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Orbilia beltraniae, a new succulenticolous species from the Canary Islands

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Abstract

Orbilia beltraniae is a new succulenticolous species from the Canary Islands associated with *Euphorbia* scrubs. Phylogenetic analyses based on rDNA sequences of ITS and partial LSU were conducted to determine the relationships of the new species to others in the genus. Macro- and micromorphological, and ecology data are provided, as well as discussion in respect to closely related species. *Orbilia beltraniae* belongs to a strongly supported clade that includes non-nematophagous species of section *Arthrobotrys*, and its closest relatives are the European species *O. rectispora* and *O. cotoneastri*.

Key words

Ascomycota, ITS, LSU, morphology, *Orbiliaceae*, phylogeny, taxonomy

Introduction

Orbilia is by far the most specious genus of the *Orbiliomycetes* (Baral 2015, Baral in Jaklitsch et al. 2016). In the past its diversity was overlooked, mainly for the lack of exploration in drylands, as well as due to the morpho-taxonomical methods used (Baral 1992, 2015). Species occur in most ecosystems (from humid to arid, from subarctic to tropical) and most types of substrate (wood and bark, leaves, dung) and exposure (xeric, hygric, aquatic). In a monograph of *Orbiliomycetes*, more than 400 species are recognized in the genus (Baral 2015, Baral et al. in prep.).

Investigations on desiccation-tolerant fungi in their natural habitats are rare, although drylands occur on every continent and cover approximately 40% of the world's land area (UN 2011). Usually, such dry ecosystems have a low number of species, but a high amount of endemism (Lacoste and Salanon 1981, UN 2011, Davies et al. 2012). The studies done by E. Beltrán-Tejera and collaborators in drylands of Macaronesia have increased the knowledge of several groups, for example: (1) Basidiomycota with two new species and 23 new records, (2) Ascomycota with four new species and 10 new reports, and (3) Myxomycota with four new species and three new records (Beltrán-Tejera and Rodríguez-Armas 1999, Lado et al. 1999, Mosquera et al. 2000a, 2000b, 2003, Lado et al. 2007, Telleria et al. 2008, Beltrán-Tejera et al. 2010, Telleria et al. 2012, Quijada et al. 2012, Beltrán-Tejera et al. 2013, Quijada et al. 2014, 2015a, 2015b, 2015c).

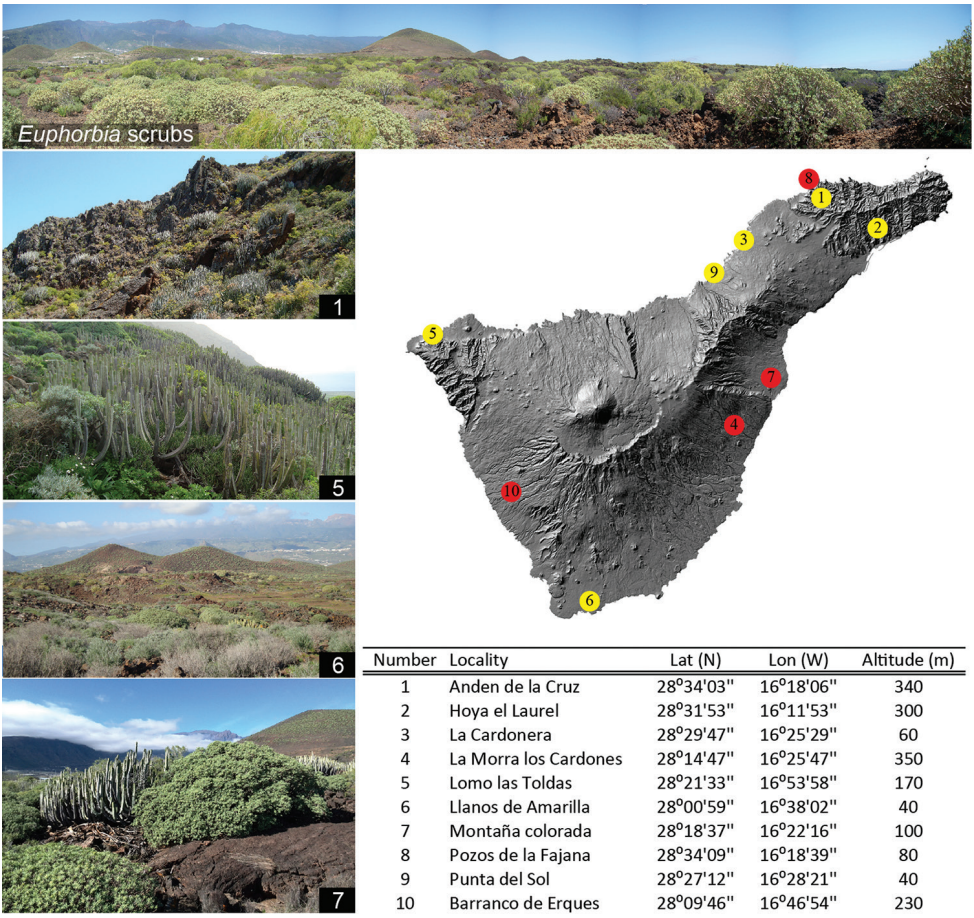
Euphorbia scrubs represents the native vegetation of drylands at lower elevations in the Canary Islands. These scrub lands are mainly composed of succulent plants (*Aeonium* Webb & Berthel, *Ceropegia* L., *Euphorbia* L., and *Kleinia* Mill) accompanied by other woody plants (*Artemisia* L., *Periploca* L., *Rubia* L.), with a high number of endemic species (>50%) (Del Arco et al. 2010). Since 2012, three new succulenticolous species of *Orbilia* have been published from this ecosystem and this type of substrate: *Orbilia asomatica* Baral, Quijada & Beltrán-Tej., *O. pisciformis* Baral, Quijada & Beltrán-Tej. and *O. succulenticola* Quijada, Baral & Beltrán- Tej. (Quijada et al. 2012, 2014). The aim of this investigation is to describe a new species of *Orbilia* that develops on the succulent remains of *Euphorbia canariensis*.

