PAUP* (Swofford, 2002). The most likely tree was computed with the sumt command in MrBayes and visualized in PAUP*. Additional support values were obtained using a maximum likelihood (ML) approach with RAxML. The GTRCAT model was applied to all partitions (ITS, mtSSU and each codon position of *mcm7*). Support values were obtained using a fast bootstrap analysis of 1000 pseudoreplicates. Both datasets were deposited in TreeBase (ID16208).

Table 2. Primers and PCR conditions used for the amplification and sequencing of the three phylogenetic markers ITS, *mcm7* and mtSSU.

Gene region	Primer name	Primer reference	Initial denaturation	Denaturation	Annealing	Elongation	Final elongation	Cycle number
ITS	ITS1F ITS4	Gardes & Bruns (1993) White & al. (1990)	94°C, 5 min	94°C, 1 min	53°C, 1 min	72°C, 2 min	72°C, 7 min	35
mcm7	Mcm7-709for Mcm7-1348rev	Schmitt & al. (2009) Schmitt & al. (2009)	94°C, 10 min	94°C, 45 s	56°C, 50 s	72°C, 1 min	72°C, 5 min	38
mtSSU	mtSSU1 mtSSU3R	Zoller & al. (1999) Zoller & al. (1999)	94°C, 3 min	94°C, 1 min	52°C, 1 min	72°C, 1.5 min	72°C, 7 min	35

RESULTS

Morphological delimitation between species of *Bagliettoa*. — Clear morphological differences were observed between eight species or species groups within *Bagliettoa* (Table 3). Additional work needs to be done to clarify delimitation within *B. parmigera* s.l., between *B. baldensis* and *B. operculata* (P.M.McCarthy) P.M.McCarthy, and between *B. marmorea* and *V. rubrocincta*.

Variability of phylogenetic markers. — The sequencing of three loci of the mycobiont was attempted for a total of 79 specimens of *Bagliettoa* and *Parabagliettoa*, and 75 sequences were recovered for ITS, 55 for *mcm7* and 40 for mtSSU (Appendix 1). A total of 31 specimens of *Bagliettoa* had sequences for all three markers, which, when aligned and after exclusion of introns and ambiguously aligned regions, yielded the data described in Table 4.

The most likely trees obtained with RAxML are presented at the same scale in Fig. 2. Among the three markers, *mcm7* showed the highest genetic variation (215 distinct alignment patterns, Table 4; longest branch lengths, Fig. 2) and provided 13 well-supported nodes (bootstrap support greater than 70%). Genetic variation within the ITS region was lower (143 distinct alignment patterns, Table 4) but the number of well-supported nodes (14) was higher for this marker than for *mcm7*. Finally, mtSSU showed the lowest genetic variation (90 distinct alignment patterns, Table 4; shortest branch lengths, Fig. 2) and provided low resolution and support to the phylogeny (only eight well-supported nodes). Moreover, the success rate of the amplification of this marker was rather low (only 40 sequences obtained from a total of 79 specimens of *Bagliettoa* and *Parabagliettoa*).

Molecular delimitation between species of *Bagliettoa*. — The congruence test detected one major conflict with the mtSSU sequence of *B. limborioides* CG1752, which clustered with *B. parmigera*. This sequence, most likely resulting from a lab contamination, was removed from the alignment. One other hard conflict was detected in the placement of *B. marmorea*

Table 3. Morphological and anatomical characters for the eight main species of *Bagliettoa*.

	B. cazzae (Zahlbr.) Vězda & Poelt	*B. marmorea* (Scop.) Gueidan & Cl.Roux[a]	*B. parmigerella* (Zahlbr.) Vězda & Poelt	*B. calciseda* (DC.) Gueidan & Cl.Roux	*B. baldensis* (A.Massal.) Vězda[b]	*B. limborioides* A.Massal.	*B. quarnerica* (Zahlbr.) Vězda	*B. parmigera* s.l.[c]
Thallus color	pale pink to purple-pink	purple pink to sometimes grey with purple pigment only in and around the perithecia	bluish to greenish grey, with a green pigment present in the upper cortex	white to light grey	white to light grey	white to light grey	white to light grey	white to light grey
Involucrellum	shield-shaped and with star-shaped aperture	absent	shield-shaped and with star-shaped aperture	absent	shield-shaped and with star-shaped aperture	shield-shaped and with star-shaped aperture, often prominent	shield-shaped and with star-shaped aperture	shield-shaped and with star-shaped aperture
	0.2 mm in diameter		0.1–0.2 mm in diameter		0.2–0.3 mm in diameter	0.3–0.4 mm in diameter	± 0.2 mm in diameter	0.2–0.3 mm in diameter
	diameter as large as or larger than the diameter of the excipulum		diameter smaller than the diameter of the excipulum		diameter smaller than the diameter of the excipulum	diameter as large as or larger than the diameter of the excipulum	diameter as large as or larger than the diameter of the excipulum	diameter as large as or larger than the diameter of the excipulum
Excipulum	colorless to pale brown	colorless but with the upper part darkened with purple pigments	colorless to brown	entirely or partly brown to black	colorless to pale brown at the bottom	colorless to brown	entirely or partly brown to black	entirely colorless to entirely or partly brown to black
	0.1–0.2 mm in diameter	0.2–0.3 mm in diameter	0.2–0.3 mm in diameter	0.2–0.3 mm in diameter	0.3–0.4 mm in diameter	0.3–0.35 mm in diameter	up to 0.16 mm in diameter	0.2–0.3 mm in diameter
Ascospores	not known	13–30 × 9–15 µm	15–25 × 7–12 µm, often absent or not well developed	13–25 × 7–12 µm	not known	17–35 × 10–13 µm, rarely one-septate	12–15 × 5–6 µm	13–26 × 7–15 µm

[a] including *B. rubrocincta* (Breuss) Gueidan & Cl.Roux from North-America.

