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Emendation of *Marcelleina* Brumm., Korf & Rifai (Ascomycota, Pezizales) and proposal of *Iodomarcelleina obscura* gen. nov., sp. nov. and *Scotopezia pseudoanthracina* gen. nov., comb. nov. for the lineages outside it

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ABSTRACT
Iodomarcelleina obscura gen. nov., sp. nov. is described from Malta. This species is morphologically close to *Marcelleina* Brumm., Korf & Rifai but differs from species of this genus in the size and ornamentation of its ascospores and its amyloid asci, a taxonomic character lacking in species of *Marcelleina*. Ecologically *I. obscura* gen. nov., sp. nov. seems to prefer calcareous soils, fruiting in open sunny grounds as well as shaded areas. Genetically, it is nested in the *Marcelleina-Ionopezia* lineage of Pezizaceae Dumort., but outside of the *Marcelleina s.str.* clade. *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov., another species not supported within *Marcelleina s.str.*, differs from *I. obscura* gen. nov., sp. nov. by its smaller and ornamented ascospores, as well as its genetic profile. Finally, the newly obtained data suggest that *Marcelleina mediterranea* Lantieri & Pfister is a synonym of *M. tuberculispora* K.Hansen & Sandal. As emended herein, the genus *Marcelleina* excludes species with amyloid asci.

KEY WORDS
Mediterranean mycobiota, Pezizaceae, new genera, new species, new combination, new synonym.

RÉSUMÉ
Amendement de Marcelleina *Brumm., Korf & Rifai (Ascomycota, Pezizales) et proposition de* Iodomarcelleina obscura *gen. nov., sp. nov. et* Scotopezia pseudoanthracina *gen. nov., comb. nov. pour les lignées exclues. Iodomarcelleina obscura* gen. nov, sp. nov. est décrite de Malte. Cette espèce est proche morphologiquement de *Marcelleina* Brumm., Korf & Rifai, mais diffère des espèces de ce genre par la taille et l'ornementation de ses ascospores, et ses asques amyloïdes, une caractéristique absente chez les *Marcelleina*. Écologiquement, *I. obscura* gen. nov., sp. nov. semble préférer les sols calcaires, fructifiant aussi bien sur terrains ensoleillés qu'ombragés. Génétiquement, elle se place au sein de la lignée *Marcelleina-Ionopezia* des Pezizaceae Dumort., mais en dehors du clade des *Marcelleina s.str*. *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov., une autre espèce non supportée au sein des *Marcelleina s.str.*, diffère de *I. obscura* gen. nov., sp. nov. par ses ascospores plus petites et ornementées, ainsi que par son profil génétique. Enfin, les nouvelles données obtenues suggèrent que *Marcelleina mediterranea* Lantieri & Pfister est un synonyme de *M. tuberculispora* K.Hansen & Sandal. Tel qu'amendé ici, le genre *Marcelleina* exclut les espèces avec des asques amyloïdes.

MOTS CLÉS
Mycobiote méditerranéen, Pezizaceae, genres nouveaux, espèce nouvelle, combinaison nouvelle, synonyme nouveau.

INTRODUCTION

During a field investigation trip to the northern part of Malta an unusual small, dark brown, disc shaped discomycete was collected and studied. The specimen was initially determined as a species of *Marcelleina* Brumm., Korf & Rifai, but the amyloid reaction of asci was unusual for this genus, only known in *Marcelleina pseudoanthracina* (Donadini) R.Kristiansen & J.Moravec which produces an ambiguous reaction. The sample was subsequently sequenced along with several other collections of *Marcelleina s.str.* and one species of *Delastria* Tul. & C.Tul., to evaluate the phylogenetic structure of the whole group.

The genus *Marcelleina* was erected in the monographic work on *Ascobolus* Pers. ex J.F.Gmel. and *Saccobolus* Boud. by Brummelen (1967) to accommodate a part of the type collection of *Ascobolus persoonii* P.Crouan & H.Crouan, in which two different but quite similar species where found. As the plate accompanying the description of *A. persoonii* shows reticulate ascospores (Crouan & Crouan 1867: fig. "4"), Brummelen (1967) retained this epithet for the species named *Marcelleina persoonii* (P.Crouan & H.Crouan) Brumm., and designated the other part of material with the newly coined name *Marcelleina atroviolacea* (Delile ex De Seynes) Brumm., now considered a posterior synonym of *Smardaea planchonis* (Dunal ex Boud.) Korf & W.Y.Zhuang (Moravec 1987; Benkert 2005; Van Vooren 2009). Later, *Marcelleina* was considered a synonym of *Pulparia* P.Karst. by Korf (1972). This decision was followed by Donadini (1979) but not by Pfister (1985) who reinstated *Marcelleina*. Finally Dissing (1988) suggested that *Pulparia* was a synonym of *Pulvinula* Boud., based on the examination of two authentic collections, including the holotype.

Based on the diagnosis proposed by Brummelen (1967), *Marcelleina* is defined as follows: apothecia sessile, terricolous, first small, then expanded, with a soft flesh. Disc plane-convex, violet, violaceous brown, brownish or blackish, without carotenoid pigments. Asci cylindric, operculate, with inamyloid wall. Ascospores globose, smooth or ornamented. Paraphyses thin, widened at the top, sometimes curved.

A revision of the genus was published by Moravec (1987) who retained seven species (including their synonyms): *M. persoonii*, *M. rickii* (Rehm) Graddon, *M. georgii* (Svrček) J.Moravec, *M. brevicostatispora* J.Moravec, *M. pseudoanthracina*, *M. benkertii* J.Moravec, and *M. donadinii* Astier & J.Moravec. *M. chopraiana* (L.R.Batra) S.C.Kaushal (Kaushal *et al.* 1981) was not cited by Moravec. Moravec (1987) also amended the definition of the genus, especially for defining the excipulum structure and the spore content ("containing a single large guttule").

Later, two new species were published: *M. tuberculispora* K.Hansen & Sandal (Hansen *et al.* 1998) and *M. mediterranea* Lantieri & Pfister (Lantieri & Pfister 2010). Another species, *M. parvispora* E.Rubio, Tabarés & Alej.Martínez (Rubio *et al.* 2011), was erroneously assigned to *Marcelleina*, but later placed in synonymy with *Kallistoskypha incarnata* (Duvernoy & Maire) Pfister, Agnello, Lantieri & LoBuglio based on molecular data and morphological comparison (Pfister *et al.* 2013).

Marcelleina was classified in the family Pyronemataceae (Korf 1972, as *Pulparia*) based on, amongst other features, the inamyloidity of its asci, but Hansen *et al.* (2001) considered the genus as a member of the Pezizaceae based on preliminary molecular data. This result was later confirmed by Hansen *et al.* (2005). Similar results have been obtained by Van Vooren (2020), also suggesting that *M. pseudoanthracina* belongs to an independent clade. Until now no molecular study focused on this genus has been carried out.