Methods

Specimens were collected in Tenerife (Canary Islands, Spain) during 2008–2014. Ten localities of *Euphorbia* scrubs were monitored in both the rainy season (September to May) and dry season (June to August), along an altitudinal transect (40–350 m) including both northern and southern slopes (Fig. 1). The sampling was restricted to the largest branches lying on the ground. Species of the following native succulent genera were sampled: *Aeonium*, *Ceropegia*, *Euphorbia*, and *Kleinia*.

Macro- and microscopic studies

Observations were made with a Motic stereomicroscope SMZ140, and with a Motic B1 light microscope. Microphotographs were taken with an USB Moticam 2500 camera and processed with the software Motic images Plus 2.0. Specimens were studied in both the living and dead state. Collection data and measurements followed methods of Quijada et al. (2012, 2014). Cell walls were sometimes contrasted with Congo Red



Number	Locality	Lat (N)	Lon (W)	Altitude (m)
1	Anden de la Cruz	28°34'03"	16°18'06"	340
2	Hoya el Laurel	28°31'53"	16°11'53"	300
3	La Cardonera	28°29'47"	16°25'29"	60
4	La Morra los Cardones	28°14'47"	16°25'47"	350
5	Lomo las Toldas	28°21'33"	16°53'58"	170
6	Llanos de Amarilla	28°00'59"	16°38'02"	40
7	Montaña colorada	28°18'37"	16°22'16"	100
8	Pozos de la Fajana	28°34'09"	16°18'39"	80
9	Punta del Sol	28°27'12"	16°28'21"	40
10	Barranco de Erques	28°09'46"	16°46'54"	230

Figure 1. Monitored localities in *Euphorbia* scrubs: number of locality, place name for locality according to IDECanarias visor 3.0 (http://visor.grafcan.es/visorweb/), latitude, longitude, and altitude. In yellow the localities where *Orbilia beltraniae* was found.

(CR). Potassium hydroxide 5% (KOH) as mountant was used to measure dead cells for comparison with old herbarium specimens of related species. The following abbreviations were adopted (Baral 1992): * = living state; † = dead state; *† = living and dead state (no difference noted); SCBs = KOH-soluble cytoplasmic bodies; SBs = spore bodies; VBs = refractive vacuolar bodies; LBs = lipid bodies. Colour coding refers to Anonymous (1976).

DNA extraction, PCR amplification, and DNA sequencing

For complete details about DNA extraction, PCR amplification, PCR purification, and cycle sequencing see Baral et al. (2017b). Sequences were obtained of the complete

Table 1. Specimens used in this study with voucher information and GenBank accession numbers. *Orbilia beltraniae* sequences are indicated in bold. Species with asterisk have at present unpublished names (Baral et al. 2017a).