[b] including *B. operculata* (P.M.McCarthy) P.M.McCarthy from Australia.

[c] including *B. parmigera* (J.Steiner) Vězda & Poelt, *B. steineri* (Kušan) Vězda and *B. suzaeana* (Servìt) Gueidan & Cl.Roux.

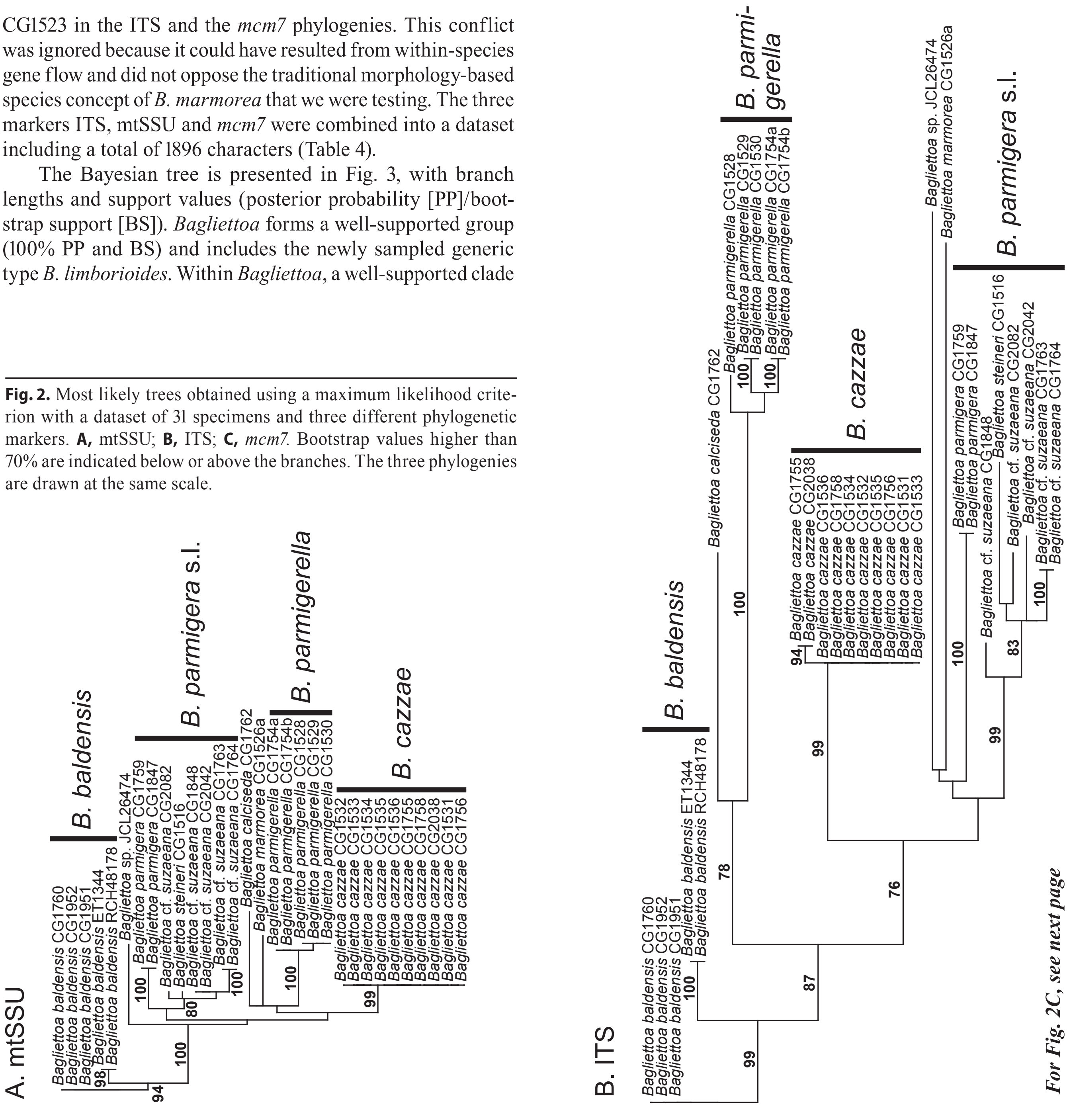
Table 4. Description of the informativeness and completeness of the two datasets used in this study.

Dataset	Speci-men	Dataset description	ITS	*mcm7*	mtSSU	3-gene combined
		Total number of included characters	599	614	768	–
Dataset 1	31	Number of distinct alignment patterns	143	215	90	–
		Percentage of gaps and completely undeterminated characters	6.52	0.42	2.26	–
		Total number of included characters in the combined dataset	526	614	756	1896
Dataset 2	82	Total number of distinct alignment patterns in the combined dataset	214	284	125	623
		Percentage of gaps and completely undeterminated characters in the combined dataset	11.88	12.25	44.96	32.24

CG1523 in the ITS and the *mcm7* phylogenies. This conflict was ignored because it could have resulted from within-species gene flow and did not oppose the traditional morphology-based species concept of *B. marmorea* that we were testing. The three markers ITS, mtSSU and *mcm7* were combined into a dataset including a total of 1896 characters (Table 4).

