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Phylogenetic and morphological circumscription of the *Orbilia aurantiorubra* group

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Abstract

The phylogeny of *Orbilia aurantiorugra* and related species is inferred from ITS sequence data. *Orbilia aurantiorubra* is redefined according to vital taxonomy. Integrated analyses of molecular and morphological data, and ecological (e.g. substrate) and geographical origin suggest the existence of three new species, which are described in this paper: *Orbilia xanthoguttulata* from Europe, *O. succulenticola* from the Canary Islands, and *O. jugulospora* from Ethiopia (Africa) and Taiwan (Southeast Asia).

Key words: Biodiversity, Europe, Canary Islands, Orbiliomycetes, taxonomy

Introduction

The genus *Orbilia* Fr. (*Orbiliaceae* Nannf.) was traditionally placed in the order *Helotiales* Nannf., and considered as a low diversity group (Spooner 1987). The advantages of using living cells (Baral 1992) for the taxonomic study of the *Orbiliaceae* have dramatically improved the knowledge of this family in the last two decades. Indeed, the family *Orbiliaceae* has undergone many changes since Baral & Marson (2001) introduced the genus *Hyalorbilia,* based on a small group of species previously included in *Orbilia*. Thus, in 2001 the *Orbiliaceae* comprised two genera and ~35 spp. (Kirk *et al*. 2001). In 2003 the family was segregated from the *Leotiomycetes* to constitute an order and class of its own, the *Orbiliales* and *Orbiliomycetes* (Eriksson *et al*. 2003). Further molecular studies found that this class formed an early diverging major clade within *Pezizomycotina* (Spatafora *et al*. 2006).

Thus far, *Orbilia* is represented by ca. 58 species worldwide, principally wood-saprobic fungi, particularly common in temperate regions (Kirk *et al*. 2008, Cannon & Kirk 2007). However, the forthcoming world monograph of Orbiliomycetes (Baral *et al*. in prep.) will recognize about 400 species within this genus, most of which are adapted to semihumid to arid, subtropical to tropical environments. Despite this considerable increase in species number, there is still a substantial lack of knowledge concerning host or habitat preferences and biogeography for many of the recognized species. Not surprisingly, this current lack of elementary data has impeded the assessment of many important aspects bearing on the evolutionary biology and systematic of this group.

Orbilia aurantiorubra Boud. is easily recognizable by its bright orange-red apothecia, and geniculate based helicoid spores containing narrowly tear-shaped spore bodies. It is briefly described by Boudier (1907) and appears in his *Icones mycologicae* (Boudier 1904–10). This species tolerates desiccation, and occurs throughout the year on different substrates in temperate, continental to Atlantic areas of Europe and West Africa (rarely in Mediterranean areas), between 0–1650 m (Baral *et al*. ined.). Specimens related to *O. aurantirubra* have ascospores variation between (7.5–)8–14.5(–16) × 1.1–1.8 µm and spores bodies between (1.8–)2.5–4.5(–5.5) × (0.5–)0.7–1.1(–1.3) µm. Some variation in spore dimensions correlated with host or geographical origins in *O. aurantiorubra* were noticed (Karasch *et al*. 2005, Priou 2005, Spooner 2001). Species considered as widespread fungi, usually showed a restricted distribution after taxonomical review (Stadler et al 2004; Baral 1984). The correlation between ascospore size and host has been evidence for species differentiation (Petrini *et al.* 1987). Generally, morphological variation observed in species can

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now be assessed using molecular phylogenetic approaches; recent application in ascomycetes taxonomy include, e.g., Hustad *et al*. (2013); or Zhao-Qing & Zhuang (2013) and Hyde *et al.* (2013).

In this general context, the aims of this investigation are (a) to elucidate the number of species related to *Orbilia aurantiorubra*, (b) to infer their phylogenetic relationships, (c) to define the geographical distribution and host specificity, and (d) to find morphological characters to differentiate each putative species.

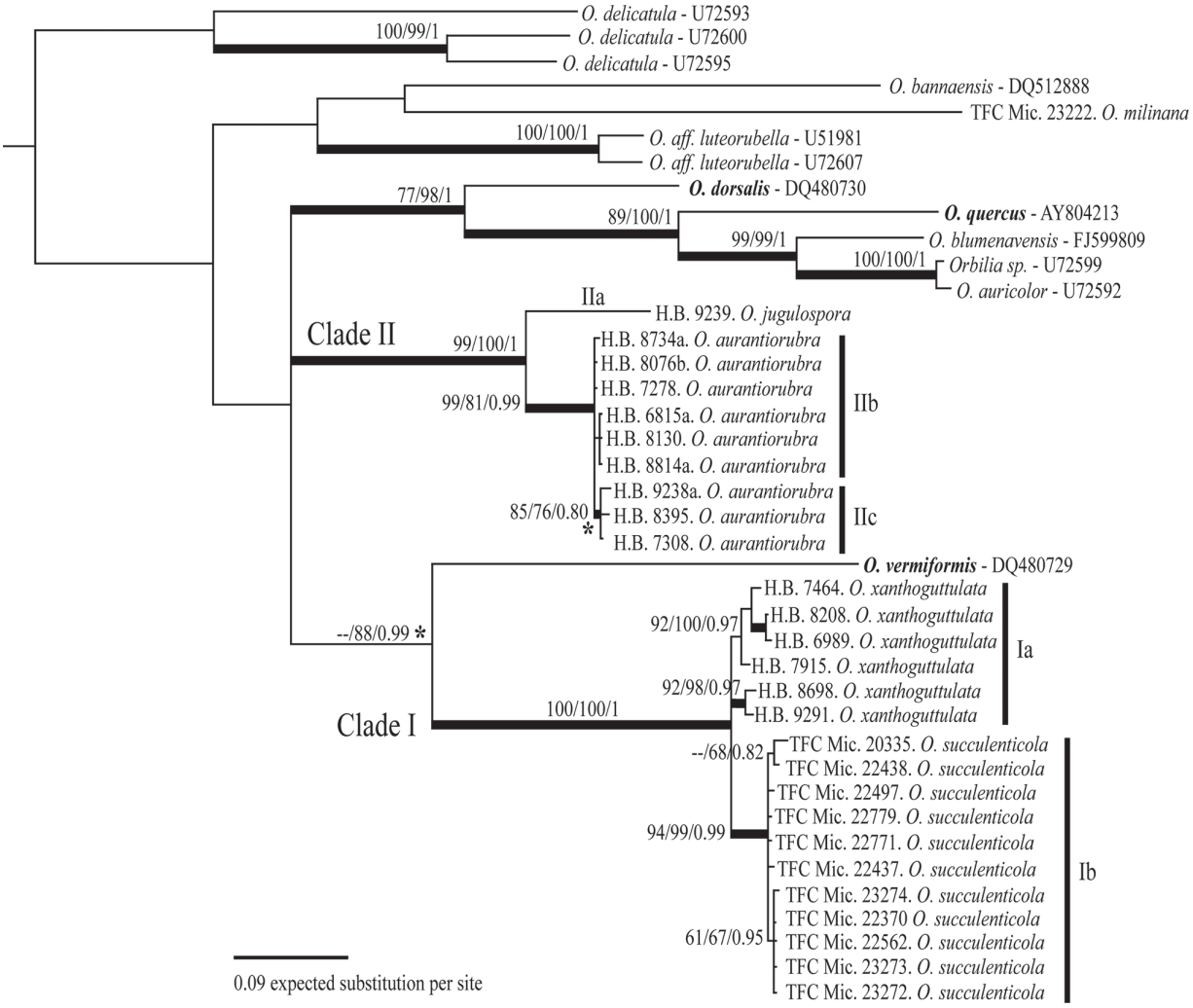

FIGURE 1. Bayesian majority-rule consensus tree. Bold branches are those which were well supported (see Methods) by MP/ML/BI methods. Asterisks indicate a branch supported by only two of these methods. Extype strains are in bold.

Material and Methods

Morphological studies

Microscopic features were mainly described in the living state, which exhibits clear biometric differences in relation to observations in the dead state. The abbreviations used follow Baral (1992). The methods for the study of apothecia follow Quijada *et al*. (2012). Measurements are given as follows: (smallest single measurement–) smallest mean–largest mean (–largest single measurement). The small and large means are based on ≥10 measurements on individual specimens. The number of specimens studied for each character is indicated between brackets.

The variation in ascospore size and shape was morphometrically measured in all the specimens included in the molecular phylogenetic analyses, except for the type of *O. jugulospora* (H.B. 9239) due to paucity of material, and two specimens from the Canary Islands (TFC Mic. 22771, 23222) that lacked apothecia. The individual metrics indicated in Clopton (2004) for deltoid and semifalciform shape series were used in side views of dead ascospores. Ten measures for seven quantitative spore characters (described in Fig. 2) were measured from each collection. Thus, our basic data matrix contains 25 specimens with 250 measures for each quantitative character. After checking that the distribution of our data did not deviate significantly from normality (Kolmogorov-Smirnov test), a principal component analysis (PCA; McCune & Grace 2002) was used to elicit the characters that greatest influence on discrimination. The morphometric differences

were represented by a graphical test (box-plots containing medians and percentiles). Given the wide heterogeneity in sample sizes, the differences were tested with the non-parametric Mann-Whitney test. All statistical analyses were carried out with SPSS 15.0 (SPSS Inc., Chicago, IL, USA) and CANOCO 4.5 (Ter Braak & Šmilauer 2002). Type specimens are deposited and preserved at the herbarium of the University of La Laguna (TFC) section mycology (Mic), in the personal herbarium of H.-O. Baral in Tübingen, in the Botanische Staatssammlung München (M), Germany and in the herbarium of the University of Alcalá de Henares (AH), Spain.