Species	Collection	Section	GenBank number
Orbilia dryadum	H.B. 6876a	*Orbilia*	KT215281
Orbilia leucostigma	H.B. 6810c	*Orbilia*	KT215282
Orbilia polyspora	H.B. 7243b	*Ovoideae*	KT215276
*Orbilia sphaerospora**	H.B. 9129	*Ovoideae*	KT222429
*Orbilia ovoidea**	H.B. 6489a	*Ovoideae*	KT215275
*Orbilia canadensis**	H.B. 6826	*Ovoideae*	KT215277
Orbilia asomatica	TFC Mic. 21258	*Arthrobotrys*	KT222399
Orbilia auricolor	H.B. 6664	*Arthrobotrys*	KT215294
Orbilia beltraniae	**TFC Mic. 24363**	***Arthrobotrys***	**KT222405**
Orbilia beltraniae	**TFC Mic. 23890**	***Arthrobotrys***	**KT222406**
Orbilia cardui	H.B. 9891	*Arthrobotrys*	KT222402
Orbilia cotoneastri	CBS 116281	*Arthrobotrys*	KT215288
Orbilia quercus	HMAS 88783	*Arthrobotrys*	AY804213/DQ656669
Orbilia rectispora	H.B. 7142	*Arthrobotrys*	KT215289
*Orbilia xinjiangensis**	H.B. 9646	*Arthrobotrys*	KT222435
*Orbilia mammillata**	H.B. 7165c	*Arthrobotrys*	KT215290
Gamsylella gephyropaga	ATCC 96677	*Arthrobotrys*	EF445990
Arthrobotrys oligospora	ATCC 96709	*Arthrobotrys*	EF445989
Drechslerella brochopaga	ATCC 96710	*Arthrobotrys*	EF445987
Drechslerella doedycoides	ATCC 96778	*Arthrobotrys*	EF445992
Orbilia aristata	H.B. 6713	*Hemiorbilia*	KT596782
*Orbilia clavuliformis**	H.B. 6714	*Hemiorbilia*	KT215271
*Orbilia subaristata**	H.B. 6685a	*Hemiorbilia*	KT215270
Orbilia flavida	H.B. 6716	*Lentiformes*	KT215228
*Orbilia subocellata**	H.B. 6474	*Lentiformes*	KT215227
*Orbilia cucumispora**	H.B. 6762a	*Lentiformes*	KT215231
Orbilia gambelii	H.B. 6466	*Habrostictis*	KT215249
*Orbilia subvitalbae**	H.B. 6504a	*Habrostictis*	KT215250
*Orbilia microserpens**	H.B. 6519a	*Habrostictis*	KT215251
*Orbilia cisti**	H.B. 6500	*Habrostictis*	KT215251
Orbilia aurantiorubra	H.B. 6815a	*Aurantiorubrae*	KF741595
Orbilia comma	H.B. 6639b	*Aurantiorubrae*	KT215258
Orbilia phragmotricha	H.B. 7535a	*Aurantiorubrae*	KT215259
*Orbilia denticulata**	H.B. 6725	*Aurantiorubrae*	KT215256
*Orbilia brachychitonis**	H.B. 7578a	*Aurantiorubrae*	KT215257
Orbilia sinensis-1	YMF1.01843	*Helicoon*	DQ480727/DQ480728
Orbilia sinensis-2	HMAS 96782	*Helicoon*	DQ656642/DQ656676
Orbilia sarraziniana	H.B. 7235	*Helicoon*	KM199780
*Orbilia rosea**	H.B. 6756a	*Helicoon*	KM199779
*Orbilia fusiformis**	YMF1.01848	*Helicoon*	EF026114/EF026115
Hyalorbilia polypori	H.B. 7557a	outgroup	KT215223

internal transcribed spacer region (ITS, ~500 bp), comprising ITS1, the 5.8S ribosomal subunit, and ITS2, and the partial nuclear large subunit (LSU, D1-D2 region, ~630 bp). All sequences are deposited in GenBank (Table 1).