The Bayesian tree is presented in Fig. 3, with branch lengths and support values (posterior probability [PP]/bootstrap support [BS]). *Baglilettoa* forms a well-supported group (100% PP and BS) and includes the newly sampled generic type *B. limborioides*. Within *Baglilettoa*, a well-supported clade

Fig. 2. Most likely trees obtained using a maximum likelihood criterion with a dataset of 31 specimens and three different phylogenetic markers. **A,** mtSSU; **B,** ITS; **C,** *mcm7*. Bootstrap values higher than 70% are indicated below or above the branches. The three phylogenies are drawn at the same scale.



For Fig. 2C, see next page

For Fig. 2A–B, see previous page

(97% PP and 93% BS) includes *B. parmigera* and *B. steineri*, as well as a group of specimens with intermediate excipulum characters (referred to as *Bagliettoa* cf. *suzaeana* (Servìt) Gueidan & Cl.Roux). All other sampled species (*B. baldensis*, *B. calciseda*, *B. cazzae*, *B. limborioides*, *B. marmorea*, *B. parmigerella*) are found together in a well-supported group (98% PP and 83% BS), and each of these species is well supported as monophyletic. High genetic variation is found within *B. marmorea*, with the two North American specimens on a long branch and sister to the rest of the specimens. A North American specimen morphologically close to *B. limborioides* (JCL26474) but with a smaller involucrellum (0.2–0.3 mm in diameter) is sister to *B. limborioides*. Two specimens of *B. baldensis* (SFL607, SFL608b) for which sequences were obtained from Favero-Longo & al. (2009) are nested within *B. parmigerella* and were most likely originally misidentified. The original specimens used to obtain cultures and sequences were not available for study.

■ DISCUSSION

Bagliettoa is commonly found on limestone in many parts of the world. Although an important component of the microbial flora of karstic systems and monument, these species have generally been overlooked due to their confusing taxonomy and the lack of efficient tools for their identification (Tretiach & Geletti, 1997). In contrast to the most recent revision of this group (Halda, 2003), our molecular and morphological study supports the recognition of twelve species and provides the basis for the re-delimitation of species in *Bagliettoa*.

Finding phylogenetic markers for species delimitation in *Bagliettoa*. — Among the six genes recently investigated as potential barcodes for fungi (nuLSU, nuSSU, ITS, *RPB1*, *RPB2*, *mcm7*), ITS had the highest probability of successful identification for the broadest range of fungi and was ultimately chosen as the universal barcode for fungi (Schoch & al., 2012). In this study by Schoch & al. (2012), protein-coding genes such as *mcm7* had a greater resolving power than ITS, but could not be selected as a universal barcode because of the high incidence of amplification and sequencing failure. First detected as a potentially efficient single-copy