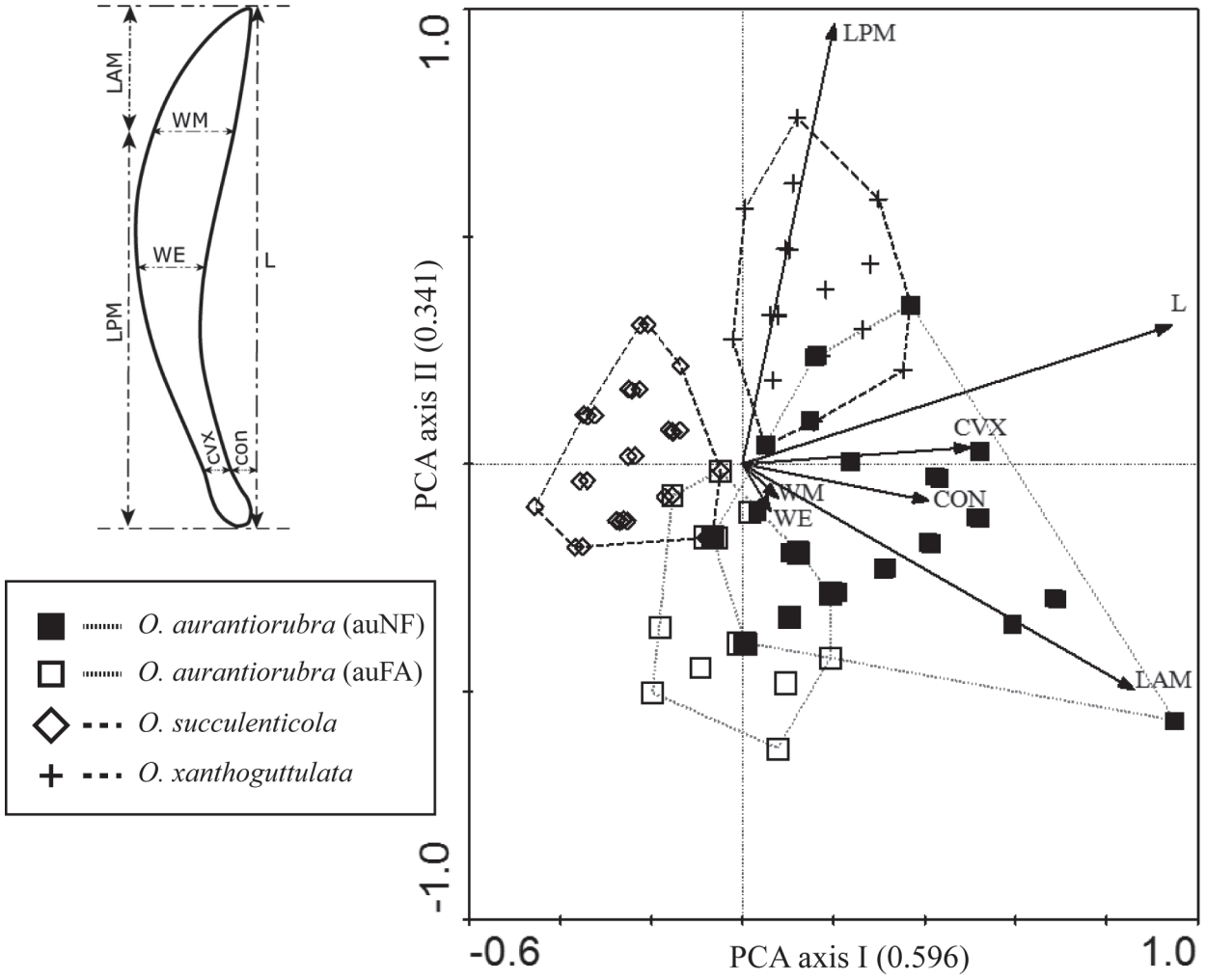

FIGURE 2. Schematic representation of the biometric traits of dead ascospores used in this study (left), and results of the PCA carried out with these measures (right). CON= concavity, CVX= convexity L= length, LAM= anterior distance to maximum width, LPM= posterior distance to maximum width, WE= equatorial width, WM= maximum width. The most important characters according to the length of their vectors are shown in the plot.

Molecular studies

Four dried apothecia were taken from each specimen. Prior to DNA isolations, apothecia were crushed in a mixer mill using glass balls, and the powder obtained was incubated in lysis buffer overnight at 55°C according to Telleria *et al*. (2012). Genomic DNA was isolated using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the maufacturer's instructions. Sequences of the internal transcribed spacer region (ITS; ~400–800 bp), comprising ITS1, the 5.8S rDNA ribosomal subunit, and ITS2 were obtained using the primers ITS4 and ITS5 (White *et al*. 1990). PCR reactions were prepared for a final volume of 25 µl, containing 2.5 µl of DNA template, 0.5 µl of each primer (0.4 µM), 21 µl of Premix Taq (TaKaRa Taq Version 2.0) and 1 µl of distilled water. The amplification profile for a touchdown PCR was as follows: 3 min of initial denaturation at 94°C followed by 10 cycles of 30s at 94°C, 45s at 60°C (–1°C each cycle), 1 min at 72°C, and then another 25 cycles of 30s at 94°C, 45s at 50°C, 1 min at 72°C; and a final extension of 7 min at 72°C. PCR products were electrophoresed on a 1% agarose gel, stained with SYBR safe, and visualized with UV light. Bidirectional sequencing PCR products were sequenced in both directions at the DNA Sequencing Service of Macrogen in Korea.

TABLE 1. Samples used in this molecular study and their GenBank accession numbers. All extype strains are in bold. Abbreviation on the table: TM = TFCMic (Herbarium of the University of La Laguna), H.B. (personal herbarium of H.-O Baral in Tübingen), C.I.= Canary Islands, E= Europe, A= Africa. Identity of species with asterisk is different from previous reports, according to unpublished results (Baral *et al*. *in prep*). The termination of the two underlined epithets was changed to avoid improper latin declension.

Species	Collection	Geographic origin	Substrate	Accession number
O. delicatula	DHP.120	USA, New York	?	U72593
O. delicatula	DHP.108	USA, Maryland	?	U72595
*O. delicatula**	DHP.91	USA, Massachusetts	?	U72600
O. bannaensis	OT003	China, Yunnan, Xishuangbanna	*Broussonetia sp.*	DQ512888
*O. aff. luteorubella**	CBS. 917.72	?	?	U51981
*O. aff. luteorubella**	DHP.146	USA, Massachusetts	?	U72607
O. dorsalis	YMF1.01835	China, Yunnan, Xishuangbanna	bark of *Euphorbiaceae*	DQ480730
O. quercus	AS 3.6762	China, Beijing, Huairou	*Quercus sp.*	AY804213
O. blumenavensis	YMFT 1.03002	China, Yunnan, Yuxi	unidentified angiosperm	FJ599809
Orbilia sp*	DHP.60	USA, Massachusetts	?	U72599
O. auricolor	DHP.90	USA, Massachusetts	?	U72592
O. vermiformis	YMF1.01842	China, Yunnan, Pu'er	unidentified angiosperm	DQ480729
O. milinana	TM. 23222	C.I., Tenerife, La Matanza	*Euphorbia canariensis*	KF696674
O. jugulospora	H.B. 9239	A, Ethiopia, Oromia	unidentified angiosperm	KF741594
O. aurantiorubra	H.B. 8814a	E, France, Deux-Sèvres	*Ulmus sp.*	KF741596
O. aurantiorubra	H.B. 8076b	E, France, Deux-Sèvres	*Ulmus minor*	KF741597
O. aurantiorubra	H.B. 8395	E, Germany, Nordrhein- Westfalen	*Cytisus scoparius*	KF741598
O. aurantiorubra	H.B. 8734a	E, Germany, Oberbayern	*Salix sp.*	KF741599
O. aurantiorubra	H.B. 7308	E, Great Britain, East England	*Lupinus arboreus*	KF741600
O. aurantiorubra	H.B. 7278	E, Austria, Kärnten	*Rhamnus frangula*	KF741601
O. aurantiorubra	H.B. 8130	E, France, Poitou-Charentes	*Salix aurita*	KF741602
O. aurantiorubra	H.B. 6815a	E, Luxembourg, Gutland	*Salix caprea*	KF741595
O. aurantiorubra	H.B. 9238a	E, France, Drôme	*Cercis siliquastrum*	KF741603
O. xanthoguttulata	H.B. 9291	E, France, Drôme	*Fraxinus excelsior*	KF768634
O. xanthoguttulata	H.B. 8698	E, Germany, Brandenburg	*Quercus robur*	KF768635
O. xanthoguttulata	H.B. 8208b	E, Sweden, Skåne	*Ulmus glabra*	KF768636
O. xanthoguttulata	H.B. 6989a	E, Luxembourg, L'Oesling	*Salix aurita x caprea*	KF741604
O. xanthoguttulata	H.B. 7915b	E, France, Savoie	*Salix sp.*	KF768637
O. xanthoguttulata	H.B. 7464	E, Spain, Andalucía	*Salix sp.*	KF768638
O. succulenticola	TM. 22779	C.I., Tenerife, Arico	*Euphorbia canariensis*	KF768639
O. succulenticola	TM. 22771	C.I., Tenerife, Arico	*E. canariensis*	KF768640
O. succulenticola	TM. 22497	C.I., Tenerife, Teno	*E. lamarckii*	KF768641
O. succulenticola	TM. 22562	C.I., Tenerife, La Matanza	*E. canariensis*	KF768642
O. succulenticola	TM. 22437	C.I., Tenerife, Arico	*E. balsamifera*	KF768643
O. succulenticola	TM. 23272	C.I., Tenerife, Punta Hidalgo	*E. canariensis*	KF768644
O. succulenticola	TM. 23273	C.I., Tenerife, Punta Hidalgo	*E. canariensis*	KF768645
O. succulenticola	TM. 23274	C.I., Tenerife, Punta Hidalgo	*E. lamarckii*	KF768646
O. succulenticola	TM. 22370	C.I., Tenerife, Punta Hidalgo	*Opuntia maxima*	KF768647
O. succulenticola	TM. 22438	C.I., Tenerife, Arico	*E. balsamifera*	KF768648
O. succulenticola	TM. 20335	C.I., Tenerife, Punta Hidalgo	*E. canariensis*	KF768649

The 40 sequences used (Table 1) were aligned using the L-INS-i algorithm (Katoh & Toh 2008) with MAFFT v7.017 (Katoh *et al*. 2002). This first alignment was 615 bp long. Ambiguously aligned regions were identified and eliminated with Gblocks v. 0.91b (Castresana 2000), using the following relaxed settings (Talavera & Castresana 2007): minimum number of sequences for a conserved or a flanking position = 21; maximum number of contiguous non-conserved position = 8; minimum length of a block = 5; and gaps in an alignment column allowed in up to half the number of included sequences. The final alignment used for phylogenetic analysis contained 523 bp (85% of the first alignment length). The data matrix has been deposited in TreeBase as accession 14981, is also deposited as a genetic diversity digest with code D-DNASE-91 (Quijada *et al*. 2013) in the Demiurge information system (http://www.demiurge-project.org). The general time-reversible model of nucleotide substitution with substitution rate heterogeneity modelled using a gamma distribution (GTR+G) was identified as the optimal model using jModelTest (Posada 2008; http://darwin.uvigo.es), based on the Akaike information criterion (Akaike 1974). Maximum likelihood (ML) analyses were performed with RAxML v.7.6.6 (Stamatakis 2006) in a hybrid version at the CIPRES science gateway (http://www.phylo.org/index.php/portal/). For heuristic searches we used the GTRCAT DNA substitution model, with a final optimization of inferred trees using GTR+G. Branch support was inferred from 5000 rounds of rapid bootstrap (Stamatakis *et al*. 2008), with bootstrap trees also used as starting trees for heuristic searches on the original alignment (-a option). Bayesian inference (BI) analysis was performed with the Markov Chain Monte Carlo method in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Four simultaneous Markov chains were run from random starting trees for 6 million generations, trees were sampled every 300th generation using the default priors and saved to a file. Two independent analyses were performed synchronously to control chain convergence. The first 25% of the sampled trees of each run were discarded as burn-in; the remaining trees from the two runs were pooled and used for generating a consensus tree, and for calculating the posterior probabilities (PP) to estimate clade support. Maximum Parsimony (MP) analysis was conducted using a heuristic search with the tree bisection reconnection (TBR) algorithm in the software TNT (Goloboff *et al*. 2008). Starting trees were Wagner trees with a random seed of 1; 9,999 replicates were used, with 10 trees saved per replicate; all characters were treated as equally weighted and unordered. Gaps were treated as missing data. Branch support was calculated using 10,000 standard bootstrap replicates. We only considered clades as well supported for ML and MP bootstrap values ≥75% and with PP≥0.95 for BI. Phylogenetic trees were drawn with FigTree 1.4 (available at http://tree.bio.ed.ac.uk/sofware/figtree/), and artwork was prepared in Adobe Illustrator CS5.