In this context, we propose to emend the genus *Marcelleina* with the following description: ascomata epigeous, sessile, discoid, shallow cupulate to pulvinate, violet to purplish brown with age, more rarely depigmented to whitish. Flesh without latex. Asci operculate, with croziers, inamyloid. Ascospores globose, hyaline, containing a large oil drop, often accompanied by smaller droplets (in vital state), smooth or ornamented. Paraphyses containing a violaceous or brownish pigment, sometimes also with an external pigment at the top. Medullary excipulum of *textura intricata*, becoming inflated in the outer part, more or less clavate in the marginal area, and becoming a *textura globulosa* in the ectal excipulum. Anamorph unknown. Trophic status: unknown. Ecology: on soil, mostly calcareous.

MATERIAL AND METHODS

Morphological studies

Fresh specimens of *Iodomarcelleina obscura* gen. nov., sp. nov. were collected by the first author and photographed *in situ*. Fresh and dried specimens of *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov. were studied by the third author. The specimens were dried at 40°C for several hours, over two days. The *exsiccata* are stored in the herbarium of University of Lyon, France (LY). A further collection of *I. obscura* gen. nov., sp. nov. (private herbarium *C.S.1091*) was examined and compared morphologically and phylogenetically. Other collections (Table 1) were loaned from the public herbaria PRM, K(M), L and BRA, as well as private herbaria of R. Martínez, M. A. Ribes, F. J. Valencia and M. Gryc.

Squashed mounts of hand cut thin sections of fresh specimens were examined microscopically following the guidelines outlined by Dougoud (2013). They were examined in tap water, Lugol's reagent (IKI) or Melzer reagent (MLZ) for the amyloidity, and lactic acid/cotton blue (LCB) for highlighting the spore ornamentation. Dried material was re-examined in tap water or in 10% ammonia solution (NH_4OH), and treated similarly to fresh material. All measurements were taken on fresh material mounted in water with the assistance of Piximètre software v5.10 (Henriot & Cheype 2020), and are given on the basis of a number (N) of free ascospores in the mounts.

Abbreviations

Me	arithmetic mean;
Q	ratio between spore length width;
Qe	mean Q.

TABLE 1. — List of sequences used for the multi-locus phylogenetic analysis. Collections sequenced for this study are in **bold**. Symbol: *, under *Delastria supernova* A.Paz & C.Lavoise.

Species	Voucher	Country	ITS	LSU	*tub*2	*tef*1	*rpb*2
Ahmadea dalanensis Aman & Khalid	LAH 36405	Pakistan	MT645090	MT645087	–	–	–
Ahmadea dalanensis	LAH 36406	Pakistan	MT645091	MT645089	–	–	–
Ascobolus crenulatus P.Karst.	KH-02-005 (C)	United States	–	AY500527	AY513299	–	AY500462
Ascobolus denudatus	KS-94-146 (C)	Denmark	–	AY500528	AY513300	–	AY500460
Delastria evae M.Romero, A.Paz & Lavoise	AH 39235	Spain	JN048883	JN048871	–	–	–
Delastria evae	AH 39212	Spain	–	JN048870	–	–	–
Delastria faustiniana A.Paz, Lavoise & P.Juste	IC19051301	Spain	KF604908	KF604907*	–	–	–
Delastria javieri A.Paz, Lavoise & R.Molina	AH 39200	Spain	JN048884	JN048872	–	–	
***Delastria rosea* Tul. & C.Tul.**	**NV 2014.08.06 (LY)**	**France**	**OR348400**	**OR348392**	**–**	**OR338889**	**OR338887**
Delastria rosea	OSC JT17961	Spain	JN102449	JN121354	–	–	–
Delastria trispora (McLennan & Cookson) Van Vooren, P.S.Catches. & D.E.A.Catches.	AD-C 56992	Australia	MH722262	MH722262	–	–	–
Ionopezia gerardii (Cooke) Van Vooren	TL-5693 (C)	Denmark	–	AY500546	AY513332	–	AY500513
Ionopezia gerardii	DHP-02.495 (FH)	Mexico	–	AY500547	AY513333	–	AY500471
Marcelleina* cf. *benkertii	**L 4343912**	**Netherlands**	**OR348393**	**OR348384**	**–**	**–**	**OR338881**
***Marcelleina benkertii* J.Moravec**	**CVL 070123-1**	**Spain**	**–**	**OR348385**	**–**	**–**	**OR338882**
***Marcelleina mediterranea* Lantieri & Pfister**	**K(M) 164532**	**Italy**	**OR348396**	**OR348388**	**–**	**–**	**–**
***Marcelleina persoonii* (P.Crouan & H.Crouan) Brumm.**	**RM 2475**	**Spain**	**OR348394**	**OR348386**	**–**	**–**	**OR338883**
Marcelleina persoonii	**Gierczyk s.n.**	**Poland**	**OR348395**	**OR348387**	**–**	**–**	**OR338884**
Marcelleina persoonii	TL-5696 (C)	Denmark	–	AY500537	AY513311	–	AY500464
Marcelleina persoonii	KH 00.007 (C)	Denmark	–	AY500536	AY513310	–	AY500463
Marcelleina rickii (Rehm) Graddon	NV 2017.08.23 (LY)	France	–	OL832154	–	–	–
Marcelleina tuberculispora K.Hansen & Sandal	All-94-8 (C)	Denmark	DQ646535	AF335120	–	–	–
Scotopezia pseudoanthracina (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov.	KH 02.15 (C)	Norway	–	AY500538	AY513312	–	AY500509
***Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov.**	**NV 2021.10.01 (LY)**	**France**	**OR348397**	**OR348389**	**–**	**–**	**OR338885**
Stouffera gilkeyae D.G.Knapp, Zagyva, Trappe & Kovács	G2	Hungary	MN857204	MN857224	–	–	–
Stouffera gilkeyae	KIPD_07	Hungary	MN857201	MN857221	–	–	–
***Iodomarcelleina obscura* gen. nov., sp. nov.**	**NV 2022.08.00 (LY)**	**Malta**	**OR348398**	**OR348390**	**–**	**OR338888**	**OR338886**
***Iodomarcelleina obscura* gen. nov., sp. nov.**	**C.S.1091**	**Malta**	**OR348399**	**OR348391**	**–**	**–**	**–**

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with an annealing temperature of 54°C in all cases. The primers ITS1F and ITS4 (White *et al.* 1990; Gardes & Bruns 1993) were employed to amplify the ITS rDNA region, LR0R and LR5 (Vilgalys & Hester 1990; Cubeta *et al.* 1991) were used for the 28S rDNA region, bRPB2-6F2 (reverse of bRPB2-6R2) and bRPB2-7R2 for the RNA polymerase II second largest subunit (RPB2) gene (Matheny *et al.* 2007), and EF1-983F, EF1-1567R and EF1-2218R (Rehner & Buckley 2005) for the translation elongation factor 1a (*tef*1) gene. PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms.