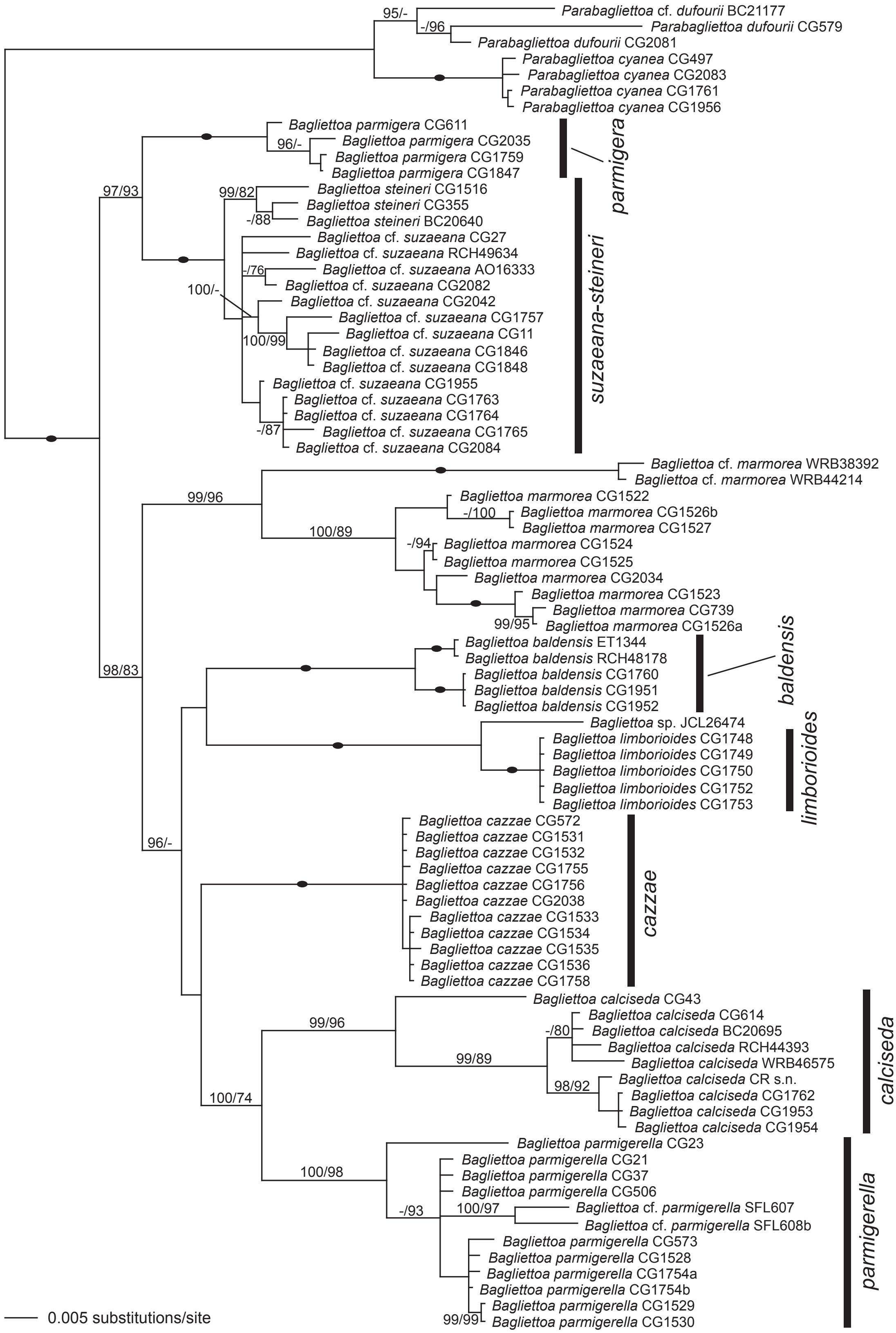


Fig. 3. Most likely tree obtained using a Bayesian approach with a combined dataset of 82 specimens and three markers (ITS, mtSSU, *mcm7*). Support values (posterior probability [PP]/bootstrap support [BS]) are shown above or below the branches. Nodes with strong support (100% PP and BS) are indicated by a black dot on the branches. Only significant values (higher than 95% PP and higher than 70% BS) are shown on the figure. *Parabagliettoa* was used as outgroup. The delimitation of sampled species of *Bagliettoa* are shown on the right.

marker for fungal phylogenies (Aguileta & al., 2008), *mcm7* was developed as a marker for genus- to class-level taxonomy in fungi (Schmitt & al., 2009; Raja & al., 2011). It was also found to be informative at the species level in various groups of lichenized fungi (e.g., Leavitt & al., 2011, 2012; Spribille & al., 2011; Sadowska-Deś & al., 2013), but so far has not been used in Verrucariales. In *Bagliettoa*, both the protein-coding marker *mcm7* and ITS showed significant genetic variation and provided good resolution and support for most species in the phylogeny. Sequencing of *mcm7* did, however, often require the use of cloning, but more genus-specific primers could be designed to avoid this problem. As for the mitochondrial marker mtSSU, it was both the least informative marker at the species level and the most difficult to amplify. Many mtSSU sequences obtained were obvious contaminants (e.g., Dothideomycetes or Chaetothyriales) and, despite cloning, the mycobiont's sequences were sometimes not recovered. Used in combination, ITS and *mcm7* form an excellent tool for the molecular identification of species of *Bagliettoa*.

Genus and species delimitations. — Although not accepted in revisions and recent Floras (e.g., Halda, 2003; Smith & al., 2009), *Bagliettoa* is a well-characterized genus that was recognized before molecular data were available for this group (Poelt & Vězda, 1981). Molecular data confirmed that most species of *Bagliettoa* formed a single lineage (Gueidan & al., 2007, 2009) and the present study confirms the placement of the type species *B. limborioides* within this lineage. The number of species recognized within *Bagliettoa* has also been controversial, with British authors using a broad concept for *B. baldensis* (Purvis & al., 1992, Smith & al., 2009). The most recent revision of the group introduced further synonyms and recognized only four species (Halda, 2003). In contrast, our morphological and molecular results strongly suggest that *B. baldensis*, *B. calciseda*, *B. cazzae*, *B. limborioides*, *B. marmorea* and *B. parmigerella* are well delineated species (Table 4). Delimitation problems exist within *Bagliettoa parmigera* s.l., a species complex including *B. parmigera*, *B. steineri* and *B. suzaeana*. Although the placement of *Verrucaria suzaeana* Servìt within *Bagliettoa* is confirmed, the morphological and molecular delimitation between *B. steineri* and *B. suzaeana* will require further study with a larger gene and specimen sampling. Within *B. marmorea*, an additional species might be found in North America, as the two specimens from the eastern U.S.A. are genetically divergent from the European specimens (the two groups differ in 15 ITS positions). This additional species might in fact correspond to *Verrucaria rubrocincta*, a species described from Arizona (Breuss, 2000) and here transferred to *Bagliettoa*. This species was considered to be distinct due to the presence of an involucrellum in the type material. Our study of the type at LI revealed that an involucrellum is in fact absent. The upper part of the excipulum is however carbonized and slightly thickened, and might have been confused with an involucrellum. Additional molecular data are needed to determine whether *B. rubrocincta* (Breuss) Gueidan & Cl.Roux is a later synonym of *B. marmorea* or if all North American material corresponds to *B. rubrocincta*.

TAXONOMY

The twelve species here accepted in *Bagliettoa* are listed below. Two new combinations are effected.

1. ***Bagliettoa baldensis*** (A.Massal.) Vězda in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria baldensis* A.Massal., Ric. Auton. Lich. Crost.: 173. 1852 – Lectotype (designated by Halda in Acta Mus. Richnov., Sect. Nat. 10: 24. 2003): [ITALY]. Mt. Baldo (Mad. Corona), *A. Massalongo s.n.* (VER n.v.).

2. ***Bagliettoa calciseda*** (DC.) Gueidan & Cl.Roux in Bull. Soc. Linn. Provence 58: 187. 2007 ≡ *Verrucaria calciseda* DC. in Lamarck & Candolle, Fl. Franç., ed. 3, 2: 317. 1805 – Neotype (designated by Gueidan & Roux in Bull. Soc. Linn. Provence 58: 183. 2007): [GERMANY, Franconian Jura]. An Kalkfelsen gegenüber Kunstein bei Eichstätt, 3 Jun 1866, *Arnold 311* (ZT!).