Phylogenetic analyses

The data matrix for alignment was constructed to explore the phylogenetic relationships. 42 sequences were included, representing eight sections (*Orbilia*, *Arthrobotrys*, *Ovoideae*, *Habrostictis*, *Hemiorbilia*, *Lentiformes*, *Aurantiorubrae*, *Helicoon*) according to Baral (2015; Baral et al. 2017a). The sequences were aligned using the L-INS-i algorithm for ITS region and the G-INS-i algorithm for LSU region with MAFFT v7.017 (Katoh et al. 2002). The program GBLOCKS v. 0.91b was used to identify and eliminate ambiguously aligned regions (Castresana 2000), using the same relaxed setting as in Quijada et al. (2014). The final alignment contained 943 bp (83% of the first alignment length). The analyses were performed using the optimal model of nucleotide substitution identified with JMODELTEST (Posada 2008; http://darwin.uvigo.es), based on the Akaike information criterion (Akaike 1974). Maximum Likelihood (ML) and Bayesian Inference (BI) analysis were performed using Geneious (v. 6.1.7). Branch support in ML analyses was inferred from 1000 rounds of bootstrap. For more details about phylogenetic methods see Quijada et al. (2014). 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

Phylogenetic results

The alignment consisted of 943 bp characters, of which 322 were parsimony-informative, 415 were variable, and 528 were constant. Only the Bayesian consensus tree is shown (Fig. 2) because overall topologies of the ML and BI analyses were identical.

Each section constituted a supported clade except for section *Orbilia*, with only two species in this analysis (*O. leucostigma* and *O. dryadum*, 71.8% MLBS, 0.94 BIPP). The two sequences of *O. beltraniae* are completely identical in their overlapping part that includes also LSU (D1-D4) and a short part of SSU. This section is divided in several supported clades. *Orbilia beltraniae* clusters with five selected species (*Orbilia xinjiangensis*, *O. cotoneastri*, *O. rectispora*, *O. asomatica*, *O. cardui*) in one supported clade (clade I, 89.3% MLBS, 0.99 BIPP). The other four clades (II–V) are represented by groups of one to three selected species. Clade II includes two species of *Drechslerella* (99.9% MLBS, 1 BIPP), clade III two species of *Arthrobotrys* (100% MLBS, 1 BIPP), clade IV two species of *Dactylellina* with low support (47.6 MLBS, 0.74 BIPP), and clade V with one species of *Gamsylella*.

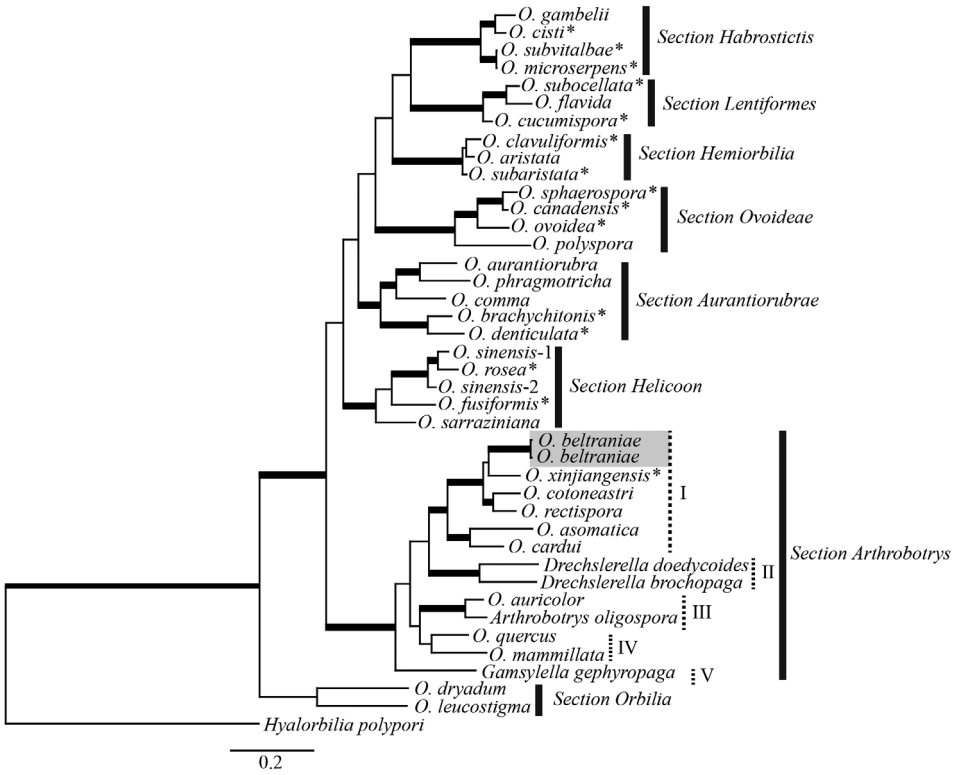


Figure 2. Bayesian 50% majority rule consensus tree based on a concatenated alignment of ITS and LSU sequences of 42 strains of *Orbiliaceae* (see Table 1). Bold branches were supported by ML bootstrap values ≥75% and BI PP≥0.95. Subgroups within section *Arthrobotrys* have been recognised by other authors in five different genera according to their asexual states and trapping devices: **I** *Dactylella* **II** *Drechslerella* **III** *Arthrobotrys* **IV** *Dactylellina* **V** *Gamsylella*. * = name at present not validly published (Baral et al. 2017a).