PHYLOGENETIC ANALYSES

A first dataset with LSU, RPB2 and β-tubulin (*tub*2) sequences was assembled in MEGA 5.0 software (Tamura *et al.* 2011), based on previous analyses of the family Pezizaceae (Van Vooren 2020). BLASTn (Altschul *et al.* 1990) was used to select the most closely related sequences retrieved from the International Nucleotide Sequence Database Collaboration (INSDC) public database (Arita *et al.* 2021), completed by sequences obtained during this study. The *tub*2 gene could not be amplified from our samples, but sequences of other species were employed anyway to provide a more robust phylogeny. Homologous sequences of genus *Ascobolus* (Ascobolaceae) were employed as outgroup (Hansen *et al.* 2005). Sequences were first aligned in MEGA v5.0 with its Clustal W application and then realigned manually as needed to establish positional homology. Aligned loci (three partitions: 932 positions from 27 LSU sequences, 1697 positions from 14 *rpb*2 sequences, and 745 positions from eight *tub*2 sequences, without significant conflicts between them when analysed independently) were loaded in MrBayes v3.2.6 (Ronquist *et al.* 2012), where a Bayesian analysis was performed with a GTR + G + I model for LSU and *rpb*2, GTR + G for *tub*2 as determined by MrModeltest2 (Nylander 2004), and two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation, until the average split frequencies between the simultaneous runs fell below 0.01 after 2.42 M generations. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML v8.2.12 (Stamatakis 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and

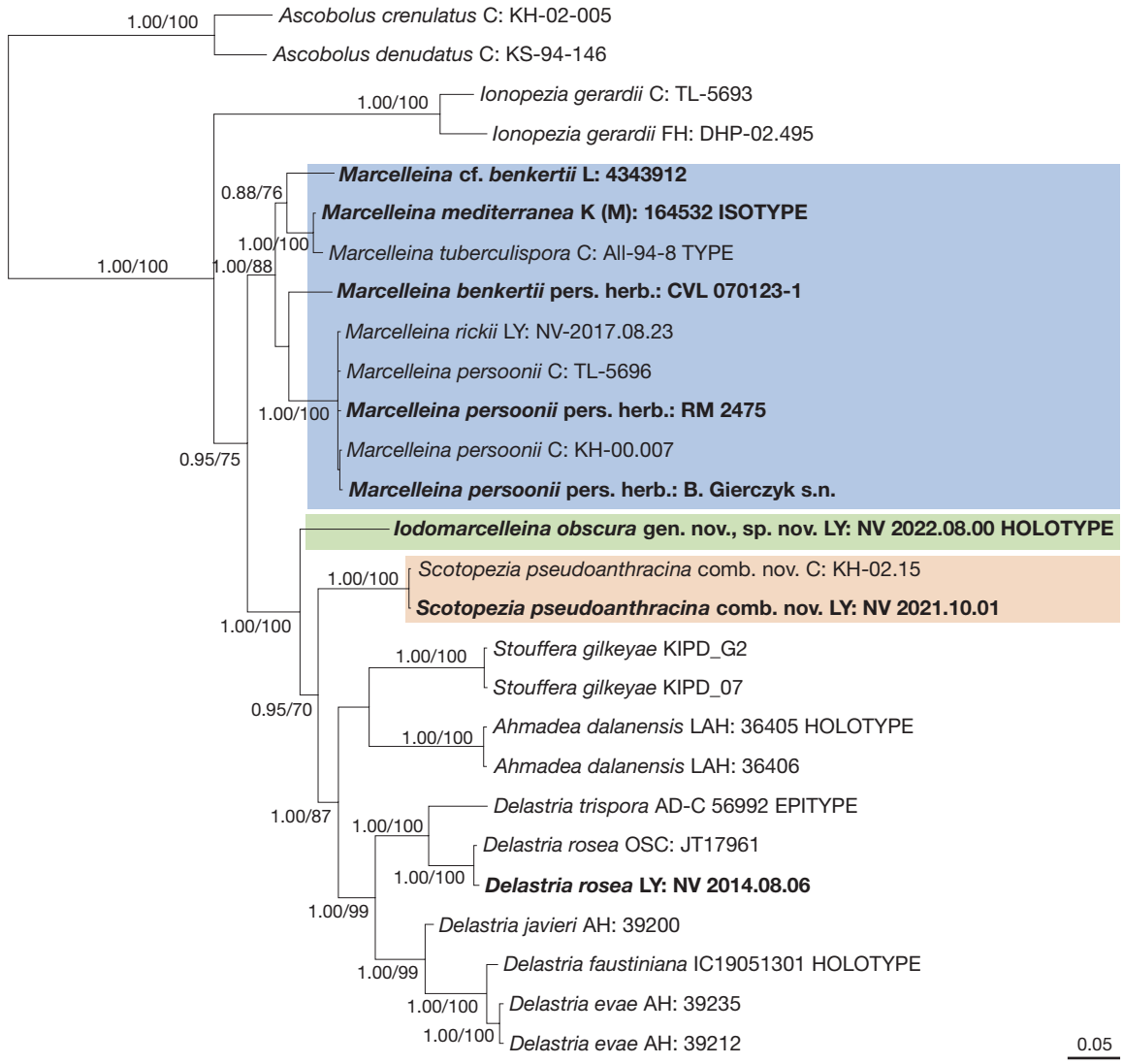

Fig. 1. — 50% majority rule consensus 28S rDNA–RPB2–β-tubulin phylogram of selected Pezizaceae of the *Marcelleina-Ionopezia* lineage obtained in MrBayes. Nodes supported by >0.95 Bayesian posterior probability (PP) or >70% maximum likelihood (ML) bootstrap proportion (BP) are shown annotated. Terminals in **bold** are newly generated sequences. *Ascobolus* Pers. species served as the outgroup.

70% bootstrap proportions (BP). A second dataset was built using ITS sequences only, aligned and analysed in the same way (637 positions from 50 ITS sequences, no ambiguous reads excluded, GTR + G + I model). In this case, Bayesian convergence was met after 0.24 M generations.

RESULTS

Both the ITS and the multi-locus analyses show that samples identified as *Marcelleina persoonii* (the type of *Marcelleina*) are not significantly different from a sample identified as *M. rickii*, but this can result from a misidentification of the latter (see also our comments in the Discussion chapter). A specimen identified as *M. tuberculispora* and two different taxa identified as *M. benkertii* (one from the Netherlands, the other from Spain) are more distantly related to the type taxon inside the clade identified here as *Marcelleina s.str.* The genus *Marcelleina* is not supported as monophyletic when it includes *M. pseudoanthracina*, but is highly supported as monophyletic when only *M. persoonii*, *M. benkertii*, *M.* cf. *benkertii*, and *M. tuberculispora* (synonym: *M. mediterranea*) are included.