Results

Morphological analyses

Relevant morphological differences among species are shown in Table 2. *Orbilia jugulospora* and *O. aurantiorubra* present a high quantity of KOH-soluble cytoplasmic bodies, while as *O. xanthoguttulata* and *O. succulenticola* have a high quantity of yellow lipid bodies, especially in paraphyses and excipular tissues. Both groups also show differences in glassy processes and the length of the spore body. *Orbilia jugulospora* is separated from *O. aurantiorubra* by its short asci and rather narrow spore bodies. The biometric differences between *Orbilia aurantiorubra*, *O. xanthoguttulata* and *O. succulenticola* are explained below.

In the PCA analysis (25 specimens measured, Fig. 2), the first two axes explained 59.6% and 34.1% of the total variance, respectively (93.7% cumulative variance), and resolved four distinct clusters of specimens. In the middle left area of the PCA (Fig. 2), a first compact cluster contained specimens of *O. succulenticola* on succulent *Euphorbia* scrubs in the Canary Islands. In the top right area, a second cluster consisted of *O. xanthoguttulata* specimens from Europe on woody substrates. A third cluster on the bottom left area was formed by specimens of *O. aurantiorubra* that grew on different species of *Fabaceae* (abbreviated in Fig. 3 as auFA): *Cercis sp.* (France), *Cytisus sp.* (Germany) and *Lupinus sp.* (United Kingdom). Finally, a fourth cluster in the middle-bottom right area contained all specimens of *O. aurantiorubra* collected on substrates other than *Fabaceae* (*Salix*, *Ulmus*, *Rhamnus;* abbreviated in Fig.3 as auNF) from Austria, France, Germany and Luxembourg. The two latter groups (auFA, auNF) are the most overlapped. Four morphometric characters had low weights (WM, WE, CVX, CON). The two characters L and LAM had a stronger discriminating power in the first axis, and distinguished *O. aurantiorubra* (auNF, auFA) from *O. succulenticola*. However, while LAM distinguished the group of *O. aurantiorubra* (auNF, auFA with LAM = 5.3 ± 1.0 µm) from that of *O. succulenticola* and *O. xanthoguttulata* (LAM = 3.1 ± 0.6 µm), the differences in L were not significant between *O. aurantiorubra* (auNF) and *O. xanthoguttulata* (Fig. 3). The character LPM separated *O. xanthoguttulata* from the remaining species, but it did not show significant differences between *O. aurantiorubra* (auNF) and *O. succulenticola*. The groups defined by vital characteristics (morphology of spore bodies, size of asci, presence or absence of KOH-soluble cytoplasmic bodies and lipid bodies) and biometrics (spore length, anterior and posterior length to maximum width) were also strongly supported by our phylogenetic analyses (Fig. 1).

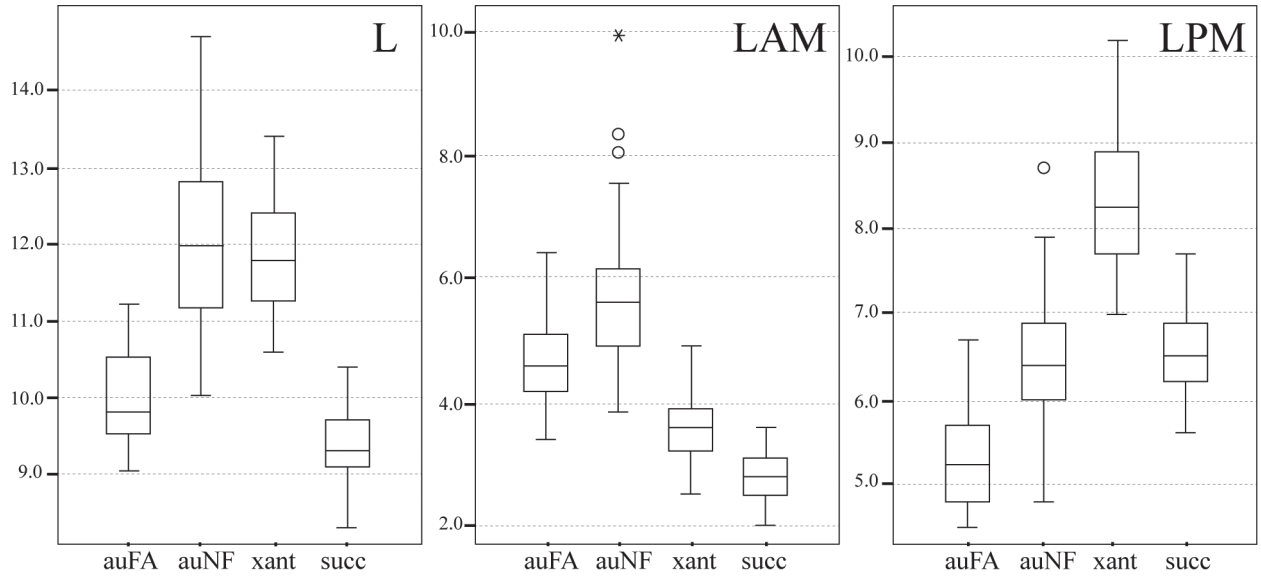

FIGURE 3. Box plots of the significant variables obtained in the PCA (L, LAM, LPM). Line within box: median; upper and lower lines are the 75th and 25th percentile, respectively. Length of vertical bar indicates the 95% confidence interval of the median. Circles are outliers, and the asterisk is an extreme outlier. L= length of ascospores, LAM= anterior distance to maximum width, LPM= posterior distance to maximum width. auFA = *O. aurantiorubra* on *Fabaceae*, auNF= *O. aurantiorubra* on non-*Fabaceae*, xant= *O. xanthoguttulata*, succ= *O. succulenticola*.

TABLE 2. Morphology and living state measurements taken in *Orbilia aurantiorubra* and related specimens (the ranges do not include maximum and minimum measures).

Orbilia species		*O. aurantiorubra*	*O. jugulospora*	*O. xanthoguttulata*	*O. succulenticola*
Asci (µm)	Length	52–70	46–50	53–74	55–63
	Width	4.5–5.3	4–4.2	4.2–5	4.2–4.8
Spores (µm)	Length	11–14.5	10.5–12	11–14.5	9.5–11
	Width	1.2–1.5	1.1–1.2	1.3–1.6	1.2–1.5
Spore bodies (µm)	Length	3–5	3.5–4.5	2–3	2–2.2
	Width	0.7–1	0.3–0.8	0.7–1.3	0.8–1.2
	Morph.	Narrow tear-shaped to subulate	Subulate	Tear-shaped	Tear-shaped
Paraphyses terminal cell (µm)	Length	14–30	26–27	11–31	21–28
	Width	2.5–4	3–4	3–4.5	3–4
	Morph.	Capitate-clavate	Capitate-clavate	Clavate-spathulate	Clavate-spathulate
Ectal cells on margin (µm)	Length	6–15	7.5–10	7–11	9–12
	Width	3.5–5	3.5–5	4–7	4–4.6
Glassy processes (µm)		Absent (rarely 1.5–6)	Absent	1–5(–9)	8–18
Lipid bodies		Very sparse in paraphyses and excipulum	Very sparse in paraphyses, medium abundant in excipulum	Sparse to abundant in excipulum and paraphyses	Sparse to abundant in excipulum and paraphyses
KOH-soluble cytoplasmic bodies		Globose and ring- to irregularly lasso-shaped	Globose and ring- to irregularly lasso-shaped	Mostly absent, never ring- to lasso-shaped	Mostly absent, never ring- to lasso-shaped
Apothecial colour		Orange or rose	(Yellow-) orange(-rose)	(Cream-)yellow (-orange)	Yellow-ochraceous

Phylogenetic analyses

The alignment consisted of 523 characters, of which 278 were parsimony-informative, 57 were variable, and 245 were constant; the MP analyses resulted in 12 equally parsimonious trees with 1353 steps each, consistency index (CI) = 0.54, and retention index (RI) = 0.79. Since the overall topologies of the MP, ML and BI analyses were identical, we only show the Bayesian consensus tree (Fig. 1).

These analyses identified two clades (Fig. 1, clades I and II). Clade I contains 17 specimens belonging to two species, all of them sharing high contents of lipid bodies in paraphyses and excipular tissues, short, tear-shaped spore bodies, and anterior distance to maximum width lower than 5 µm (average higher than 4 µm). Eleven specimens constituted a well-supported sub-clade (Ib in Fig. 1: 94% MPBS, 99% MLBS, 0.99 BIPP), consisting of *Orbilia* specimens from the *Euphorbia* scrubs in the Canary Islands. Two diagnostic characters (Table 3, positions: 130 and 404) were identified that distinguish the members of sub-clade Ib. Sub-clade Ia (branch support < 50%) consisted of six specimens, with two well-supported groups (Ia in Fig. 1: 92% MPBS, 100% MLBS, 0.97 BIPP for H.B.8208b-6989a; 92% MPBS, 98% MLBS, 0.97 BIPP for H.B. 8698-9291). Molecular diagnostic characters were identified for sub-clade Ib (Table 3, position 34) and, for group H.B. 8208b-6989a (Table 3, position 488).

Clade II contained ten specimens belonging to two species sharing high contents of KOH-soluble cytoplasmic bodies in excipular tissues, long, narrowly tear-shaped to subulate spore bodies, and average anterior distance to maximum width higher than 4 µm. Sub-clade IIb was well-supported (see Fig. 1; 99% MPBS, 81% MLBS, 0.99 BIPP), sub-clade IIc was well-supported only with MP and ML (see Fig. 1; 85% MPBS, 76% MLBS, 0.80 BIPP), both sub-clades (IIb + IIc) contained all the specimens of *Orbilia aurantiorubra* from Europe. We found four diagnostic characters for *O. aurantiorubra* (Table 3, clades IIb + IIc, positions: 389, 390, 398 and 480), and only one for the sub-clade of *O. aurantiorubra* on *Fabaceae* (Table 3, auFA, position: 355), and two for specimens that live on non-*Fabaceae* (Table 3, auNF, positions: 60 and 61). For *O. jugulospora* (Fig. 1, sub-clade IIa), which is a species closely related to *O. aurantiorubra*, six diagnostic characters were found (Table 3, positions 361, 385, 398, 412, 463 and 480). These differences are reflected in the length of the *O. jugulospora* branch, which was about 10-fold longer than that of *O. aurantiorubra*.