3. ***Bagliettoa cazzae*** (Zahlbr.) Vězda & Poelt in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria cazzae* Zahlbr. in Ann. Mycol. 12: 335. 1914 – Lectotype (designated by Halda in Acta Mus. Richnov., Sect. Nat. 10: 116. 2003): [CROATIA]. Insel Cazza, Felsen des Dadiu, 27–29 May 1911, *J. Brunnthaler & A. Ginzberger s.n.* (W No. 1912-4574!).

4. ***Bagliettoa limborioides*** A.Massal., Memor. Lichenogr.: 147. 1853 ≡ *Thrombium limborioides* (A.Massal.) Zschacke in Rabenh. Krypt.-Fl., ed. 2, 9(1,1): 555. 1934 ≡ *Verrucaria limborioides* (A.Massal.) Clauzade & Cl.Roux in Bull. Soc. Bot. Centre-Ouest, n.s., 7: 830. 1985 – Holotype: [ITALY], vive sulle roccie calcaree nel Bosco Bagato presso Genova, *F. Bagliettoa s.n.* (VER n.v.).

5. ***Bagliettoa marmorea*** (Scop.) Gueidan & Cl.Roux in Mycol. Res. 111: 1157. 2007 ≡ *Lichen marmoreus* Scop., Fl. Carniol., ed. 2, 2: 367. 1772 – Neotype (designated by Halda in Acta Mus. Richnov., Sect. Nat. 10: 113. 2003): [SLOVENIA], montes Julijske Alpe, in declivibus vallis "dolina Triglavskih jezer" supra Veliko jezero, secus viam, 1830 m, 23 Jun 2000, *J. Halda & Š. Haldová s.n.* (PRM No. 900619!).

6. ***Bagliettoa operculata*** (P.M.McCarthy) P.M.McCarthy in Australas. Lichenol. 63: 18. 2008 ≡ *Verrucaria operculata* P.M.McCarthy in Muelleria 7: 324. 1991 – Holotype: South Australia, Eyre Peninsula, 17 km S of Cowell, by the Lincoln Highway, on limestone, 23 Oct 1970, *R.B. Filson 11794* (MEL n.v.).

   Note. – This species is morphologically very similar to *B. baldensis*. Molecular data are needed to test whether these two species are synonymous. *Bagliettoa baldensis* has also been reported from Australia (McCarthy, 2008), but these reports were based on the broad concept used for this species in Purvis & al. (1992) and Smith & al. (2009).

7. ***Bagliettoa parmigera*** (J.Steiner) Vězda & Poelt in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria parmigera* J.Steiner in Verh. Zool.-Bot. Ges. Wien 61: 34. 1911 – Lectotype

(designated by Halda in Acta Mus. Richnov., Sect. Nat. 10: 51. 2003): [BELGIUM], Spa, *W. Nylander s.n.* (PRM No. 758127!).

Note. – This species belongs to *B. parmigera* s.l., a species complex in need of revision. It is characterized by a brown to entirely black excipulum and densely and regularly distributed perithecia (see note on *B. suzaeana*).

8. ***Bagliettoa parmigerella*** (Zahlbr.) Vězda & Poelt in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria parmigerella* Zahlbr. in Oesterr. Bot. Z. 68: 64. 1919 – **Lectotype (designated here):** [CROATIA], Dalmatien, Ost-Meleda, beim Dorfe Prožura, ca. 200 m, an Kalkgestein, 14 Mar 1910, *J. Baumgartner s.n.* (W No. 1913-4273!).

Note. – In the original description of *V. parmigerella*, Zahlbruckner (1919) mentioned two specimens as type material. Although one of these two specimens (W-1913-4273) was later labeled as the holotype by Servít, the lectotypification is not valid because it was never published. In Halda (2003), the other specimen (W-1913-14275) is mentioned as the holotype, but no reference was done to a lectotypification. His citation of the specimen as the holotype cannot be corrected to lectotype as stated in Art 9.9 of the *International Code of Nomenclature for algae, fungi and plants* (McNeill & al., 2012) because the term "designated here" was not used as required by Art 7.10 for publications after 2001. Here, we are officially designating the specimen W No. 1913-4273 as lectotype.

9. ***Bagliettoa quarnerica*** (Zahlbr.) Vězda in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria quarnerica* Zahlbr. in Oesterr. Bot. Z. 68: 63. 1919 – Holotype: [CROATIA], Istria, Ins. Lošinj (Lusin), ad pedem montis Osor (Ossero), in vicinitate oppidi Osor (Ossero), secus viam ad saxa calcarea, 1914, *J. Baumgartner s.n.* (W No. 1916-3421 n.v.).

10. ***Bagliettoa rubrocincta*** (Breuss) Gueidan & Cl.Roux, **comb. nov.** [MycoBank no.: MB 809809] ≡ *Verrucaria rubrocincta* Breuss in Bryologist 103: 707. 2001 – Holotype: U.S.A., Arizona, Gila County, Tonto National Forest, N of Payson, Houston Mesa Road, Whispering Pines, slope of hill with *Pinus ponderosa*, sandstone and limestone, 11 Jul 1997, *Breuss 13.176* (LI!).