Based on the multi-locus analysis (Fig. 1), samples identified as *M. pseudoanthracina*, as well as *Iodomarcelleina obscura* gen. nov., sp. nov., are strongly supported in a clade sister to *Marcelleina* containing the hypogeous genera *Stouffera* Kovács & Trappe (Kovács *et al.* 2011), *Delastria* (Alvarado *et al.* 2011; Paz *et al.* 2018) and *Ahmadea* Aman, Khalid & Moncalvo (Aman *et al.* 2020). This result is also supported in the phylogeny based on the single ITS locus (Fig. 2). The hypogeous genera *Temperantia* K.Hansen, Healy & Kovács (Kovács *et al.* 2011), *Babosia* D.G.Knapp, Zagyva, Trappe & Kovács (Knapp *et al.* 2020) and *Hydnobolites* Tul. & C.Tul. (not shown in Fig. 1) also belong to this clade based on works of Aman *et al.* (2020) and Knapp *et al.* (2020).

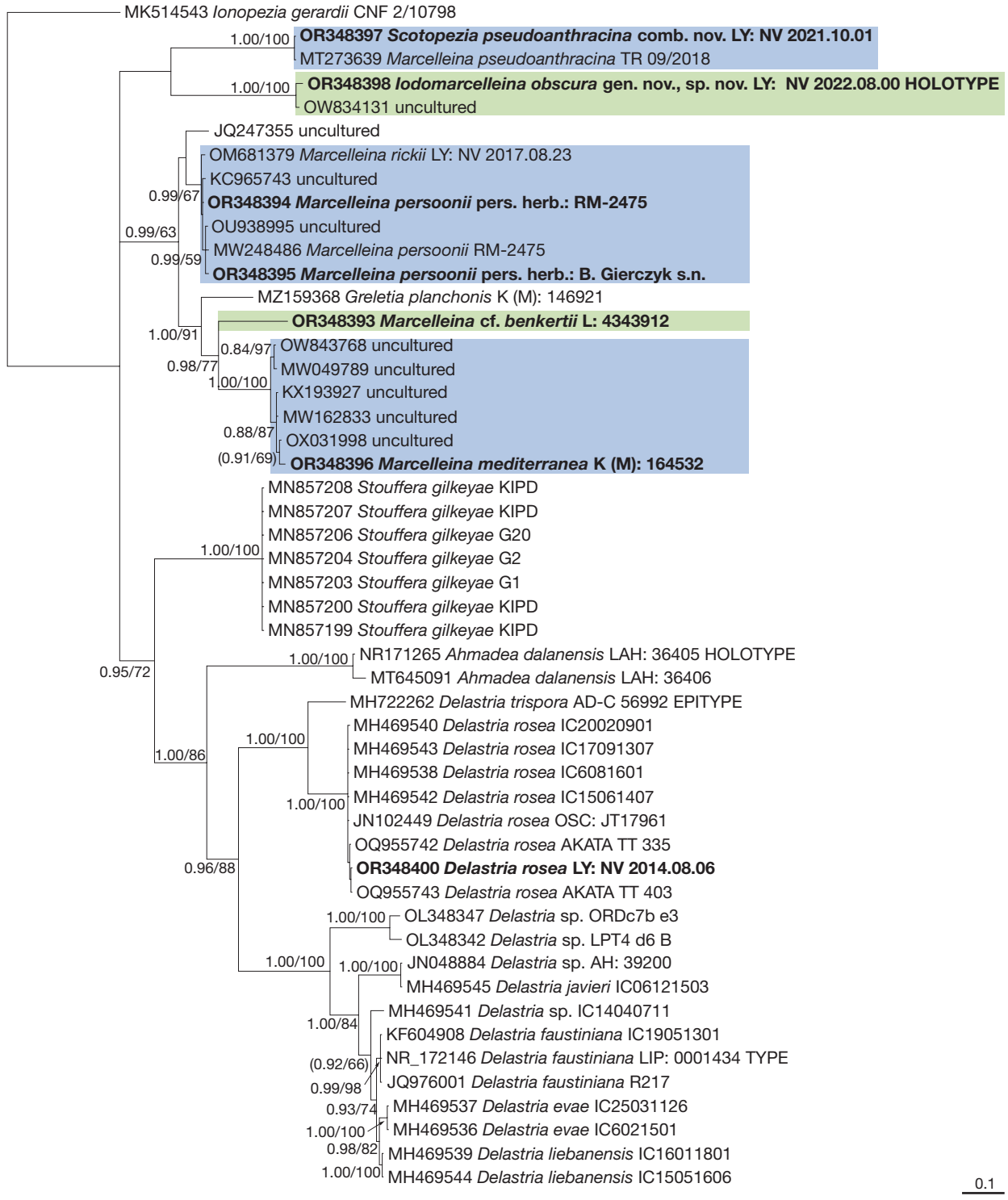

Fig. 2. — 50% majority rule consensus ITS phylogram of selected Pezizaceae of the *Marcelleina-Ionopezia* lineage obtained in MrBayes. Nodes supported by >0.95 Bayesian posterior probability (PP) or >70% maximum likelihood (ML) bootstrap proportion (BP) are shown annotated. Terminals in **bold** are newly generated sequences. *Ionopezia gerardii* (Cooke) Van Vooren served as the outgroup.

Based on molecular results and morphological studies (e.g. amyloid reaction of asci, smooth ascospores; see Taxonomy chapter), the collection from Malta is here proposed to represent an undescribed species, which is accommodated in a new genus named *Iodomarcelleina* gen. nov. Similarly, *Marcelleina pseudoanthracina* is accommodated into a separate genus, named *Scotopezia* Van Vooren, Sammut & P.Alvarado, gen. nov., due to its lack of support within *Marcelleina*, its isolated position in the multi-gene analysis, and some of its morphological characters (e.g. amyloid reaction).