TABLE 3. Diagnostic characters (unique combination of nucleotides) found in the alignment of ITS sequences for *Orbilia* species identification. (auFA= *O. aurantiorubra* on *Fabaceae*, auNF= *O. aurantiorubra* on non-*Fabaceae*). Dots represent without nucleotide changes in this position respect to variability observed in *Orbilia delicatula*, positions with zero represent gaps.

	Nucleotide position (bp) in the alignment														
Orbilia taxa	34	60	61	130	355	361	385	389	390	398	404	412	463	480	488
O. delicatula	G	C	A / C	A	T / 0	0	0	A / 0	T	A / G	G	G	T	G	C
O. bannaensis	0	.	.	C	.	C	.	.	.	C	.	.	A	.	G
O. milinana	.	T	G	.	.	.	C	C	G	.	.	.	.	.	.
O. cf. *luteorubella*	0	.	.	.	.	.	T	.	.	.	.	.	.	T	.
O. dorsalis	0	.	.	.	.	.	C	.	A	.	.	.	.	.	.
O. quercus	.	.	.	.	.	.	G	C	A	C	.	.	.	.	.
O. blumenaviensis	T	.	.	.	.	.	.	G	G	.	.	.	.	.	.
Orbilia sp.	C	.	.	.	.	.	.	G	.	.	.	.	C	.	.
O. auricolor	C	.	.	.	.	.	.	G	.	.	.	.	C	.	.
O. jugulospora	C	.	.	.	.	**A**	**A**	C	.	**T**	.	**T**	**G**	**C**	.
O. aurantiorubra (auNF)	C	**0**	**0**	.	.	G	T	**T**	**C**	**C**	.	.	.	**A**	.
O. aurantiorubra (auFA)	T	T	.	.	**C**	G	T	**T**	**C**	**C**	.	.	.	**A**	.
O. vermiformis	.	.	.	.	.	.	G	.	A	.	.	.	C	.	.
O. xanthoguttulata	**A**	.	.	C	.	G	.	G	.	.	.	A	0	0 /.	0 / **A** / T
O. succulenticola	.	.	.	**T**	.	G	T	.	.	.	**A**	A	C	.	T

Taxonomy

Orbilia aurantiorubra Boudier (1907: 103). (Fig. 4 & 5)

Type:—EUROPE. FRANCE. Haute-Marte: Champagne, on *Salix sp.*, 110 m, 22 February 1877, Rinchon (holotype PC!).

Apothecia rehydrated (0.2–)0.4–1.2(–1.7) mm diam., 0.14–0.25(–0.35) mm high (receptacle 0.14–0.18 mm), pale or usually light to bright orange-apricot (to brick-red), more rarely yellow-orange or salmon-rose(-orange), hardly to medium translucent, round, strongly undulating when large, scattered to often ± densely gregarious in smaller or larger groups; disc slightly concave, soon flat, margin distinct, 0–5 µm protruding, smooth to finely rough; broadly sessile or with a narrow stipe-like base 0.03–0.13 × 0.18–0.2 mm, superficial; dry bright to deep ochraceous-orange or mostly orange-rose-apricot to brick- or blood-red, with thick protruding margin. *Asci* *(45–)52–70(–82) × (4.2–)4.5–5.3(–5.5) µm {10}, †(42–)45–70(–75) × (3.8)4–4.5(–5.2) µm {3}, 8-spored, spores *4-seriate, 3–5 lower spores inverted {12} (rarely or often mixed: sometimes 1–3 apical spores inverted, sometimes also lower spores not inverted), pars sporifera *22–38 –> 18 µm long; apex (†) strongly truncate (rarely with slight dent and lateral inflation), hemispherical in profile view, thin-walled; base with short to often very long, thin, flexuous stalk, L- Y- or h-shaped. *Ascospores* *9–12.5 {19} or 11–13.5 {16} or (12–)13–15(–17.2) {19} × (1.1–)1.2–1.5(–1.6) µm {50} (in situ), †(9–)9.5–13 {8} or (10.5–)12–14.5(–15) {11} × (1.1–)1.2–1.4(–1.5) µm {19}, narrowly fusiform with (sub-) cylindrical middle part, with gradually tapering, acute to acuminate apex, base slightly to medium attenuated (tail-like), often very slightly inflated at the end, distinctly helicoid (looking falcate in profile view), near base medium to mostly strongly geniculate; *SBs* *(2.7–)3–5(–6) × (0.5–)0.7–1 µm {22} → 2.3–3.7 × 1–1.3 µm, narrowly tear-shaped to subulate, also abruptly narrowed in a filum of ± equal length. *Paraphyses* apically uninflated to slightly or medium (rarely strongly) capitate-clavate, exceptionally spathulate or ± moniliform, (0–)3–6 µm protruding beyond dead asci, terminal cells *(10–)14–30(–35) × (2.2–)2.5–4(–5.3) µm {8}, †1.8–4(–4.7) µm wide {2}, lower cells *(8–)11–18(–22.5) × 1.3–2.7 µm {7}, near base *3–10 × 2–3.5 µm; rarely branched at upper septum, hymenium subhyaline to light orange. *Medullary excipulum* totally 50–170 µm thick, upper part 0–50 µm thick, hyaline to pale rose, of dense textura intricata with small angular inflated cells, lower part 50–120 µm thick, of dense to medium loose, large-celled t. globulosa–angularis (cells *7–20 × 6–12.5 µm), irregular to upwards oriented, near margin often forming a distinct, subhyaline to light yellow-orange-rose, horizontal t. porrecta 8–20 µm thick, partly only here sharply delimited. *Ectal excipulum* very pale to light rose or orange, of thin-walled († slightly gelatinized), vertically oriented t. angularis(–globulosa) from base to lower flanks, 25–80(–200) µm thick near base, cells *7–23(–27) × 6–15(–24) µm {6}; 15–20 µm thick on mid flanks and margin, on mid flanks of t. prismatica–angularis oriented at an 80–90° angle to the surface, at margin of t. prismatica–porrecta at 45–90°, marginal cortical cells */†6–15 × (3–)3.5–5(–6) µm {4}, glassy processes absent, exceptionally present, 1.5–6 × 3.5–4 µm {1}. *SCBs* in paraphyses globose {8}, 1.2–2(–3) µm diam.; also filiform to S-shaped {10}; excipular cells on lower and mid flanks with inconspicuous to strongly refractive, thin (rarely thick), ring-, trapezoid to S-shaped SCBs {14}, marginal cortical cells also with globose SCBs 0.8–3 µm diam. *LBs* in ectal excipulum and paraphyses minute, sparse, hyaline, rarely yellowish. *Exudate* over paraphyses (0.3–)1–2(–4) µm thick, cloddy to granular, also cap-like, hyaline to pale yellowish, firmly attached (large clods also detaching); over margin and flanks rough-cloddy, subhyaline to pale yellow-chlorinaceous, 0.5–5 µm thick.

Distribution and ecology:—In Europe, on branches rarely twigs or trunks, ± thermophilous but also ± moist, shady woods in temperate, continental to atlantic, but also Oro-Mediterranean and Mediterranean areas; –7 up to 1560 m. Phenology: I–XII. Desiccation tolerance: fully viable for at least 1 month, after 23 months some ascospores and excipular cells still alive.

Other material examined:—EUROPE. AUSTRIA. Kärnten: on *Rhamnus frangula*, 410 m, 31 December 2012, W. Jaklitsch (herb. Baral 7278!). Poitou-Charentes: Deux-Sèvres, on *Ulmus sp.*, 103 m, 17 April 2008, H.O. Baral (herb. Baral 8814a!); *ibid.,* on *Ulmus cf. minor*, 158 m, 4 March 2006, M. Hairaund and B. Coué (herb. Baral 8076b!); *ibid.,* on *Salix aurita*, 4 m, 25 March 2006, H.O. Baral (herb. Baral 8130!); Rhône-Alpes: Drôme, on *Cercis siliquastrum*, 215 m, 22 September 2009, G. Marson (herb. Baral 9238a!); Provence-Alpes-Côte d'Azur: Alpes-de-Haute-Provence, on *Spartium junceum*, 740 m, 17 August 2001, G. Marson (herb. Baral 7023!). GERMANY. Nordrhein-Westfalen: on *Cytisus scoparius*, 80 m, 30 December 2006, F. Kasparek (herb. Baral 8395!); Oberbayern: on *Salix sp.*, 535 m, 31 December 2007, T.R. Lohmeyer (herb. Baral 8734a!). GREAT BRITAIN. East England: Suffolk, on *Lupinus arboreus*, 15 m, 7 March 2003, E. Batten & S.M. Francis (herb. Baral 7308!); *ibid.*, 1 m, 15 February 2003, E. Batten (herb. Baral 7298!). LUXEMBOURG. Gutland: on *Salix caprea*, 305 m, 5 November 2000, G. Marson (herb. Baral 6815a!); *ibid.*, on *Salix caprea*, 270 m, 27 April 1993, G. Marson (herb. Baral 4868!). SPAIN. Asturias: on *Salix sp.*, 280 m, 9 September 2008, E. Rubio (herb. Baral 4578!). Canary Islands: La Palma, on *Chamaecytisus proliferus*, 820 m, 1 January 2005, J.P. Priou (herb. Priou 25001!).