Taxonomy

Orbilia beltraniae **Quijada, Baral & G. Marson, sp. nov.**
Mycobank: MB813971
Fig. 3

Type. SPAIN. Canary Islands: Tenerife, Tacoronte, La Cardonera, 28°29'47"N, 16°25'29"W, 60 m alt., on detached branch of *Euphorbia canariensis* lying on the ground, 30 Oct 2013, L. Quijada (holotype: TFC Mic. 24363, isotype TFC Mic. 24359).

Diagnosis. *Similis Orbiliae cotoneastri sed ascosporae longiores, paraphyses ad apicem leniter vel modice lanceolato-lageniformes. Habitat ad ramos* Euphorbiae canariensis *in zona subtropica (semi-)arida Macaronesiae.*

Etymology. The specific epithet refers to Esperanza Beltrán-Tejera in recognition of her many contributions to the development of Canarian mycology, of her work in education and of our friendship.

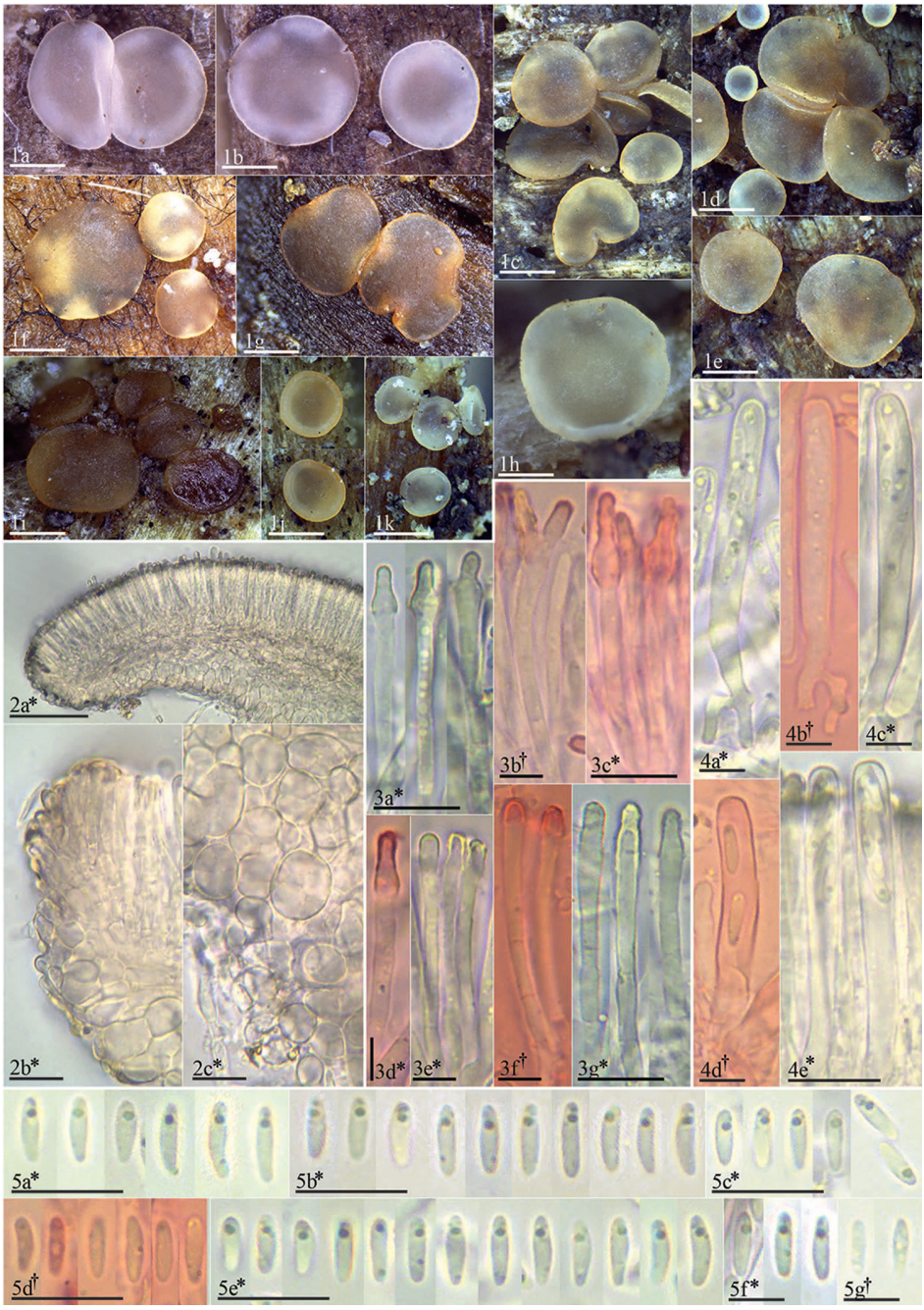


Figure 3. Morphological features of *Orbilia beltraniae*. **1** Apothecia rehydrated after 1–2 weeks **2** Excipular tissues in median section **3** Paraphyses **4** Asci **5** Ascospores. Scale bars: 500 µm= **1a–k**; 50 µm= **2a**; 10 µm= **2b–c, 3a, c, g, 4e, 5a–e**; 5 µm= **3b, d–f, 4a–d, 5f**; Mounted in: CR= **3b–d, 4b, d, 5d**; H_2O= **2a–c, 3a, e–g, 4a, c, e, 5a–c, e–g**. Photos: TFC Mic. 23771 = **1i–k, 3b–c, 4b, 5a**; TFC Mic. 23836 = **1h, 2c, 3d–e, 5b**; TFC Mic. 23890 = **1d–e, 4a, 5f**; TFC Mic. 23902 = **1f–g, 3a, 4c**; TFC Mic. 24231 = **2a, 3g, 5c, g**; TFC Mic. 24363 =**1c, 2b, 3f, 4d–e, 5d–e**; TFC Mic. 24449 = **1a–b**.

Description. *Apothecia* moist 0.4–1.2(1.5) mm diam, 0.1–0.2 mm thick (receptacle 0.06–0.07 mm), pale white (231. p White) to medium orange yellow (71. OY), rarely medium yellow brown (77. m y Br), medium translucent, round to somewhat undulating, gregarious in small groups; disc flat, margin