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Two new species in the *Micarea prasina* group from Western Europe

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Abstract: Detailed morphological and anatomical studies informed by molecular inferences with mtSSU as a marker revealed two new species of *Micarea* in Western Europe, both belonging to the core group of the genus, namely the *M. prasina* group: *M. herbarum* from the Netherlands and Poland and *M. meridionalis* from Portugal and Italy. *Micarea herbarum* looks like a small or depauperate *M. denigrata* but clearly differs by the lack of gyrophoric acid, while *M. meridionalis* is distinguished by its granular thallus and the production of micareic acid.

Key words: lichen diversity, lichenized Ascomycota, molecular phylogeny, taxonomy

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Introduction

Recently, the study of the genus *Micarea* has again attracted the attention of lichenologists. This is because the production of molecular sequences and phylogenetic reconstructions has revealed greater diversity in *Micarea*, including in Europe and Macaronesia (Czarnota & Guzow-Krzemińska 2010; van den Boom & Ertz 2014; Guzow-Krzemińska *et al.* 2016), and because a significant number of new species continue to be described from all parts of the world (van den Boom 2010; Sérusiaux *et al.* 2010; Svensson & Thor 2011; Câceres *et al.* 2013; Aptroot & Câceres 2014; Brand *et al.* 2014; Córdova-Chávez *et al.* 2014; McCarthy & Elix 2016). In Europe, the genus was investigated in a major revision (Coppins 1983) and was further studied at a smaller scale by Coppins (2009) for Great Britain and Ireland, and Czarnota (2007) for Poland.

The *M. prasina* group is known to be very variable and was suspected of containing additional species (Coppins 1983). Several studies dealing with collections within Europe (Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016) and outside Europe (Barton & Lendemer 2014; Brand *et al.* 2014) have shown that it represents a complex assemblage of species that is yet to be properly disentangled. Based on morphological, anatomical, chemical and molecular evidence, our study of the material belonging to that group available to us from Europe yielded evidence of two undescribed species. They are formally described in this paper.

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Material and Methods

The material examined was collected by the authors in Belgium, France, Germany, the Netherlands, Portugal, Romania and Spain (Canary Islands, Tenerife). Anatomical measurements were made on material mounted in dilute KOH for conidia and paraphysis width, and in water for all other characters. In each collection, *c.* 10 well-developed ascospores representing the size and shape variation detected, conidia and paraphyses were measured with a precision of 0·1 µm using *camera lucida* drawings. For thallus description, we refer to Coppins (1983) for the use of areolate- and goniocyst-type, the latter being "a finely granular thallus, composed of discrete, ± globular structures, mostly *c.* 12–40 µm diam., these ecorticate granules consist of clustered algal cells intertwined and surrounded by short-celled hyphae, and never protected by an amorphous covering layer".

Chemical compounds were studied using several methods: response to UV light, melting point determination, microcrystallization and thin-layer chromatography

(TLC) using solvent system A (toluene/1,4-dioxane/acetic acid 180:45:5) for all collections and C (toluene/acetic acid 170:30) when gyrophoric acid was suspected (Huneck & Yoshimura 1996; Orange *et al.* 2010), in both cases visualization of spots was achieved with sulphuric acid sprayed over the plates, followed by heating at 110 °C for *c.* 5 min.