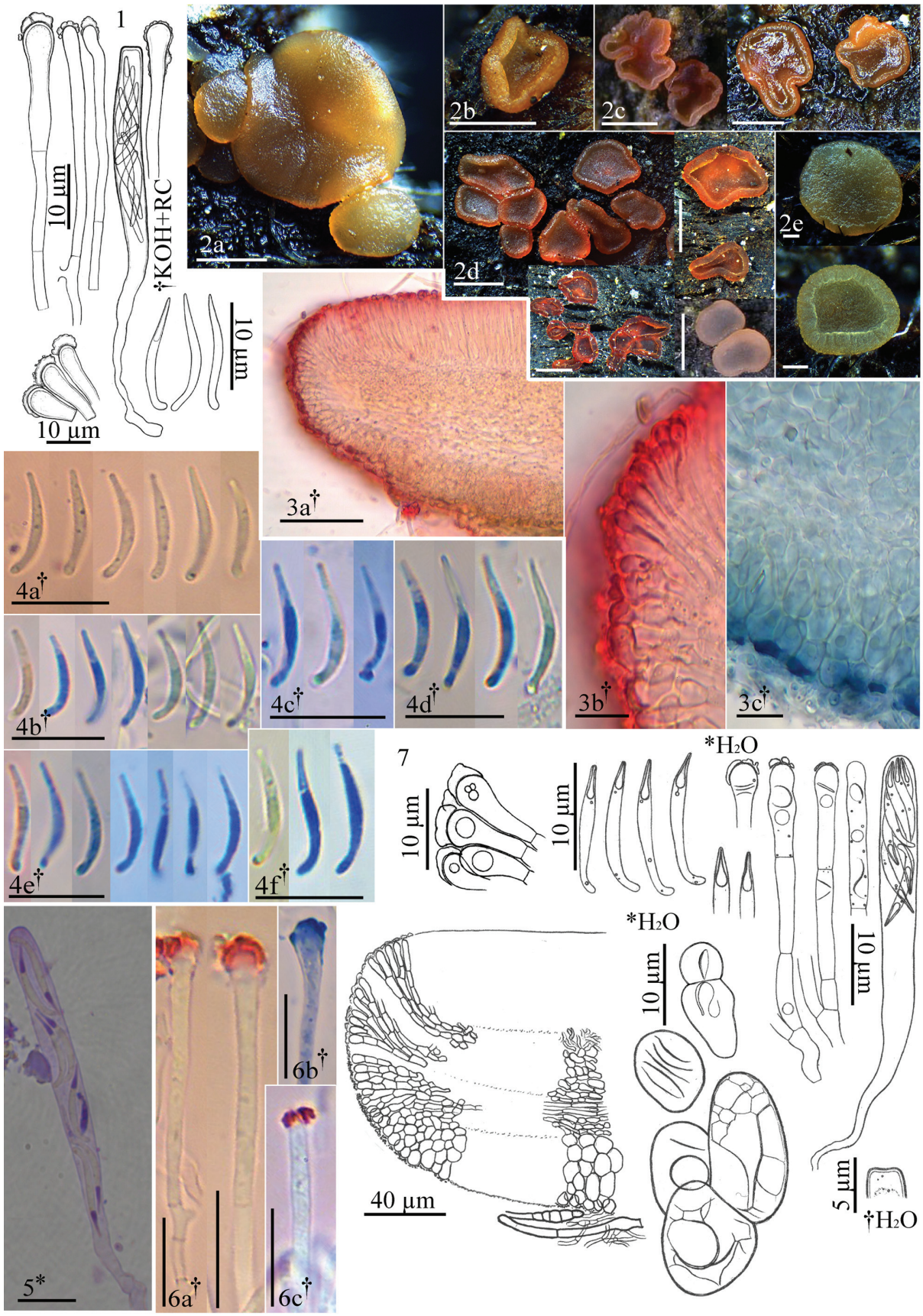

FIGURE 4. Morphological characteristics of *Orbilia aurantiorubra* on non-*Fabaceae* substrates (*Salix* spp., *Ulmus* spp., etc.). 1. Drawing of reexamined holotype. 2. Apothecia on natural substrates, scale bars 2a-d= 500 µm, 2e= 100 µm. 3. Section of apothecia and excipular characteristics, scale bars 3a= 50 µm, 3b–c= 10 µm. 4. Dead ascospores. 5. Living asci (phot. Michael Hairaud). 6. Paraphyses; scale bars 4a–f, 5a and 6a–c= 10 µm. 7. Drawings with vital characteristics for H.B. 4868. (H.B. 6815a= 4a, 6a; H.B. 7278= 2b, 4c; H.B. 8076b= 2a, 4d, 5; H.B. 8130= 2c, 4f; H.B. 8734a= 2d, 3a–b, 6b; H.B. 8814a= 2e, 4e, 6c).

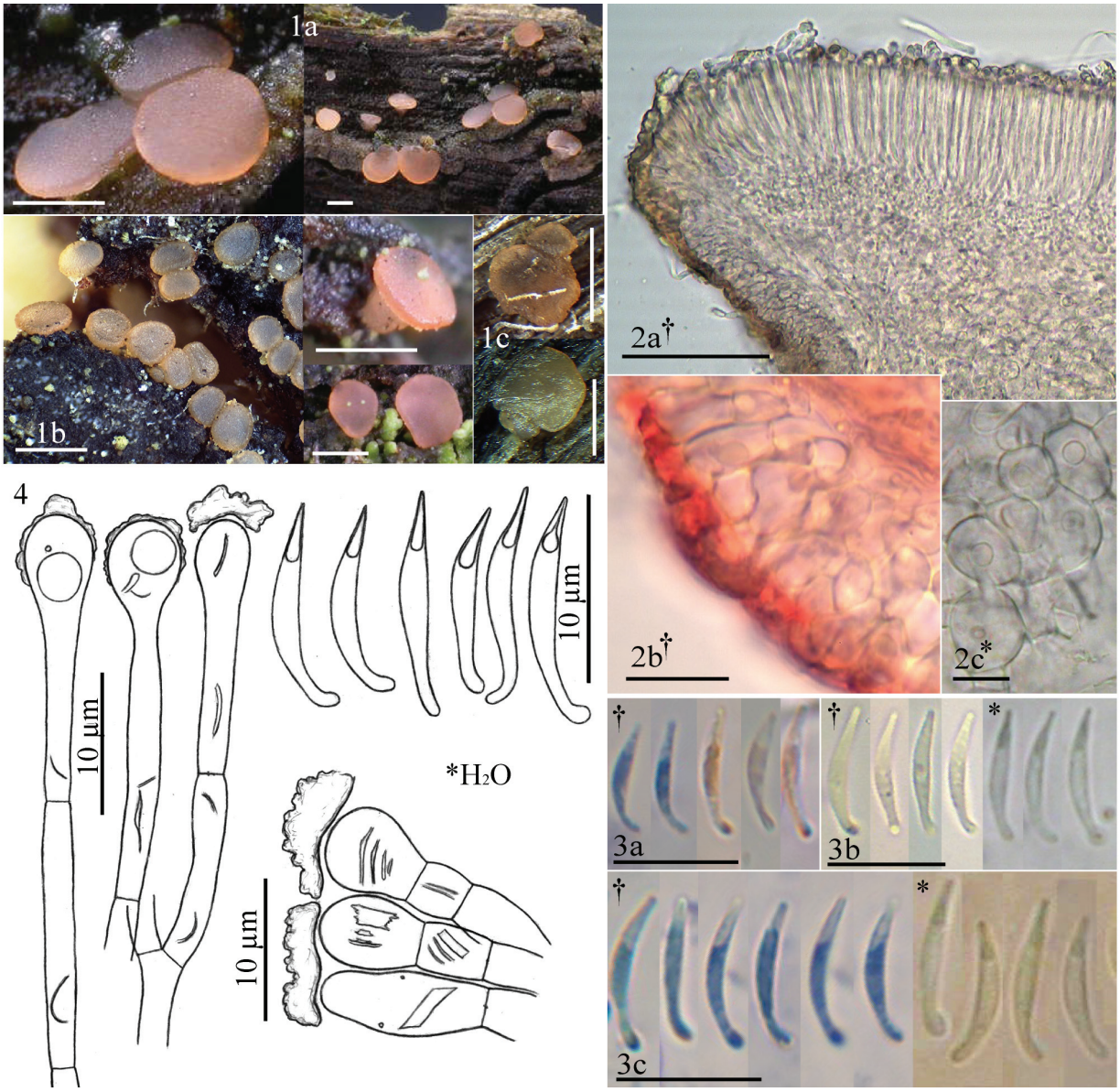

FIGURE 5. Morphological characteristics of *Orbilia aurantiorubra* on *Fabaceae* substrates. 1. Apothecia on natural substrates, scale bars 1a–c= 500 µm. 2. Section of apothecia, excipular characteristics and SCBs, scale bars 2a= 50 µm, 2b–c= 10 µm. 3. Dead (left) and living (right) ascospores, scale bars 3a–c= 10 µm. 4. Drawing of vital characteristics (ascospores, paraphyses, marginal ectal excipulum) for H.B. 7023. (H.B. 7308= 1c, 3a; H.B. 8395= 1a, 2b, 3c; H.B. 9238a= 1b, 2b, 3b).

Orbilia jugulospora Baral, *spec. nov.* (Fig. 6) MycoBank MB 806132

Differt ab Orbilia aurantiorubra ascosporis et corpusculis refringentibus angustioribus, excipulo ectali refractivis corpusculis refractivis solubilibus falcatis vel circuliformibus.

Type:—AFRICA. ETHIOPIA. Oromia: on unindentified angiosperm bark, 2520 m, 22 December 2009, Lindemann 9239 (holotype herb. Baral!, isotype M!).

Apothecia rehydrated (0.2–)0.3–0.7(–0.8) mm diam., 0.15–0.2 mm high (receptacle 0.11 mm), bright orange(-ochraceous) or rose(–pink), (semi-)translucent, round, scattered to gregarious; disc flat, margin distinct, not protruding, smooth; sessile or with a broad stipe 0.02–0.05 × 0.28 mm, superficial; dry bright orange-rose. *Asci* *46–50 × 4–4.2(5.5) {2}, †42–67 × 3.8–4.2 µm {1}, 8-spored, spores *4-seriate in two bundles, 3–5 lower spores inverted {2} (often strongly mixed, sometimes some upper spores inverted), pars sporifera *25 µm long; apex (†) strongly truncate. *Ascospores* *10.5–12(–13) × (1–)1.1–1.2(–1.3) µm {2}, †10–12 × 0.9–1.1 µm {2}, narrowly fusiform, apex acute

to acuminate, base narrowed in a tail with rounded, very slightly inflated end, strongly curved (helicoid), near base hook-like; *SBs* *3.5–4.5 {2} × 0.3–0.5 {2} or 0.5–0.8 µm, subulate, straight. *Paraphyses* apically slightly to medium clavate-capitate, terminal cells *26–27 × 3–4 µm {2}, lower cells *5.5 × 1.5–1.7 µm {2}; unbranched at upper septum, hymenium pale orange. *Medullary excipulum* hyaline to pale orange, 30–50 µm thick, of loose to dense textura intricata with inflated cells, sharply delimited from ectal excipulum (at mid flanks by a t. porrecta). *Ectal excipulum* pale orange, of (*) thin-walled, vertically oriented textura globulosa-angularis(-prismatica) from base to margin, 70–110 µm thick near base, cells *9–14 × 6–10 µm {2}; 20 µm thick at flanks, 15–20 µm at margin, oriented at a 50–80° angle to the surface, marginal cortical cells *7.5–10 × 3.5–5 µm {2}; glassy processes absent. *Anchoring hyphae* medium abundant, *1.8–2.5 µm wide, walls 0.2 µm thick {2}. *SCBs* globose, in paraphyses 1.5–2.4 µm diam, in basal and marginal ectal excipulum 2.5–3 µm diam., here also rod- to sickle- (half-moon) or ring-shaped {2}, medium to strongly refractive, subhyaline; *LBs* in ectal excipular cells at lower flanks 0.2–0.4 µm diam, in groups, bright yellow-orange. *Exudate* over paraphyses 0.2–2 µm thick, cloddy-continuous to granular, very pale yellowish, firmly attached, over margin and flanks 1–2.5 µm thick, cloddy.

Etymology:—Named after the very acute, spiky spore apex.

Distribution and ecology:—On medium decayed bark of ± xeric, corticated branches of unidentified angiosperms in a subtropical evergreen, predominantly broad-leaved subafromontane forest in the central plateau of the Ethiopian highlands, and in a subtropical evergreen, broad-leaved forest in Southeastern Asia. Phenology: unknown**.** Desiccation tolerance: ± fully viable for 3 weeks.