thin, ± smooth, not protruding, sessile on a broad base, superficial. *Asci* *(32)38–40(46) × (3)3.5–4.5 µm, †(27.5)33–35(43) × (2.5)3–3.5 µm, 8-spored, 4–6 lower spores inverted, pars sporifera *14–20 µm; apex strongly truncate (not indented, not inflated), thin-walled; base with short to medium long stalk, h- or H-shaped. *Ascospores* *(4.5)5.5–6(7) × (1.2)1.4–1.6(2) µm, †(4)4.5–5 × 1–1.5 µm, cylindrical to slightly fusoid-clavate, with rounded to obtuse ends, straight or slightly curved, only slightly (rarely medium) tapered below; *SBs* *(1)1.5–2 × 0.5–1 µm, globose, often ± eccentrical, sometimes with distinct filum. *Paraphyses* uninflated to often slightly to medium lanceolate-lageniform with rounded tip, terminal cell *(13.5)17.5–19.5(25) × 2–3 µm, 3–5 µm protruding beyond living asci, lower cells *(4.5)7–9.5(10.5) × 1.5–2.5 µm, unbranched at upper septum, rarely with a bifurcate apex. *Medullary excipulum* 20–45 µm thick, of dense *textura intricata* with inflated cells, sharply delimited. *Ectal excipulum* of thin-walled *t. globulosa-angularis*, at base 40–105 µm thick, cells *(7.5)11–13(18) × (4.5)8–9.5(13) µm, at margin 10–28 µm thick, oriented at a 40–80° angle to the surface, marginal cortical cells *(5.5)7.5–8.5(12.5) × (2.5)3.5–4.5(7) µm, not forming distinct cell rows; glassy processes absent. *Anchoring hyphae* rather sparse, *1.5–3.5 µm diam, wall 0.2 µm thick. *SCBs* only present in paraphyses, globose, numerous in each terminal cell, *VBs* absent. *Exudate* forming a thin and firmly attached layer over paraphyses, up 1 µm thick on marginal cortical cells. Asexual state unknown.

Distribution and ecology. *Orbilia beltraniae* is so far only known from Tenerife island (Canarian archipelago, Macaronesia).