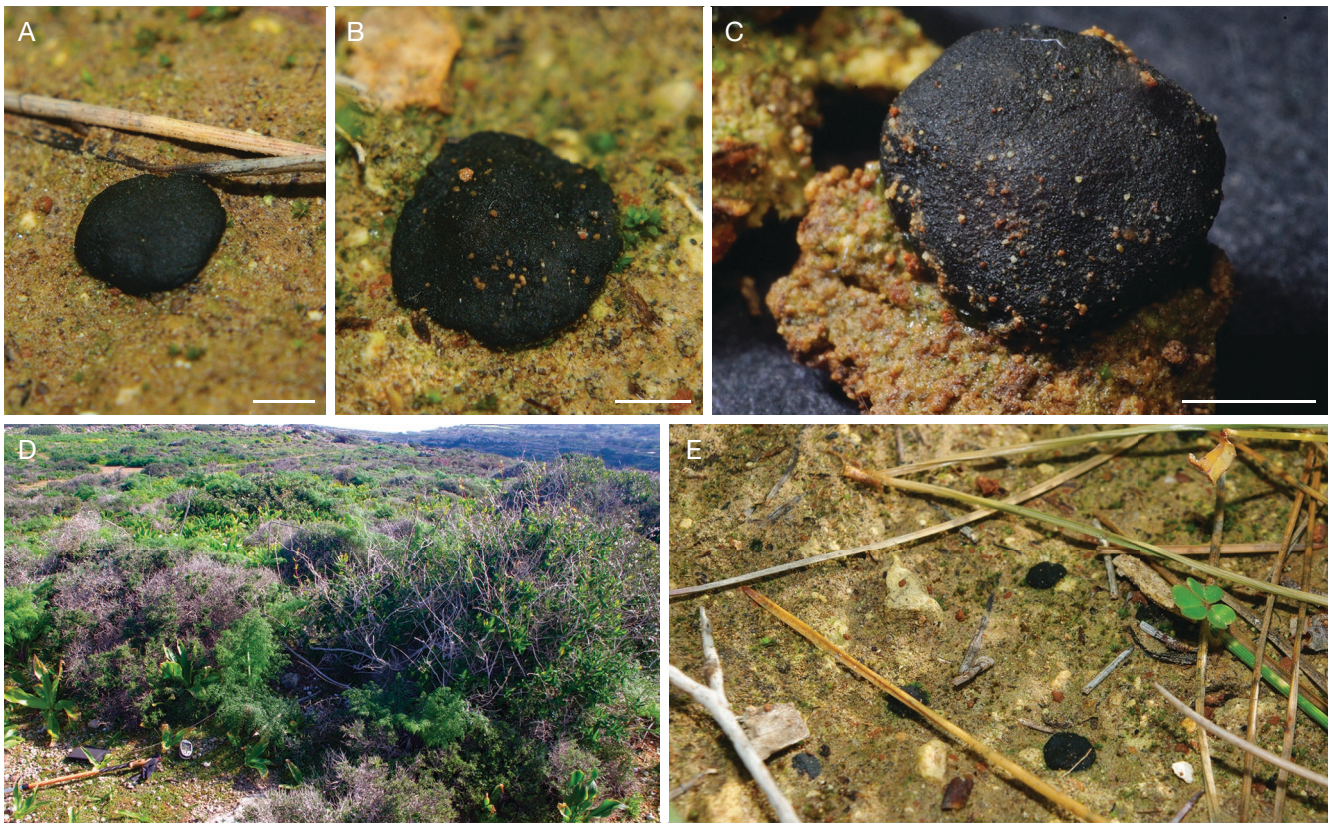

Fig. 3. — *Iodomarcelleina obscura* gen. nov., sp. nov.: **A**, **B**, apothecium *in situ*; **C**, close-up view; **D**, habitat; **E**, apothecia in their environment. All photographs from *NV 2022.08.00* (holo-, LY). Scale bars: 2 mm.

The analysis based on the ITS locus (Fig. 2) confirmed the isolated positions of species of both *Iodomarcelleina* gen. nov. and *Scotopezia* Van Vooren, Sammut & P.Alvarado, gen. nov., outside the *Marcelleina* clade. The popularity of the ITS barcode in environmental sequencing gave us the opportunity to include all highly similar environmental sequences in our ITS analysis. None of the environmental sequences included in this analysis was obtained from mycorrhizae, suggesting that species of *Marcelleina*, as well as *Iodomarcelleina* gen. nov., might be saprobic but a trophic guild is yet to be convincingly identified for this genus.

The presence of a sequence identified as *Greletia planchonis* (Dunal ex Boud.) Donadini (synonym: *Smardaea planchonis* (Dunal ex Boud.) Korf & W.Y.Zhuang) surely comes from a misidentification and may represent a collection of *M. benkertii*, the latter having smooth ascospores, as for *S. planchonis*. Members of *Smardaea* Svrček do not belong to Pezizaceae but to Pyropyxidaceae (Zeng *et al.* 2022).

TAXONOMY

Family Pezizaceae Dumort.

Genus *Iodomarcelleina* gen. nov.

Etymology. — Referring to the amyloid reaction of asci in iodine solution, and *Marcelleina*, the phylogenetically closest genus.

Type species. — *Iodomarcelleina obscura* sp. nov.

MycoBank number. — MB 849113.

Description

Ascomata epigeous, discoid, sessile, dark coloured. Flesh without latex. Asci operculate, with croziers, having a diffusely amyloid wall in iodine solution. Ascospores globose, multiguttulate, smooth. Paraphyses with an external pigment at the top. Ectal excipulum of *textura globulosal angularis*. Anamorph unknown. Trophic status unknown.

Iodomarcelleina obscura sp. nov.
(Figs 3; 4)

Differs from *Marcelleina benkertii* by the straight and inflated paraphyses and the amyloid reaction of the asci, from the other *Marcelleina* species by the amyloid reaction of the asci and lack of violaceous tinges in the apothecia, and from *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov. by its smooth ascospores.

Holotype. — **Malta**. Mellieha, Ahrax, 35°58'57.0"N, 14°21'43.3"E, 30 m a.s.l., on soil, in an open ground near *Pinus halepensis* Mill., 3.II.2017, leg. C. Sammut, *NV 2022.08.00* (holo-, LY).

Additional specimen examined. — **Malta**. Mellieha, Selmun area, 35°57'58.0"N, 14°22'35.0"E, 92 m a.s.l., in a sheltered area on