Other material examined:—ASIA. TAIWAN. Taipei: Wen Shan Qu, on unidentified angiosperm bark, 30 m, 27 September 1998, Kirschner (herb. Kirschner 405!, *duplic* herb. Baral 6250!).

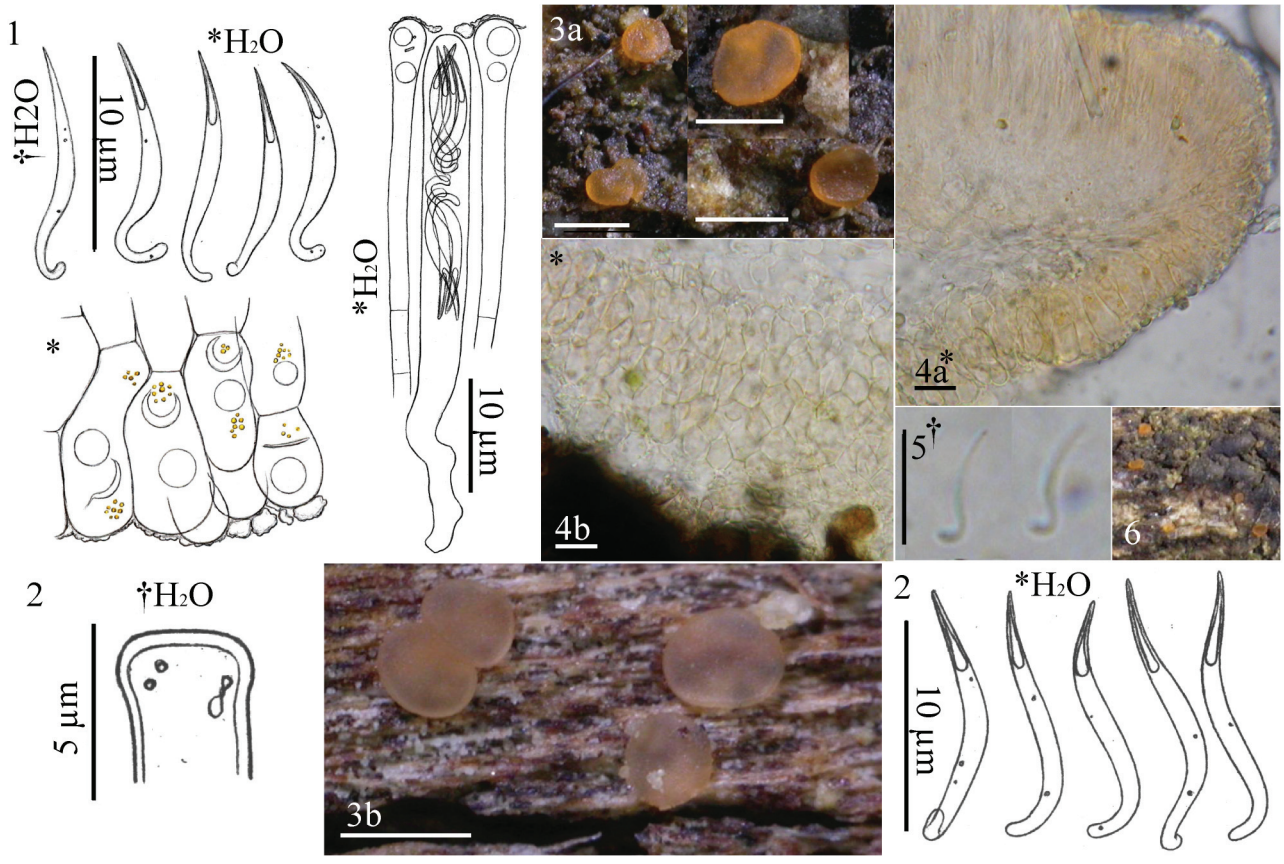

FIGURE 6. Morphological characteristics of *Orbilia jugulospora*. 1. Drawings for H.B. 9239 (ascus and paraphyses, ascospores, ectal excipulum at flanks). 2. Drawings for H.B. 6250 (ascus apex, ascospores). 3. Apothecia on natural substrate, scale bars 3a–b= 500 µm. 4. Section of apothecium, excipular characteristics, scale bars 4a–b= 10 µm. 5. Ascospores, scale bar= 10 µm. (H.B. 6250= 3b; H.B. 9239= 3a, 4a–b, 5).

Orbilia xanthoguttulata Baral, *spec. nov.* (Fig. 7) MycoBank MB 492257

Differt ab Orbilia aurantiorubra apotheciis plerumque vivide aureo-luteis, etiam ochraceo-aurantiacis, paraphysibus spathulatis, cellulis vivis terminalibus paraphysium et excipuli plerumque guttulas luteas oleosas continentibus, sine refractivis corpusculis solubilibus non globosis, margine saepe processis vitreis brevibus praedito, corpusculis refringentibus ascosporarum brevioribus. Habitat ad corticem, raro lignum, ramorum siccorum arborum angiospermarum, raro coniferarum, in zona temperata humida in Europa.

Type:—EUROPE. SPAIN. Asturias: on *Genista cf. florida*, 1625 m, 30 October 1991, A. Raitviir and R. Galán 6796 (holotype AH!, isotype herb. Baral!).

Apothecia rehydrated (0.2–)0.3–1(–1.5) mm diam., 0.19–0.31 mm high (receptacle 0.11–0.15(–0.22) mm), pale to light cream-yellowish or mostly golden yellow, also ochraceous-orange, sometimes turning deep orange to blood-red with age, somewhat translucent, round, lobate when large, scattered to gregarious; disc slightly concave to flat, margin ± thick, 0–15 µm protruding, smooth; often with a distinct, cylindrical to obconical stipe 0.05–0.2(–0.3) × 0.2–0.4(–0.8) mm, superficial or slightly erumpent from bast; dry light to deep honey-yellow-ochre to yellow-orange, orange-apricot-red, or vermilion-red. *Asci* *(47–)53–74(–82) × (4–)4.2–5(–5.3) µm {10} → 5.7–5.8 µm wide, †(40–)44–67(–79) × (3.5–)3.8–4.6(–5) µm {7}, 8-spored, spores *4-seriate, ± helicoidally twisted, (2–)3–5(–6) lower spores inverted {14} (not or rarely mixed), pars sporifera *23–30(–35) {8} or *30–36(–39) µm {1} long, †20–30 or 30–40 µm; apex (†) strongly truncate. *Ascospores* *(10–)11–14.5(–16) × (1.2–)1.3–1.6(–1.8) µm {17}, †(9.2–)11–14.5 × 1–1.5 µm {6}, narrowly fusiform with (sub-)cylindrical middle part, apex acute (to acuminate), base slightly to strongly attenuated, falcate to mostly distinctly helicoid, medium to strongly curved especially near base; *SBs* *(1.8–)2–3(–3.3) × 0.7–1.3 µm {16}, tear-shaped. *Paraphyses* apically (slightly to) medium or strongly inflated, spathulate (to sublageniform), terminal cells *11–31 × (2.5–)3–4.5(–5.7) µm {9}, †2.5–4.2 µm wide {2}, covered by glassy caps of exudates, exceeding the living or dead asci by 2–8 µm, lower cells *9–15 × 1.3–2.1 µm {4}, †1–1.7 µm wide {3}; never branched at upper septum; hymenium (pale to) light to bright yellow due to abundant yellow LBs. *Medullary excipulum* hyaline, 20–70 µm thick, sometimes up to 150 µm in centre, of medium loose to dense textura intricata with many inflated cells, very sharply delimited from ectal excipulum by a parallel 10–20(–30) µm thick layer of dense or loose t. porrecta. *Ectal excipulum* hyaline, towards margin usually light to bright yellow-orange, of thin-walled, vertically oriented t. globulosa–angularis–prismatica, 50–100(–180) µm thick at base, 30–100 µm at lower flanks, cells *10–25(–28) × 6–17(–20) µm {7}; 15–30 µm thick at mid flanks and margin, at margin of t. angularis–globulosa oriented at a 70–90° angle {8}, marginal cortical cells clavate to subglobose, *(4–)7–11(–14) × (3–)4–6(–9) µm {5}; glassy processes present {13}, (0–)1–5(–9) × 3–5(–7) µm, conical, low to high refractive, partly stratified, hyaline to pale yellowish, ± curved outwards if not very short, also ± absent {4}, often difficult to distinguish from exudate. *LBs* bright to deep golden yellow(-orange), near apex and in lower half of paraphyses 0.2–1(–2) µm diam., scattered or mostly rather abundant {>18}; in medullary excipulum 1–2.5 µm diam., sparse; in ectal excipulum scattered or abundant, small to large; deep blue-green-olive when applying IKI to water mount. *Exudate* over paraphyses forming individual, conical to convex or truncate glassy caps on nearly all apices, (0.3–)0.5–2 µm thick {>18}, smooth to warted, firmly attached, hyaline, over margin and flanks 0.2–0.5 µm thick.

Etymology:—Named according to the yellow lipid bodies in the paraphyses and marginal excipular cells.

Distribution and ecology:—From atlantic W- and subatlantic to subcontinental Central and boreal N-Europe, comprising planar or colline to subalpine, partly thermophilous forests at 4–1755 m altitude, with some records from oro- to Mediterranean Maquis in S- and SW-Europe, showing a remarkably diverse ecological amplitude, on gymno- and angiosperms, on slightly to strongly decayed bark, rarely on wood. Phenology: I–XII. Desiccation tolerance: some immature asci still viable after 4 months (Central Europe), some mature asci and excipular cells still alive after 31 months (Spain).

Other material examined:—EUROPE. FRANCE. Loire: on *Fraxinus excelsior*, 880 m, 6 May 2010, P. Chaillet (herb. Baral 9291!); Deux-Sèvres: on *Ulmus sp.*, 135 m, 1 May 2006, B. Coué (herb. Baral 8170!); Savoie: on *Salix sp.*, 1550 m, 6 May 2005, N. van Vooren (herb. Baral 7915b!); Drôme: on *Cercis siliquastrum*, 383 m, 12 September 2009, G. Marson (herb. Baral 9182!). GERMANY. Bayern: Oberbayern, on *Picea abies*, 1200 m, 30 September 1999, R. Kirschner (herb. Baral 6510!); Brandenburg: on *Quercus robur*, 55 m, 24 November 2007, R.K. Schumacher (herb. Baral 8698!). LUXEMBOURG. L'Oesling: on *cf. Populus tremula*, 310 m, 26 March 2001, G. Marson (herb. Baral 6989a!); *ibid.*, 26 April 1994, G. Marson (herb. Baral 5065!); Gutland: on *Fagus sylvatica*, 350 m, 21 August 2005, G. Marson (herb. Baral 7884!). SPAIN. Andalucía: on *Salix atrocinerea*, 663 m, 6 January 2004, F. Prieto and A. González (herb. Baral 7464!). SWEDEN. Hälsingland: on *Populus tremula*, 18 m, 23 July 2010, H.O. Baral and P. Perz (herb. Baral 9366!); Skåne: on *Ulmus glabra*, 135 m, 3 June 2006, T. Laessøe and H.O. Baral (herb. Baral 8208b!). SWITZERLAND. Glarus: on *Picea abies*, 1100 m, 6 September 1995, R. Kirschner (herb. Baral 5330!).