Note. – This species is morphologically similar to *B. marmorea*, a species that has been reported from the Ozark Highlands of eastern North America (Harris & Ladd, 2005). However, the high genetic variation among the material sequenced here suggests that the specimens from the eastern U.S.A. could correspond to separate species. Additional sampling is needed to test if the material of *B. rubrocincta* from Arizona is related to, or conspecific with, that from the eastern U.S.A.

11. ***Bagliettoa steineri*** (Kušan) Vězda in Biblioth. Lichenol. 16: 363. 1981 ≡ *Verrucaria steineri* Kušan in Acta Bot. Inst. Bot. Univ. Zagreb 5: 28. 1930 ≡ *Protobagliettoa steineri* (Kušan) Servít ex J.Nowak & Tobol., Porosty Polskie: 1119. 1975 – Type: [CROATIA], Ins. Krk, Malinska, *F. Kušan s.n.* (not found).

Note. – This species belongs to *B. parmigera* s.l., a species complex in need of revision. It is characterized by an entirely colorless excipulum that becomes dark only when the perithecium is dead.

12. ***Bagliettoa suzaeana*** (Servít) Gueidan & Cl.Roux, **comb. nov.** [MycoBank no.: MB 809810] ≡ *Verrucaria suzaeana* Servít in Beih. Bot. Centralbl., Abt. B 59: 150. 1939 – Holotype: [SLOVAKIA], Slovenský Ráj, Ztratená ad flum. Hnilec, 900 m, *J. Suza s.n.* (PRM No. 757903 n.v.).

Note. – This species belongs to *B. parmigera* s.l., a species complex in need of revision. It has an excipulum colorless to slightly pigmented (but never entirely black) and differs from *B. parmigera* s.str. by having less densely and more irregularly distributed perithecia (Servít, 1939: 116).

Identification key

1\. Thallus light pink to purple-pink, pigment K+ dark blue-green (test on the thallus) 2

1\. Thallus not light pink to purple-pink 3

2\. Perithecium with a shield-shaped involucrellum opening by a star-shaped aperture .... ***B. cazzae***

2\. Perithecia without a shield-shaped involucrellum .... ***B. marmorea*** (including *B. rubrocincta*)

3\. Thallus bluish or greenish grey; green pigment present in the upper cortex, visible on a cross section of the thallus observed with a compound microscope .... ***B. parmigerella***

3\. Thallus light grey, white or whitish; green pigment present or not in the upper cortex 4

4\. Perithecia without a shield-shaped involucrellum. Thallus often radially fissured and prominent around the perithecia .... ***B. calciseda***

4\. Perithecia with a shield-shaped involucrellum opening by a star-shaped aperture 5

5\. Involucrellum narrower than the excipulum 6

5\. Involucrellum at least as large as or larger than the excipulum 7

6\. Thallus light grey, but with green pigment in the upper cortex .... ***B. parmigerella***

6\. Thallus white or slightly greenish (color due to the algal layer) but without green pigment in the upper cortex .... ***B. baldensis*** (including *B. operculata*)

7\. Involucrellum protruding and large (0.3–0.4 mm diameter) .... ***B. limborioides***

7\. Involucrellum not or only slightly protruding, small (0.15–0.3 mm diameter) 8

8\. Excipulum always entirely colorless (except when dead) .... ***B. steineri***

8\. Excipulum entirely or partly brown to black 9

9\. Excipulum 0.1–0.2 mm, ascospores 12–15 × 5–6 µm .... ***B. quarnerica*** (not studied)

9\. Excipulum 0.2–0.3 mm, ascospores 13–25 × 7–12 µm. .... 10 (*B. parmigera* s.l.)

10\. Perithecia (involucrellum 0.2–0.25 mm) densely and regularly distributed .... ***B. parmigera*** s.str.

10. Perithecia (involucrellum 0.25–0.3 mm) less densely and irregularly distributed ………. ***B. suzaeana**** (including *V. bosniaca* Servít with larger oil cells and better-developed ascospores).

* Because of uncertainties concerning the distinction between *B. suzaeana*, *B. parmigera* s.str. and other species related to *B. parmigera* studied by Servít (1939: 116), we are using the name *B.* cf. *suzaeana* for our specimens.

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Appendix 1. Specimen voucher information and GenBank accession numbers. Missing data are indicated by dashes and newly published sequences are highlighted in bold.

Species name, locality, collector, collection number (herbarium), ITS, *mcm7*, mtSSU