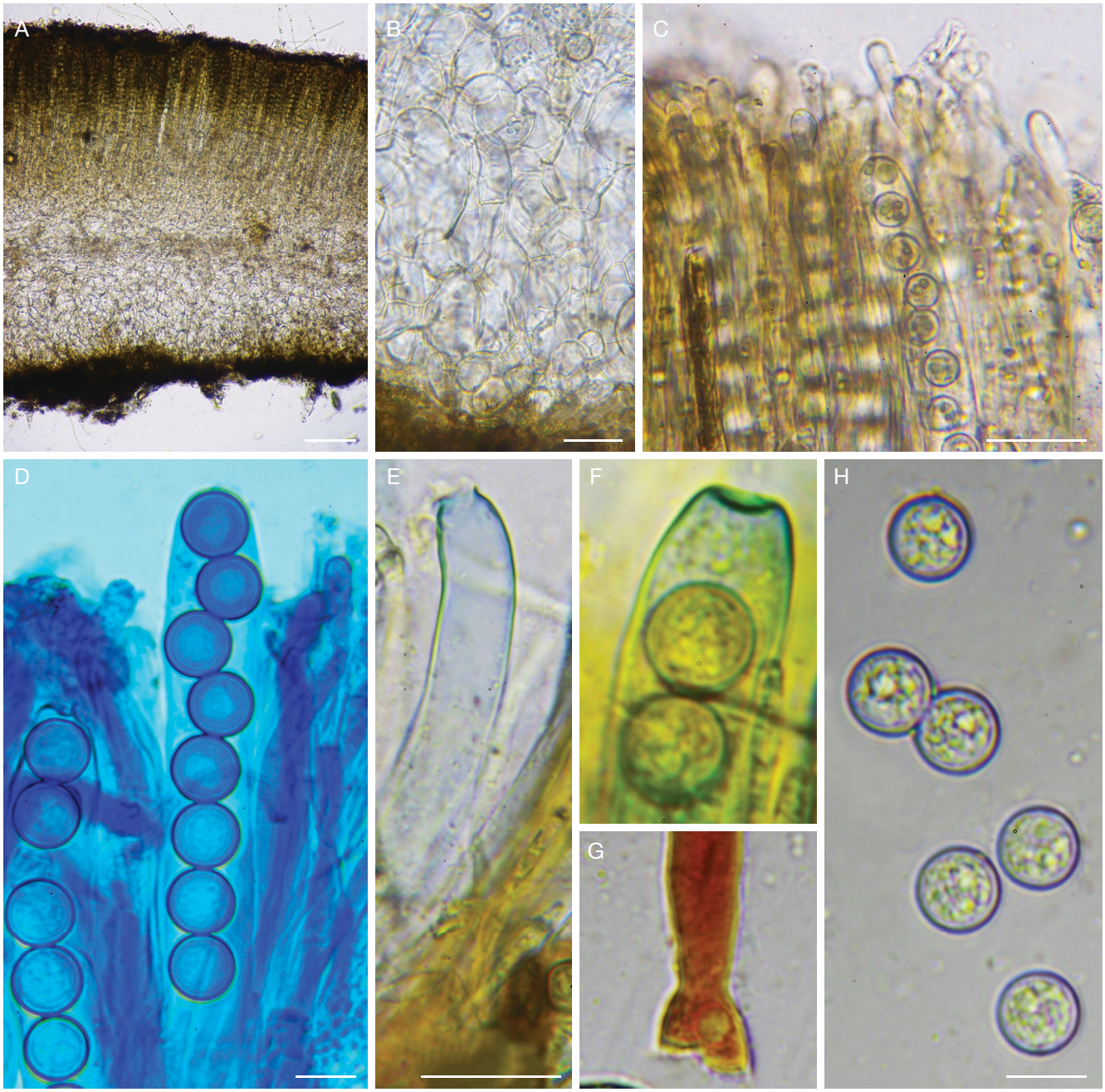

Fig. 4. — *Iodomarcelleina obscura* gen. nov., sp. nov.: **A**, section of an apothecium; **B**, inner part of the ectal excipulum; **C**, top of the asci and paraphyses; **D**, ascospores in asci (in CB); **E**, amyloid reaction of the ascus wall; **F**, detail of the amyloid reaction at the top of an ascus; **G**, ascus base; **H**, ascospores. All photographs from *NV 2022.08.00* (holo-, LY), except **E** and **F** from *C.S.1091*. Scale bars: A, 100 µm; B, C, E, 25 µm; D, H, 10 µm.

calcareous soil, near *Asparagus aphyllus* L., *Ferula melitensis* Brullo, C.Brullo, Cambria, Giusso, Salmeri & Bacch., *Teucrium fruticans* L., *Thymbra capitata* Cav., *Uriginea maritima* (L.) Baker, 16.II.2017, leg. M. Sammut, *C.S.1091*.

Etymology. — From Latin *obscurus*, meaning dark.

MycoBank number. — MB 849114.

Description

Macroscopic features (Fig. 3)

Apothecia 4-7 mm diam., sessile, discoid, shallowly patelliform to flattened, hymenium smooth and regular, dark brown to black. Margin not differentiated. Flesh up to 750 µm thick, without latex.

Microscopic features (Fig. 4)

Hymenium 260-340 µm thick, brownish, darker near the edge. Asci cylindrical, 190-250 × 14-21 µm, 8-spored, operculate, with croziers, weakly bluing in IKI (diffused along wall). Ascospores globose, 9.5-11.9 µm diam., Me = 10.8 µm, Q = 0.96-1.05, Qe = 1.00 (N = 50), smooth, thick-walled, containing several small oil bodies. Paraphyses thin, slightly claviform, inflated at the tips up to 6 (-10 µm), straight to

curved, occasionally branched, multiseptate, with external agglutinated brown pigment. Subhymenium pale brown, 30-50 µm thick, of *textura intricata*. Medullary excipulum bi-layered: the upper part 30-60 µm thick, of *textura angularis*, with cells 11-28 µm wide, thin-walled; the lower part 40-60 µm thick, of *textura intricata*, with hyphae 2.5-5 µm wide. Ectal excipulum 200-270 µm thick, of *textura globulosa/angularis*, made up of hyaline cells, 12-50 µm diam., arranged perpendicularly to the surface, becoming brownish in the outermost part. Anchoring hyphae present, hyaline, 4-8 µm in diameter.

Habitat

The area where the holotype was collected consists of a thin strip of land, about 950 m in length and a mean width of 100 m, on a sloping cliffside facing the sea. The vegetation consists of an anthropized maquis, with *Olea europaea* L., *Pinus halepensis* and *Eucalyptus* sp., transitioning to a typical Mediterranean garigue vegetation inclusive of *Thymbra capitata*, *Erica multiflora* L., *Euphorbia dendroides* L. as well as endemic *E. melitensis* Parlatore. The soil is calcareous with sparse organic matter. There are no natural water sources, and the availability of water depends on rainfalls or contributions from overnight dew. The area is also well known as a popular camping site resulting in a high degree of human activity, particularly from autumn to spring.

Genus *Marcelleina* Brumm., Korf & Rifai

Marcelleina tuberculispora K.Hansen & Sandal

Nordic Journal of Botany 18 (5): 622 (Hansen *et al.* 1998).

Marcelleina mediterranea Lantieri & Pfister, *Mycotaxon* 111 (1): 465-469 (Lantieri & Pfister 2010) **syn. nov.**

Genus *Scotopezia*
Van Vooren, Sammut & P.Alvarado, gen. nov.

Etymology. — From ancient Greek *σκότος* (skótos), meaning obscurity, darkness, and *πέζις* (pezis) meaning "fungus without stipe", referring to the dark colour of apothecia.

Type species. — *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov.

MycoBank number. — MB 849115.

Description

Ascomata gregarious, epigeous, discoid, sessile, brown to black coloured. Flesh without latex. Asci operculate, with croziers, having a diffusely but inconstantly amyloid wall in iodine solution. Ascospores globose, uniguttulate, warted. Paraphyses embedded at the top with a yellowish-brown exudate. Excipulum of *textura globulosa/subglobulosa*. Anamorph unknown. Trophic status unknown.

Scotopezia pseudoanthracina
(Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov.
(Fig. 5)

Basionym: *Peziza pseudoanthracina* Donadini, *Documents mycologiques* 12 (46): 6 (Donadini 1982).

Specimens examined. — **France**. Eure, Martot, 49°16'56.5"N, 1°04'45.7"E, 22 m a.s.l., in a former quarry, numerous specimens on clay soil, 11.X.2021, leg. A. Delannoy, *NV 2021.10.01* (LY). **Germany**. Nordwestmecklenburg, Dechow, Woitendorfer Wald, 53°43'22.0"N, 10°57'35.8"E, 70 m a.s.l., 18.VIII.2019, leg. and det. T. Richter, duplicate of FuRi 19/287.