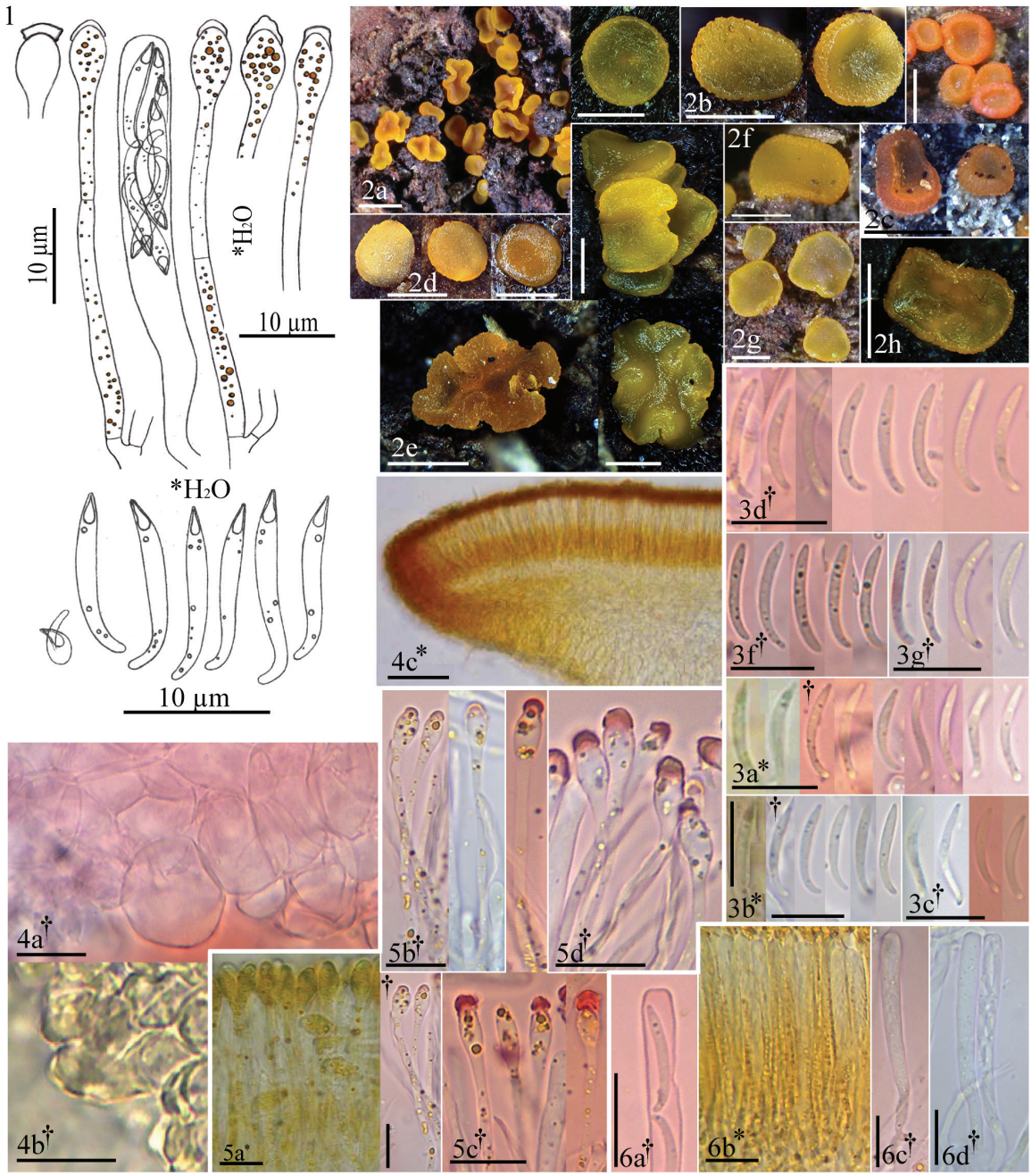

FIGURE 7. Morphological characteristics of *Orbilia xanthoguttulata*. 1. Drawing with vital characteristics for the isotype (H.B. 5092). 2. Apothecia on natural substrates, scale bars 2a–h= 500 µm. 3. Living and dead ascospores, scale bars 3a–g= 10 µm. 4. Section of apothecia, 4a at flanks, 4b at margin, with glassy processes, scale bars 4a–b= 10 µm, 4c= 50 µm. 5. Paraphyses. 6. Asci; scale bars 5a–d and 6a–d= 10 µm. (H.B. 6989a= 2a, 3d, 6a; H.B. 7464= 2b, 3g, 4a, 5d; H.B. 7915b= 2e, 3f, 4b; H.B. 8170= 2g, H.B. 8208= 2d, 3b, 4c, 5b, 6d; H.B. 8698= 2c, 3c, 5c, 6b; H.B. 9182= 2f; H.B. 9291= 2h, 3a, 5a, 6c).

Orbilia succulenticola L. Quijada, Baral & Beltrán-Tej., *spec. nov.* (Fig. 8) MycoBank MB 805598

Similis Orbiliae xanthoguttulatae sed ascosporis brevioribus, processis vitreis longioribus. Habitat ad ramos putridos siccos Euphorbiae spp. in zona semi-arida subtropica in Macaronesia.

Type:—EUROPE. SPAIN. Canary Islands. Tenerife: Punta Hidalgo-Chinamada, on *Euphorbia canariensis*, 350 m, 20 October 2008, L. Quijada & E. Rodriguez 20335 (holotype TFC Mic!, isotype herb. Baral!).

Apothecia rehydrated (0.3–)0.6–1(–1.2) mm diam., (0.18–)0.23–0.3(–0.37) mm high (receptacle 0.14–0.15 -> 0.1–0.12 mm), light yellowish-ochraceous, slightly translucent, ± round, scattered to gregarious in small groups; disc flat to slightly convex, margin thin, not protruding, very finely crenulate; sessile on an obconical stipe up to 0.07 × 0.35 mm, superficial; dry slightly concave, deep ochraceous-brownish(-orange). *Asci* *(51–)57–60(–67) × (4–)4.1–4.3(–4.8) µm

{6}, †(41–)48–51(–57) × (3.1–)3.4–3.7(–4) µm {10}, 8-spored, spores *3–4-seriate, (2–)3–4(5) lower spores inverted {6} (partly slightly mixed), pars sporifera *20–22 µm long; apex (†) strongly truncate. *Ascospores* *(9–)9.5–11(–12) × (1–)1.2–1.4(–1.6) µm {6}, †(8.3–)9.1–9.6(–10.4) × (0.9)–1–1.3 µm {10}, fusiform-clavate, apex acute, base medium to strongly attenuated in a ± distinct tail ca. ¼–⅓ of spore length, inequilateral to slightly curved in upper part, medium (to strongly) curved in region of tail, slightly helicoid; *SBs* *(1.7–)2–2.4(–2.6) × 0.8–1.2 µm {8}, tear-shaped, with a short, hardly visible filum. *Paraphyses* apically slightly to medium (to strongly) clavate-spathulate, terminal cell *(16.5–)21.4–24.5(–28) × (2.4)3–3.5(–4.3) µm {6}, (†) 1–8 µm longer than asci, lower cells *(5–)9.1–11(–14) × 1.5–2 µm {6}; rarely branched at upper septum, hymenium pale yellowish-ochraceous on surface and in lower part**.** *Medullary excipulum* hyaline, 70–124 µm thick, of dense textura intricata, horizontally oriented t. porrecta on flanks, with ± many inflated cells, sharply delimited from ectal excipulum mainly on flanks. *Ectal excipulum* hyaline, of (†) thin-walled, vertically oriented t. prismatica–angularis at base and flanks, 50–77 µm thick near base, cells *(8.4–)13.4–16.4(–28) × (6.6–)9–12(–18.5) µm {6}; 10–22 µm thick near margin, of light yellowis-ochraceous t. prismatica oriented at a 30–60° angle to the surface, marginal cortical cells *(7.7–)9.3–12(–13) ×(3.6–)4–4.6(–6) µm {6}; glassy processes 8–17.6 × 3–5 µm {6}, on flanks 2–10 × 4–6 µm, medium refractive, indistinctly stratified, consistently curved downwards, covered by yellowish exudate. *SCBs* in paraphyses and ectal excipulum absent, sometimes indistinct small globose SCBs seen in paraphyses. *LBs* bright to deep golden yellow(-orange), near apex and in lower half of paraphyses 0.4–0.7 µm diam., usually scattered {>10}; in medullary excipulum sparse more abundant near subhymenium; in ectal excipulum abundant near margin; exudate over paraphyses (0.3–)1.1–1.5(–2.1) µm thick, granular-cloddy, sometimes cap-like, pale yellowish-ochraceous, firmly attached, over margin and flanks 1.5–3.5 µm thick, cloddy.

Etymology:—The name refers to the succulent substrates on which the apothecia grows, *Euphorbia spp.* and *Opuntia maxima*.

Distribution and ecology:—In arid-semiarid succulent vegetation of the Canary Islands, *Euphorbia* scrubs, always on decorticated and dead branches near the ground, never on bark. Phenology: VIII–I. Desiccation tolerance: ectal and medullary excipulum, also some paraphyses and mature asci viable after 1 month.

Other material examined:—EUROPE. SPAIN. Canary Islands: Tenerife, Abades, Montaña Centinela, on *Euphorbia canariensis*, 167 m, 23 January 2010, L. Quijada (TFC Mic 22771!, 22779!); *ibid.*, Abades, Barranco la Vera, on *Euphorbia balsamifera*, 39 m, 17 October 2009, L. Quijada (TFC Mic 22437!, 22438!); *ibid.*, El Escobonal, Barranco de Erques, on *Euphorbia atropurpurea*, 216 m, 5 February 2010, L. Quijada (TFC Mic 22837!, 22822!); *ibid.*, La Matanza, Puntillo del Sol, on *Euphorbia canariensis*, 44 m, 21 November 2009, L. Quijada (TFC Mic 22562!); *ibid.*, Mesa del Mar, Hoya las Higueras, on *Euphorbia canariensis*, 74 m, 26 November 2009, L. Quijada (TFC Mic 22632!); *ibid.*, Punta Hidalgo, sendero a Chinamada, on *Euphorbia canariensis*, 402 m, 21 August 2011, L. Quijada (TFC Mic 23272!, 23273!); *ibid.*, on *Euphorbia lamarckii*, 402 m, 21 August 2011, L. Quijada (TFC Mic 23274!); *ibid.*, on *Opuntia maxima*, 362 m, 11 October 2009, L. Quijada (TFC Mic 22370!); *ibid.*, Teno, el Tosconito, on *Euphorbia lamarckii*, 216 m, 8 November 2009, L. Quijada (TFC Mic 22497!); *ibid.*, Teno, Cuchillo el Balo, on *Euphorbia balsamifera*, 111 m, 16 November 2009, L. Quijada (TFC Mic 22115!, 22118!, 22119!).