OUTGROUP: *Parabagliettoa cyanea* (A.Massal.) Gueidan & Cl.Roux, Le Planas, Vallon-Pont-d'Arc, Ardèche, France, *Gueidan 497* (DUKE), **KM371422,** –, –; ***Parabagliettoa cyanea*,** combe de Vaumale, Lioux, Vaucluse, France, *Gueidan 1761* (BM), **KM371423, KM371497, KM371552; *Parabagliettoa cyanea*,** combe de Vaumale, Lioux, Vaucluse, France, *Gueidan 1956* (BM), –, **KM371498,** –; ***Parabagliettoa cyanea*,** combe de Vaumale, Lioux, Vaucluse, France, *Gueidan 2083* (BM), **KM371424, KM371499,** –; ***Parabagliettoa dufourii*** (DC.) Gueidan & Cl.Roux, massif de la Ste-Baume, Plan-d'Aups-Ste-Baume, Var, France, *Gueidan 579* (DUKE), **KM371425,** –, FJ225684; ***Parabagliettoa dufourii*,** Brecon Beacons National Park, Carmarthenshire, Wales, U.K., *Gueidan 2081* (BM), –, **KM371500, KM371553; *Parabagliettoa*** cf. ***dufourii*,** Rassal wood, Wester Ross, North West Highlands, Scotland, U.K., *Coppins 21177* (E), **KM371426, KM371501, KM371554. — INGROUP: *Bagliettoa baldensis*** (A.Massal.) Vězda, Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1760* (BM), **KM371427, KM371502, KM371555; *Bagliettoa baldensis*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1951* (BM), **KM371428, KM371503, KM371556; *Bagliettoa baldensis*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1952* (BM), **KM371429, KM371504, KM371557; *Bagliettoa baldensis*,** Lawrence county, Alabama, U.S.A., *Tripp 1344* (NY), **KM371430, KM371505, KM371558; *Bagliettoa baldensis*,** Valley View Glades Natural Area, Missouri, U.S.A., *Harris 48178-A* (NY), **KM371431, KM371506, KM371559; *Bagliettoa calciseda*** (DC.) Gueidan & Cl.Roux, massif de la Ste-Baume, Nans-les-Pins, Var, France, *Gueidan 43* (MARSSJ), **KM371432,** –, –; ***Bagliettoa calciseda*,** Les Rousses, Jura, France, *Gueidan 614* (DUKE), **KM371433,** –, FJ225667; ***Bagliettoa calciseda*,** Sivergues, Vaucluse, France, *Gueidan 1762* (BM), **KM371434, KM371507, KM371560; *Bagliettoa calciseda*,** Sivergues, Vaucluse, France, *Gueidan 1953* (BM), **KM371435, KM371508,** –; ***Bagliettoa calciseda*,** Sivergues, Vaucluse, France, *Gueidan 1954* (BM), **KM371436, KM371509,** –; ***Bagliettoa calciseda*,** Mendip Hills, North Somerset, England, U.K., *Coppins 20695* (E), **KM371437,** –, –; ***Bagliettoa calciseda*,** Mirabeau, Vaucluse, France, *Roux s.n.* (BM), **KM371438,** –, –; ***Bagliettoa calciseda*,** Gruber Wildlife Management Area, Oklahoma, U.S.A., *Buck 46575* (NY), **KM371440, KM371511,** –; ***Bagliettoa calciseda*,** Schermerhorn Park, Cherokee county, Kansas, U.S.A., *Harris 44393-A* (NY), **KM371439, KM371510,** –; ***Bagliettoa cazzae*** (Zahlbr.) Vězda & Poelt, fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 572* (DUKE), **KM371441,** –, FJ225668; ***Bagliettoa cazzae*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1531* (BM), **KM371442, KM371512, KM371561; *Bagliettoa cazzae*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1532* (BM), **KM371443, KM371513, KM371562; *Bagliettoa cazzae*,** Venasque, Vaucluse, France, *Gueidan 1533* (BM), **KM371444, KM371514, KM371563; *Bagliettoa cazzae*,** Venasque, Vaucluse, France, *Gueidan 1534* (BM), **KM371445, KM371515, KM371564; *Bagliettoa cazzae*,** vallon du Mestrallet, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1535* (BM), **KM371446, KM371516, KM371565; *Bagliettoa cazzae*,** vallon du Mestrallet, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1536* (BM), **KM371447, KM371517, KM371566; *Bagliettoa cazzae*,** Venasque, Vaucluse, France, *Gueidan 1755* (BM), **KM371448, KM371518, KM371567; *Bagliettoa cazzae*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1756* (BM), **KM371449, KM371519, KM371568; *Bagliettoa cazzae*,** vallon du Mestrallet, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1758* (BM), **KM371450, KM371520, KM371569; *Bagliettoa cazzae*,** col de Teghime, Bastia, Haute Corse, France, *Gueidan 2038* (BM), **KM371451, KM371521, KM371570; *Bagliettoa limborioides*** A.Massal., fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1748* (BM), **KM371452, KM371522, –; *Bagliettoa limborioides*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1749* (BM), **KM371453, KM371523,** –; ***Bagliettoa limborioides*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1750* (BM), **KM371454, KM371524,** –; ***Bagliettoa limborioides*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1752* (BM), **KM371455, KM371525,** –; ***Bagliettoa limborioides*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1753* (BM), **KM371456, KM371526,** –; ***Bagliettoa marmorea*** (Scop.) Gueidan & Cl.Roux, Nîmes-le-Vieux, causse Méjean, Lozère, France, *Gueidan 739* (DUKE), **KM371458,** –, –; ***Bagliettoa marmorea*,** Venasque, Vaucluse, France, *Gueidan 1522* (BM), –, **KM371528,** –; ***Bagliettoa marmorea*,** Venasque, Vaucluse, France, *Gueidan 1523* (BM), **KM371459, KM371529,** –; ***Bagliettoa marmorea*,** Venasque, Vaucluse, France, *Gueidan 1524* (BM), **KM371460, KM371530,** –; ***Bagliettoa marmorea*,** Venasque, Vaucluse, France, *Gueidan 