The species has been found between 40–340 m alt., from the coast to lowland elevations, on the northern and southern slopes where *Euphorbia* scrubs develop. All specimens have to date been collected on *Euphorbia canariensis*, so it seems that the species is host specific, growing during the rainy period between autumn and spring on succulent wood of detached, dead branches lying on the ground. Its desiccation tolerance was not thoroughly explored, but it can be said that its mature asci can survive at least one or two weeks in the herbarium. Although no apothecia of *O. beltraniae* were found in summer, several specimens were found to be fully alive during dry periods without rain in winter and spring, which permits us to conclude that the apothecia tolerate desiccation and probably survive over the summer to continue growth in autumn.

Other specimens examined. *Orbilia beltraniae* (all on detached branches of *Euphorbia canariensis* lying on the ground). SPAIN. Canary Island: Tenerife, Buena Vista del Norte, Lomo las Toldas, 28°21'33"N, 16°53'58"W, 170 m alt., 27 Dec 2012, L. Quijada (TFC Mic. 23836); La Laguna, Andén de la Cruz, 28°34'03"N, 16°18'06"W, 340 m alt., 20 May 2013, L. Quijada (TFC Mic. 24231); 29 Dec 2013, L. Quijada (TFC Mic. 24449); La Matanza de Acentejo, Punta del Sol, 28°27'12"N, 16°28'21"W, 40 m alt., 2 Mar 2013, L. Quijada (TFC Mic. 23890); S/Cruz de Tenerife, Hoya el Laurel, 28°31'53"N, 16°11'53"W, 300 m alt., 5 Mar 2013, L. Quijada (TFC Mic.

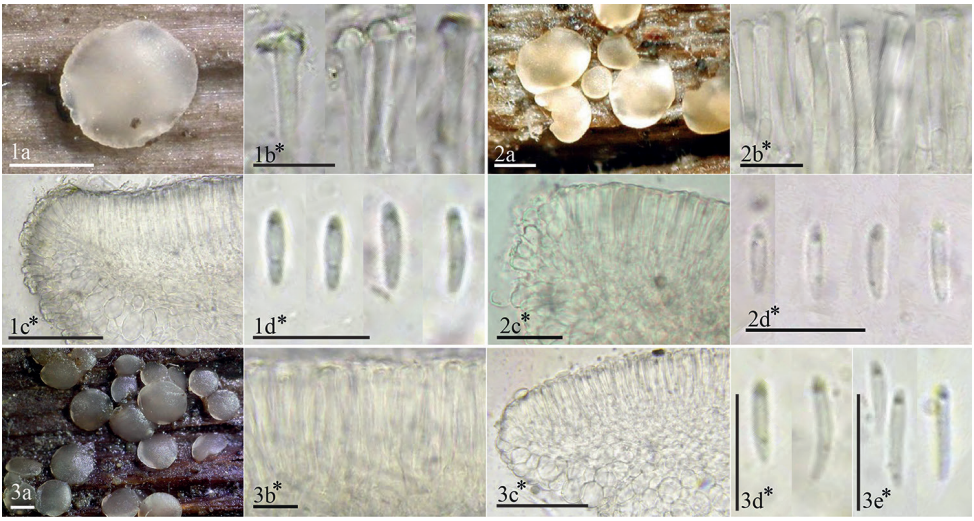


Figure 4. Morphological features of species related to *Orbilia beltraniae*. **1** *Orbilia cardui* **2** *Orbilia cotoneastri* **3** *Orbilia rectispora*. **a** Fresh apothecia **b** Paraphyses **c** Excipular tissues in median section **d–e**. Ascospores. Scale bars: 500 µm= **1a, 2a, 3a**; 50 µm= **1c, 3b**; 10 µm= **1b, d, 2b–d, 3c–e**. All mounted in H_2O. Photos: H.B. 7241a = **2a**; H.B. 9549 = **3b, d**; H.B. 9645b = **2b, d**; H.B. 9891 = **1a, c–d**; H.B. 9901 = **1b**; H.B. 9962 = **3a, c, e**; J.P.P. 28122 = **2c**.

23902); San Miguel de Abona, Llanos de Amarilla, 28°00'59"N, 16°38'02"W, 40 m alt., 16 Dec 2012, L. Quijada (TFC Mic. 23771).