Discussion

Two main groups could be recognized within the four species treated here, which clustered in two different clades in our phylogenetic analysis. So that Clade I (*Orbilia xanthoguttulata* and *O. succulenticola*) and clade II (*O. aurantiorubra*, *O. jugulospora*) are not closely related, despite their morphological similarities.

Within *Orbilia aurantiorubra* s.l., two groups could be distinguished by our data: one containing specimens on non-*Fabaceae* substrates (*Ulmus* sp., *Salix* sp., *Rhamnus frangula*) that tend to have longer spores, and another one containing specimens on *Fabaceae* that tend to have shorter spores. Only two recent papers report such correlation: in United Kingdom, Spooner (2001) gave ascospore sizes of 11–14 µm on *Acer pseudoplatanus* and *Ulmus* sp.; and in La Palma (Canary Islands) Karasch *et al*. (2005) found ascospore sizes of 9.5–11 µm on *Chamaecytisus proliferus*. Boudier's holotype collected on *Salix* sp. (1907: 103, 1904–10: 268) belongs to the long-spored individuals. Boudier gave a spore size of 15–18 × 2 µm, but re-evaluation of his measurements on the holotype by the second author of this paper gave different data (†11.5–16.5 × 1.2–1.4 µm).

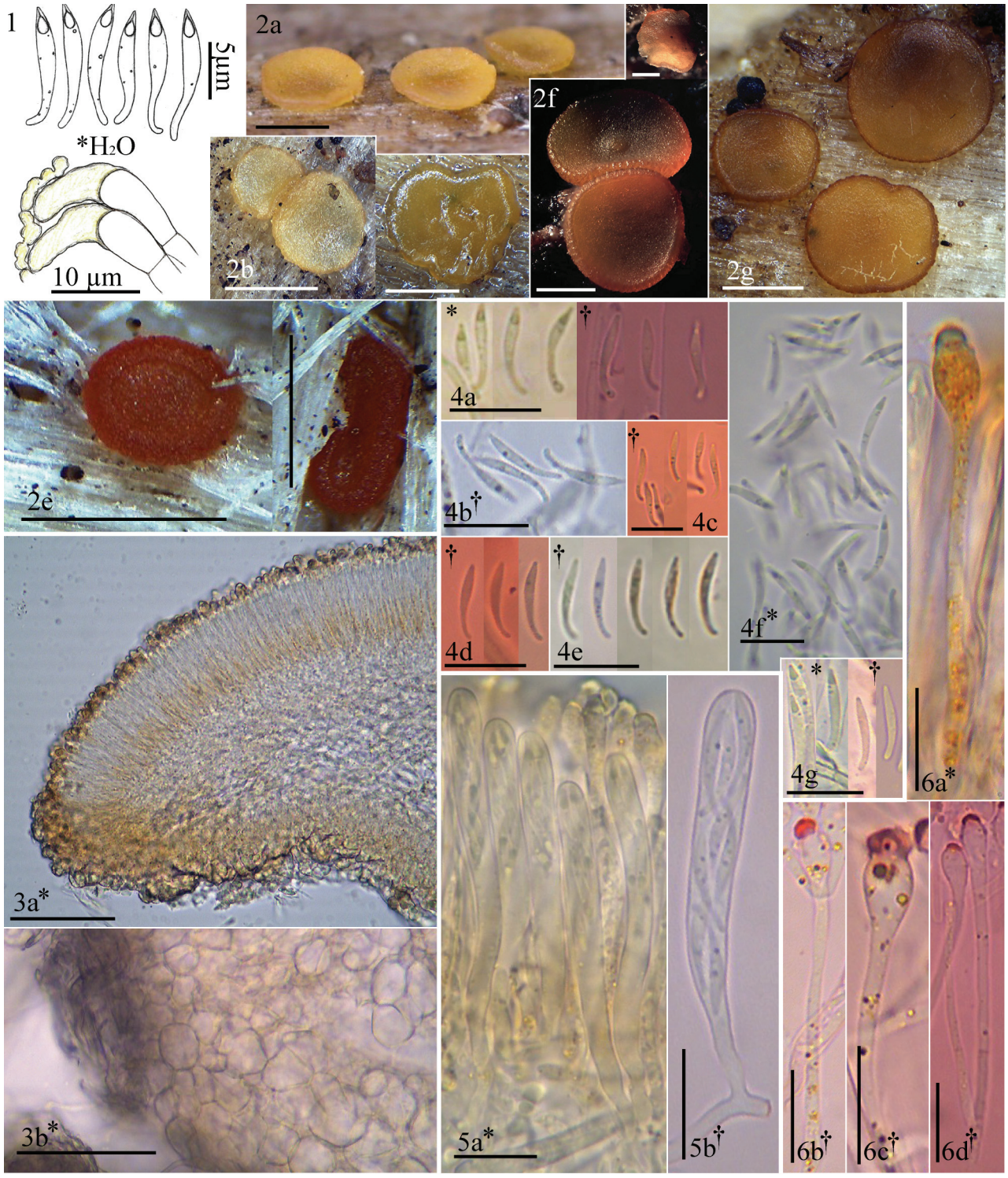

FIGURE 8. Morphological characteristics of *Orbilia succulenticola*. 1. Drawing for ascospores and marginal cortical cells with glassy processes for the isotype (H.B. 8958). 2. Apothecia on natural substrates, scale bars 2a–g = 500 µm. 3. Section of apothecia near the margin and on the base, scale bars 3a–b = 50 µm. 4. Living and dead ascospores. 5. Living and dead asci. 6. Paraphyses; scale bars 4a–g, 5a–b and 6a–d= 10 µm. (TFC Mic. 20335= 2a, 4a, 6d; TFC Mic. 22562= 2b, 3a, 4b, 6c; TFC Mic. 23272= 2e, 4g, 6b; TFC Mic. 22119= 2f; TFC Mic. 23274= 2g, 5b; TFC Mic. 23893= 3b; TFC Mic. 22779= 4c; TFC Mic. 22437= 4d; TFC Mic. 22497= 4e; TFC Mic. 24073= 4f; TFC Mic. 24074= 5a; TFC Mic. 23912= 6a).

We found significant biometrical differences between *O. aurantiorubra* on *Fabaceae* and on non-*Fabaceae* (Fig. 3), and both groups are phylogenetically well-supported (Fig. 1). However, two facts prevent us from suggesting the segregation of *O. aurantiorubra* until a greater number of collections is assessed by molecular methods: (1) the low number of clear nucleotide differences found between the two substrate types; and (2) the finding (H.-O. Baral, in

prep.) that some populations of *O. aurantiorubra* on *Salix* sp. (H.B. 8130, H.B. 8734a) tend to have comparatively short ascospores (10.5–13 µm), whereas some specimens on *Fabaceae* were found to have a similar intermediate spore length [e.g., H.B. 7298 on *Lupinus sp.* from England: 11–13 µm; Priou (2005) on *Cytisus scoparius* from France and Portugal: 9–13 µm].

Orbilia xanthoguttulata and *O. succulenticola* are easily recognized by their clavate-spathulate paraphysis apices which mostly contain rather abundant yellow lipid bodies. These are also found in the lower part of the paraphyses and in the marginal excipular tissue, and give the distinct yellow(-orange) colour to the apothecia. The morphology of ascospores is similar to *O. aurantiorubra* and *O. vermiformis* (Yu *et al*. 2007), but the latter two species differ from *O. xanthoguttulata* and *O. succulenticola* in apothecial colour, in paraphyses and excipular margin morphology and content. Although bootstrap support values for the two groups within sub-clade Ia were high (*O. xanthoguttulata* clade), we refrain from distinguishing more species, because the samples were morphologically rather uniform, and we did not expect such molecular variability. We found ten diagnostic characters shared by all specimens of *O. xanthoguttulata*, three for supported group H.B. 8208b-6989a, and two for H.B. 8698-9291, respectively (additional material, Table 4).

Orbilia succulenticola has shorter ascospores than *O. xanthoguttulata*, with sizes similar to those of *O. aurantiorubra* on Fabaceae (Fig. 3, L), but the spore bodies of *O. succulenticola* are distinctly shorter than in *O. aurantiorubra* s.l., and slightly shorter than in *O. xanthoguttulata* (Table 2). *Orbilia succulenticola* could be confused with *O. euphorbiae* (Henn.) Svrček, which also occurs in Euphorbia scrubs in the Canary Islands. However, the latter species has shorter spores (mainly *7–9 µm) with rounded to obtuse apices, and much smaller spore bodies (0.8–1.3 × 0.3–0.5 µm).

TABLE 4. Diagnostic characters (unique combination of nucleotides) found in the clade I of ITS sequences.

	Nucleotide position (bp) in the alignment																	
Orbilia taxa	25	34	38	93	94	99	130	147	350	359	360	373	385	395	404	477	488	507
O. vermiformis	G	G	A	0	C	C	A	G	G	0	0	T	G	A	G	G	C	C
O. xanthoguttulata	**T**	**A**	A	C	T	**A**	**C**	**C**	**C**	C	**C**	T	**0**	**T**	G	**0 / A**	0	0
O. xanth. group																		
H.B. 8208-6989	**T**	**A**	A	C	T	**A**	**C**	**C**	**C**	**T**	**C**	T	**0**	**T**	G	A	**A**	**A**
O. xanth. group																		
H.B. 8698-9291	**T**	**A**	A	**G**	**G**	**A**	**C**	**C**	**C**	C	**C**	T	**0**	**T**	G	A	T	T
O. succulenticola	A / C	G	**G**	C	T	C	**T**	**A**	**T**	C	**T**	**C**	**T**	**C**	**A**	G	T	T

Key to the species

1. Paraphyses and excipular cells containing bright yellow-orange globose LBs; SCBs absent or globose, SBs mean length shorter than *3 µm 2
– Paraphyses and often also excipular cells without LBs, SCBs present, globose to ring-shaped to trapezoid SCBs; SBs mean length longer than *3 µm 3
2. Spores larger than 11 µm; mean length of lower part of spore (below maximum width) longer than 7.5µm; on angio- and gymnosperm bark (rarely wood) in Europe ..... *O. xanthoguttulata*
– Spores usually up to 10.5 µm, mean length of lower part of spore (below maximum width) shorter than 7.5 µm; on *Euphorbia* scrubs in the Canary Islands ..... *O. succulenticola*
3. Spores *1–1.3 µm wide, SBs *0.3–0.8 µm wide; on angiosperm bark, NE-Africa, SE-Asia ..... *O. jugulospora*
– Spores *1.2–1.5 µm wide, SBs *(0.5–)0.7–1 µm wide; on bark (rarely wood) in the Canary Islands and Europe *O. aurantiorubra*

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