1525* (BM), **KM371461, KM371531, –; *Bagliettoa marmorea*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1526a* (BM), **KM371462, KM371532, KM371572; *Bagliettoa marmorea*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1526b* (BM), **KM371463, KM371533,** –; ***Bagliettoa marmorea*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1527* (BM), **KM371464, KM371534, –; *Bagliettoa marmorea*,** vallée du Golo, Francardo, Haute Corse, France, *Gueidan 2034* (BM), **KM371465, KM371535,** –; ***Bagliettoa*** cf. ***marmorea*,** Huckleberry Ridge Conservation Area, Missouri, U.S.A., *Buck 38392* (NY), **KM371466,** –, **KM371573; *Bagliettoa*** cf. ***marmorea*,** Huckleberry Ridge Conservation Area, Missouri, U.S.A., *Buck 44214* (NY), **KM371467,** –, **KM371574; *Bagliettoa parmigera*** s.str. (J.Steiner) Vězda & Poelt, Les Rousses, Jura, France, *Gueidan 611* (MARSSJ), **KM371469,** –, –; ***Bagliettoa parmigera*** s.str., fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1759* (BM), **KM371471, KM371537, KM371575; *Bagliettoa parmigera*** s.str., fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1847* (BM), **KM371473, KM371538, KM371577; *Bagliettoa parmigera*** s.str., vallée du Golo, Francardo, Haute Corse, France, *Gueidan 2035* (BM), **KM371475,** –, **KM371579; *Bagliettoa parmigerella*** (Zahlbr.) Vězda & Poelt, massif de la Ste-Baume, Nans-les-Pins, Var, France, *Gueidan 21* (DUKE), **KM371476,** –, –; ***Bagliettoa parmigerella*,** massif de la Ste-Baume, Plan-d'Aups-Ste-Baume, Var, France, *Gueidan 23* (MARSSJ), **KM371477,** –, FJ225666; ***Bagliettoa parmigerella*,** massif de la Ste-Baume, Nans-les-Pins, Var, France, *Gueidan 37* (MARSSJ), **KM371478,** –, –; ***Bagliettoa parmigerella*,** Le Grand Champs, Onnion, Haute-Savoie, France, *Gueidan 506* (MARSSJ), **KM371479,** –, –; ***Bagliettoa parmigerella*,** massif de Marseilleveyre, Marseille, Bouches-du-Rhône, France, *Gueidan 573* (DUKE), **KM371480,** –, FJ225669; ***Bagliettoa parmigerella*,** fontaine de Voire, massif des Calanques, Bouches-du-Rhône, France, *Gueidan 1528* (BM), **KM371481, KM371540, KM371580; *Bagliettoa parmigerella*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1529* (BM), **KM371482, KM371541, KM371581; *Bagliettoa parmigerella*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1530* (BM), **KM371483, KM371542, KM371582; *Bagliettoa parmigerella*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1754a* (BM), **KM371484, KM371543, KM371583; *Bagliettoa parmigerella*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1754b* (BM), **KM371485, KM371544, KM371584; *Bagliettoa*** cf. ***parmigerella*** (deposited as ***V. baldensis***), Duino-Aurisina, Trieste, Italy, *Favero-Longo 607* (TO), EF369522, –, –; ***Bagliettoa*** cf. ***parmigerella*** (deposited as ***V. baldensis***), Duino-Aurisina, Trieste, Italy, *Favero-Longo 608b* (TO), EF369521, –, –; ***Bagliettoa steineri*** (Kušan) Vězda, Le Planas, Vallon-Pont-d'Arc, Ardèche, France, *Gueidan 355* (MARSSJ), **KM371487,** –, FJ225670; ***Bagliettoa steineri*,** Brecon Beacons National Park, Carmarthenshire, Wales, U.K., *Gueidan 1516* (BM), **KM371488, KM371545, KM371585; *Bagliettoa steineri*,** Mendip Hills, North Somerset, England, U.K., *Coppins 20640* (E), **KM371495,** –, **KM371591; *Bagliettoa*** cf. ***suzaeana*** (Servìt) Gueidan & Cl.Roux, massif de la Ste-Baume, Nans-les-Pins, Var, France, *Gueidan 11* (MARSSJ), **KM371468,** –, –; ***Bagliettoa*** cf. ***suzaeana*,** massif de la Ste-Baume, Plan-d'Aups-Ste-Baume, Var, France, *Gueidan 27* (MARSSJ), **KM371486,** –, –; ***Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1757* (BM), **KM371470, KM371536,** –; ***Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1763* (BM), **KM371489, KM371546, KM371586; *Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1764* (BM), **KM371490, KM371547, KM371587; *Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1765* (BM), **KM371491,** –, –; ***Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1846* (BM), **KM371472,** –, **KM371576; *Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 1848* (BM), **KM371474, KM371539, KM371578; *Bagliettoa*** cf. ***suzaeana*,** Buoux, Vaucluse, France, *Gueidan 1955* (BM), –, **KM371548, KM371588; *Bagliettoa*** cf. ***suzaeana*,** col de Teghime, Bastia, Haute Corse, France, *Gueidan 2042* (BM), **KM371492, KM371549, KM371589; *Bagliettoa*** cf. ***suzaeana*,** Carreg Cennen Castle, Carmarthenshire, Wales, U.K., *Gueidan 2082* (BM), **KM371493, KM371550, KM371590; *Bagliettoa*** cf. ***suzaeana*,** Le Destet, chaîne des Alpilles, Bouches-du-Rhône, France, *Gueidan 2084* (BM), **KM371494, KM371551,** –; ***Bagliettoa*** cf. ***suzaeana*,** Delaware Water Gap National Recreation Area, Pennsylvania, U.S.A., *Harris 49634* (NY), **KM371496,** –, –; ***Bagliettoa*** cf. ***suzaeana*,** Rhossili, Gower, Glamorgan, Wales, U.K., *Orange 16333* (NMW), FJ645261, –, –; ***Bagliettoa*** sp., Hell Creek Natural Area, Arkansas, U.S.A., *Lendemer 26474* (NY), **KM371457, KM371527, KM371571**