MycoBank number. — MB 849116.

DISCUSSION

The newly found species, *Iodomarcelleina obscura* gen. nov., sp. nov., is morphologically similar to *Marcelleina benkertii* (Moravec 1987) because of its habit, smooth ascospores and spore size (9-12 µm), but differs in some other important characters. The paraphyses in *Iodomarcelleina obscura* gen. nov., sp. nov. are mostly straight (with a few bent) and inflated at the tips, whilst *M. benkertii* has curved paraphyses, only slightly inflated (Moravec 1987; Di Meo & Lalli 1993). The apothecia are brown-black without violaceous tinges contrary to *M. benkertii* (Ribes *et al.* 2015) and the ascospores of *M. benkertii* contain one large guttule in living state. The only other known species of *Marcelleina* with smooth ascospores is *M. chopraina* (Batra 1961; Kaushal *et al.* 1981) from Mussoorie (India), but the apothecia are differently coloured, "sanford brown", "amber brown" to "madder brown", and the ascospores are uniguttulate and larger, 14-18 µm diameter. Genetically, the two samples of *M. benkertii* analysed (L4343912 and CVL070123) are highly supported within *Marcelleina s.str.* but are genetically different from each other. Although the exact identity of *M. benkertii* cannot be resolved in the present work, it is clear that both samples are unrelated to *Iodomarcelleina* gen. nov.

The other species of *Marcelleina* differ from *Iodomarcelleina obscura* gen. nov., sp. nov. in spore ornamentation: pustulate to tuberculate in *M. mediterranea* (Lantieri & Pfister 2010) and *M. tuberculispora* (Hansen *et al.* 1998), warted in *M. pseudoanthracina* (Donadini 1981; Dougoud 2002) and *M. georgii* (Moravec 1987), with low ridges in *M. brevicostatispora*, *M. persoonii* (Moravec 1987) and *M. rickii* (Graddon 1976; Perić 2009).

In the present work, we revised some types of the species described by Moravec (1987) and also an isotype of *M. mediterranea*. The latter was described from a collection made in Sicily and considered mainly different from *M. tuberculispora* due to its spore ornamentation. After the revision of the types, we disagree with this opinion. The figure depicted by Lantieri & Pfister (2010: fig. 1D) does not conform to the configuration of the warts we observed. We did not see a morphological difference with the configuration shown by

FIG. 5. — *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov. Photograph from *NV 2021.10.01* (LY). Scale bar: 2 mm.

M. tuberculispora (Hansen *et al.* 1998: fig. 20) because the warts are more rounded than drawn in the protologue. The size of ascospores is (12.5) 13-14.5 (16) µm diam. (some ascospores larger than 16 µm were observed, but we think they were overmature), Me = 13.7 µm in our revision vs (13) 14-18 µm in the protologue. Regarding the excipulum structure, the drawing of the protologue does not show significant differences between the medullary and the ectal parts. The description reports some differences, but our revision shows that the medullary excipulum is a mix of small globose cells and elongated hyphae, whilst the ectal excipulum has larger globose cells, reaching 60 µm diam. Finally, the LSU sequence obtained from this isotype (GenBank[OR348388]) shows a 99.16% similarity with that of the type of *M. tuberculispora* (GenBank[AF335120]).

We also revised type collections of *M. georgii* and *M. brevicostatispora*. These two species are microscopically very similar, with few differences in spore size, respectively 9-11 µm diam., Me = 10 µm, and 8-10 µm, Me = 9.1 µm. We also did not observe a real difference in spore ornamentation, although the figures of Moravec (1987: 481) show a variation of ornaments rather important between these two species. We suspect these two names actually apply to the same species, maybe showing two states of maturity. Unfortunately, we failed to get DNA sequences from these collections, so we are unable to confirm our hypothesis.

Similarly, only tiny differences exist between *M. persoonii* and *M. rickii*, the latter showing a spore ornamentation formed by an incomplete network, with higher ridges, compared to the first (Moravec 1987). Confusions between these two taxa in the literature were noticed by Moravec (1987). We must confess that naming collections from mountainous regions is always challenging, to the point that, in some cases, we find it impossible to choose between *M. persoonii* and *M. rickii*. We failed to find a modern collection of *M. persoonii* coming from lowlands, either in the original area (Bretagne) or other regions in France, that could be used as an epitype. Type material of *M. rickii* is housed in S fungarium (as *Barlaea rickii*) but it is unavailable for a loan. Collected at the end of the 19th century, it is probably too old to get sequences. Genetically, modern samples identified with both names seem not significantly distinct from each other, although a larger study including more samples and more gene regions should be conducted to check their status more thoroughly.

After revising the type of *M. donadinii*, we found that the smooth ascospores described by Moravec (1987) are actually mixed with low-warted ascospores, and some immature spores are ornamented with sparse large pustules. The ascospores of *M. donadinii* measure 12.5-14.5 (15) µm in diam. Phylogenetically, *M. donadinii* seems to be outside the family Pezizaceae on the basis of LSU data obtained from a paratype (data not shown). Its taxonomic status will be treated in another paper.