Orbilia cardui. EUROPE. Germany: Sachsen, 6 km NNE of Chemnitz, 1 km E of Glösa, Indianerteich, 50°53'10"N; 12°57'23"E, 330 m alt., on stem of *Angelica sylvestris*, 15 May 2014, B. Mühler (H.B. 9891); Luxembourg: Esch-sur-Alzette, 2 km NNE of Dudelange, 1.5 km S of Bettembourg, Triage, 49°30'15"N; 06°05'50"E, 280 m alt., on herbaceous stems of undetermined dicotyledoneous, 25 Jul 2014, G. Marson (H.B. 9901).

Orbilia cotoneastri. EUROPE. France: Bretagne, Morbihan, 12 km S of Auray, 1.6 km SW of Locmariaquer, Breneguy, 47°33'35"N; 02°57'42"W, 5 m alt., on wood of branch of *Ulex europaeus*, 3 Nov 2002, J.P. Priou (H.B. 7241a); 5.3 km S of La Gacilly, 2 km N of St.- Vincent-sur-Oust, La Provostaie, 47°43'04"N; 02°08'50"W, 5 m alt., on stems of *Fallopia sachalinensis*, 3 Jun 2008, J.P. Priou (J.P.P. 28122); Galicia: La Coruña, 18 km SE of Coruña, SE of Betanzos, N of Calle de Concepción Arenal, 43°16'34"N; 08°12'21"W, 40 m alt., on bark of branch of *Quercus robur*, 31 Dec 2011, B.A. Rodríguez (H.B. 9645b).

Orbilia rectispora. EUROPE. Germany: Mecklenburg-Vorpommern, 0.5 km SE of Rehna, Radegasttal, 53°46'30"N; 11°03'30"E, 20 m alt., on culms of *Sparganium erectum*, 11 Jul 2015, T. Richter (H.B. 9962); Great Britain: Yorkshire, South Yorkshire, 3.5 km S of Barnsley, 1.5 km SW of Worsbrough, Worsbrough Country Park, 53°31'15"N; 01°28'55"W, 70 m alt., on leaves of *Typha latifolia*, 20 May 2011, H.O. Baral (H.B. 9549).

Discussion

Section *Arthrobotrys* will be proposed in the monograph of *Orbiliomycetes* (Baral et al. in prep.) as a subgroup of *Orbilia* to accomodate species with narrowly sickle-shaped, rod-shaped, or ellipsoid ascospores, desiccation-tolerant or -sensitive apothecia, and asexual states that either form various types of organs for trapping nematodes or other invertebrates or do not form trapping devices in culture when nematodes are added. *Orbilia beltraniae* is phylogenetically close to *O. cotoneastri* and *O. rectispora*, which belong to the group of section *Arthrobotrys* in which trapping organs are not formed, and that are currently referred to the asexual state genus *Dactylella* Grove. *Orbilia cotoneastri* has ascospores and spore bodies very similar to those of *O. beltraniae*, but differ in paraphysis morphology, showing uninflated or rarely capitate-clavate apices; in *O. beltraniae* they are uninflated to medium lanceolate-lageniform with rounded tips. *Orbilia rectispora* has longer ascospores than *O. beltraniae* [up to 9.5(11) µm vs. up to 6.5(7) µm]. In addition, the three species have a very different ecology: *O. beltraniae* occurs on succulent substrates in semiarid places of the Canary Islands, *O. cotoneastri* occurs on wood, bark, and herbaceous stems in moist places of Europe, and *O. rectispora* occurs on leaves of monocots in wet places of Europe (Baral et al. in prep).

Orbilia cardui is phylogenetically more distantly related to the above species, but more similar to it in size of asci, ascospores, and spore bodies. Also here, the paraphyses shape distinguishes *O. beltraniae* from *O. cardui* which has slighty capitate paraphyses. *Orbilia cardui* has a wide ecological spectrum in Europe, growing in shady forests and open ruderal places or wetlands on wood, bark, herbaceous stems, and even on other fungi (Baral et al. in prep), whereas *O. beltraniae* is apparently restricted to wood of the Canarian endemic succulent *Euphorbia canariensis*.

Orbilia beltraniae is the fourth species so far found exclusively in the *Euphorbia* scrubs of the Canary Islands. Although it seems to be host specific on *Euphorbia canariensis*, the three previously described species (*Orbilia asomatica*, *O. pisciformis*, *O. succulenticola*) share this substrate but they also develop on other succulent species like *E. balsamifera*, *E. lamarckii*, and *E. atropurpurea* (Quijada et al. 2012, 2014, 2015c).

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