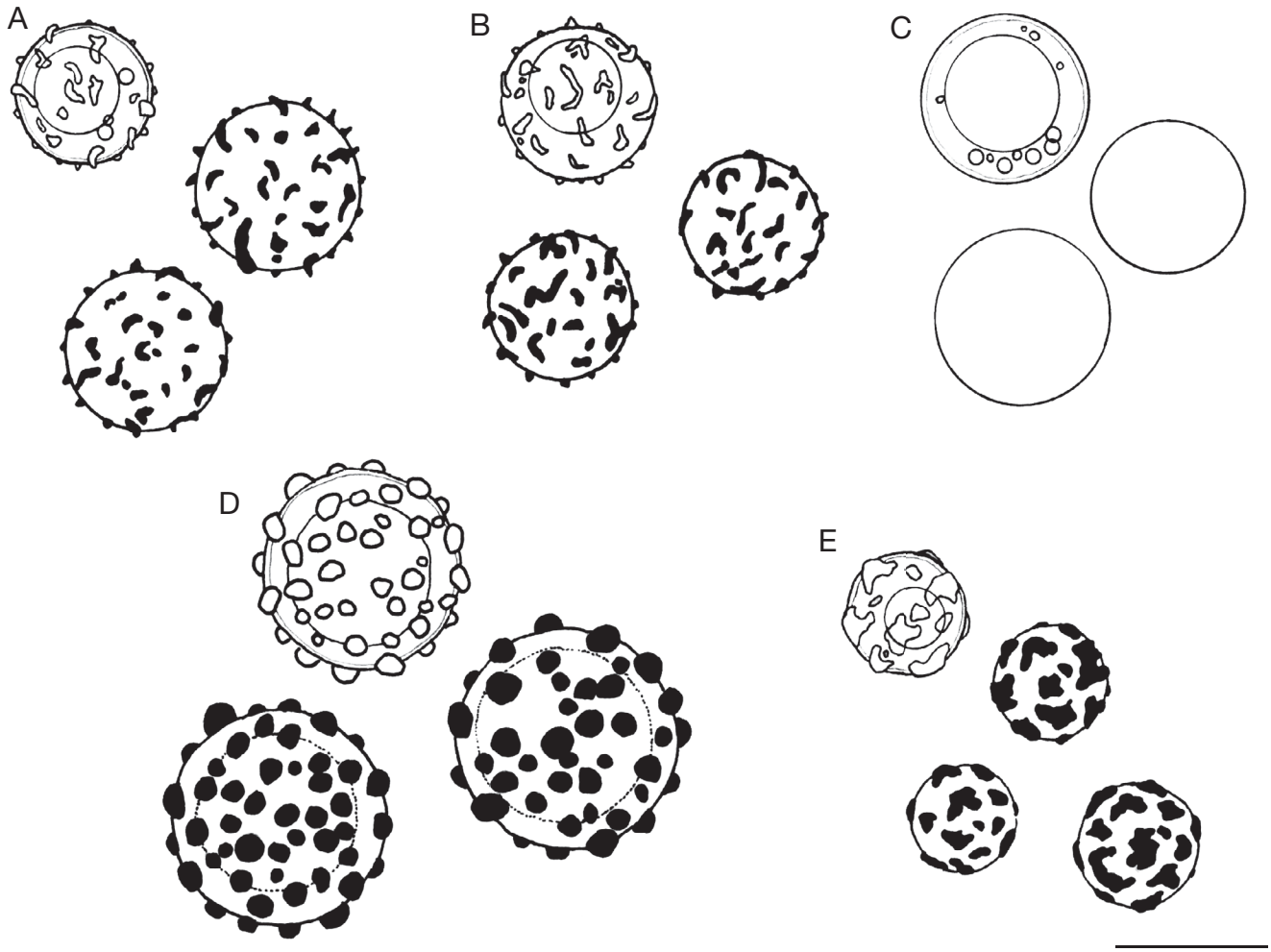

Fig. 6. — Ascospores of *Marcelleina* Brumm., Korf & Rifai and allies: **A**, *Marcelleina georgii* (Svrček) J.Moravec, from BRA-F-123 (neotype); **B**, *M. brevicostatispora* J.Moravec, from PRM 710001 (holotype); **C**, *M. benkertii* J.Moravec, from BRA F-127 (holotype); **D**, *M. mediterranea* Lantieri & Pfister, from K(M) 164532 (isotype); **E**, *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P.Alvarado, comb. nov., from *NV2021.10.01* (LY). All ascospores seen in LCB, except the top one of each group, in water. Drawings N. Van Vooren. Scale bar: 10 µm.

Another important difference between *Iodomarcelleina obscura* gen. nov., sp. nov. and other species of *Marcelleina* is the amyloid reaction of asci. Indeed, the newly discovered species possesses amyloid asci, with a diffusely blue reaction along the wall, similar to the reaction seen in some Pezizaceae genera such as *Ruhlandiella* Henn., defined as "W type" in Van Vooren (2020), occasionally slightly stronger near the tip ("WT type" in Van Vooren 2020). This reaction seems stronger in the mature asci and is more conspicuous in empty asci. It is observed in both fresh samples and rehydrated exsiccata. Although *Marcelleina* belongs to Pezizaceae (Hansen *et al.* 2005), the amyloid reaction was considered to be a relict character in this genus (Van Vooren & Mauruc 2020).

Marcelleina pseudoanthracina shows an inconstant amyloid reaction. Indeed, contrary to the opinion of Moravec (1987), an amyloid reaction was observed by Donadini (1981) and Dougoud (2002). This reaction is illustrated in Van Vooren (2020: fig. 3J). This feature and the absence of violet tinges in ascomata, as well as the genetic data, exclude *M. pseudoanthracina* from *Marcelleina s.str.* Therefore, we propose the new genus *Scotopezia* Van Vooren, Sammut & P.Alvarado, gen. nov. to accomodate it.

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Updated key to the species of *Marcelleina* Brumm., Korf & Rifai and allied genera

This key is based on the work of Moravec (1987) and applies to small discoid to pulvinate species, rather thick, showing violet, purple-brown, brown to black ascomata, and having globose ascospores containing at least one oil drop.

1. Ascus wall amyloid, sometimes weakly, best seen in the young asci 2
— Ascus wall always inamyloid 3

2. Ascomata brownish, dark brown to blackish, sometimes with olivaceous shades, without purplish tinges; ascospores 7-8.5(9) µm diam., ornamented with large, angular warts ..... *Scotopezia pseudoanthracina* (Donadini) Van Vooren, Sammut & P. Alvarado, comb. nov.
— Ascomata black; ascospores 9.5-11.9 µm diam., smooth ..... *Iodomarcelleina obscura* gen. nov., sp. nov.

3. Ascospores ornamented, uniguttulate, but often accompanied by smaller droplets 4
— Ascospores smooth, uniguttulate, rarely accompanied by smaller droplets 8

4. Ascospore ornamentation forming a complete or incomplete irregular reticulum 5
— Ascospore ornamentation made of isolated warts or curved irregular ribs which only rarely anastomose, appearing as ribs or spines in optical section; ascospores more than 8 µm diam. 6

5. Ascospore reticulum mostly complete, only rarely incomplete, relatively low in optical section; 9-11 µm diam. ..... *Marcelleina persoonii* (P.Crouan & H.Crouan) Brumm.
— Ascospore reticulum mostly very incomplete, only very rarely complete, irregular, comparatively higher in optical section; 8-10.7 (11) µm diam. ..... *M. rickii* (Rehm) Graddon

6. Ascospore ornamentation made of large warts, ± rounded or tuberculate in optical section; ascospores 12-16 µm diam. ..... *M. tuberculispora* K.Hansen & Sandal (synonym: *M. mediterranea*)
— Ascospore ornamentation made of smaller warts or ribs, rather low in optical section 7

7. Isolated warts and ribs 0.25-0.8(1.4) µm thick, 0.3-0.7(1) µm high; paraphyses enlarged above; ascospores (8)9-11 µm diam. ..... *M. georgii* (Svrček) J.Moravec
— Isolated warts and ribs coarser, 0.5-1.5(2.2) µm thick, 0.5-1.5(1.7) µm high; paraphyses not or very slightly enlarged above; ascospores (8)9-11 µm diam. ..... *M. brevicostatispora* J.Moravec

8. Ascomata violet to dark violet; ascospores 10-12 µm diam. ..... *M. benkertii* J.Moravec
— Ascomata light to dark brown; ascospores 14-18 µm diam. ..... *M. chopraiana* (L.R.Batra) S.C.Kaushal

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