Well-preserved specimens lacking any visible symptoms of fungal infection were selected for DNA isolation. Extraction of DNA and PCR amplification were performed following the protocol of Cubero *et al.* (1999). We used the primers mrSSU1 and mrSSU3R (Zoller *et al.* 1999) for the production of mtSSU sequences. Amplicons were sequenced by Macrogen®. Sequence fragments were assembled with Sequencher version 5.2.3 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were then subjected to MEGABLAST searches to detect potential contamination. They were included in a single matrix together with data extracted from GenBank, published in Andersen & Ekman (2005), Czarnota & Guzow-Krzemińska (2010), Guzow-Krzemińska *et al.* (2016) and van den Boom & Ertz (2014). We chose *Byssoloma leucoblepharum* and *B. subdiscordans* as outgroup following the topology obtained for the *Pilocarpaceae* by Miadlikowska *et al.* (2014). Accession data are included in Table 1. Sequences were aligned manually using MacClade version 4.08 (Maddison & Maddison 2005) and alignment was checked with the MAFTT software (Katoh & Standley 2013). Ambiguous regions were delimited using the online version of Gblocks v0.91b (Castresana 2000) at http://molevol.cmima.csic.es/castresana/Gblocks.html, allowing for gap positions within the final blocks, and carefully checked manually. The aligned matrix is available from the corresponding author on request. We inferred the maximum likelihood tree and bootstrap support values based on 1000 pseudoreplicates in the same run using RAxML HPC2 version 7.2.8 (Stamatakis 2006; Stamatakis *et al.* 2008) with the GTRCAT model and the default settings as implemented on the CIPRES portal (Miller *et al.* 2010). Phylogenetic trees were visualized using FigTree v1.2.3 (Rambaut 2009). Branch support values were considered significant when ML bootstrap (BS) > 70%. We included in our accessions for molecular inferences other specimens besides those belonging to the two species described here as new, for the following reasons: 1) we wished to assess the only phylogenetic tree available for the genus with further data (Andersen & Ekman 2005); 2) we suspect that *Micarea* is more complex in other groups than the *prasina* group and we wished to test this hypothesis.

Results

The data matrix includes 21 mtSSU sequences newly produced for the following species: *M. adnata*, *M. byssacea*, *M. denigrata*, *M. doliiformis*, *M. herbarum*, *M. lignaria* var. *lignaria*, *M. meridionalis*, *M. nowakii*, *M. micrococca*, *M. prasina*, *M. pycnidiophora*, *M. stipitata* and *M. viridileprosa*. The single most-likely tree (Fig. 1) resolves two strongly supported clades within *Micarea*. Both species newly described in this paper are resolved within a single clade and more precisely within a strongly supported (BS = 98) subclade including all accessions of the *M. prasina* group (Coppins 1983, 2009; Czarnota 2007; Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016). Within that subclade the resolution is poor, except for a strongly supported group comprising *M. byssacea*, *M. hedlundii*, *M. micrococca* A and B (*sensu* Czarnota & Guzow-Krzemińska 2010), *M. viridileprosa* and *M. xanthonica*.

Our new accessions of *M. nowakii* from Romania are identical to two accessions of the same species from Poland (Czarnota & Guzow-Krzemińska 2010). In two further accessions of the same species, also from Poland, one is resolved as nearly identical to *M. herbarum* sp. nov., and the other as sister to accessions of the same species.

All accessions of *M. prasina* from Poland (Czarnota & Guzow-Krzemińska 2010) are resolved as a lineage (as '*prasina* 1' in Fig. 1) separate to those from Belgium, France and the USA (as '*prasina* 2' in Fig. 2); thus two cryptic species might be involved and require further study. Within the *M. prasina* group, our sequence of *M. byssacea* is nearly identical to those referred to that species by Czarnota & Guzow-Krzemińska (2010), our sequence of *M. viridileprosa* is identical to those of Czarnota & Guzow-Krzemińska (2010) and finally, our sequences of *M. micrococca* belong to *M. micrococca* "A" of Czarnota & Krzemińska (2010). While our sequences of *M. adnata*, *M. denigrata*, *M. doliiformis*, *M. lignaria* var. *lignaria*, *M. nitschkeana* and *M. pycnidiophora* are almost identical to those retrieved from GenBank, this is not the case for *M. peliocarpa* and *M. stipitata*. Indeed, our accession of *M. peliocarpa* from the Netherlands is resolved with strong support as closely related to the recently described *M. usneae* from Madeira (van den Boom & Ertz 2014) while the sequence from Norway (Andersen & Ekman 2005) is quite different (26 substitutions for the mtSSU sequence). The same applies to our

TABLE 1. *Specimens and GenBank Accession numbers used in this study with their respective voucher information. New sequences in bold.*

Name	Country	Collector, year, collection number, herbarium and DNA sample number where appropriate	Publication	GenBank Acc. no.
Byssoloma leucoblepharum	Portugal	*S. Ekman*, -, 3502, BG	Andersen & Ekman 2005	AY567778
B. subdiscordans	USA	*T. Tønsberg*, -, 25968, BG	Andersen & Ekman 2005	AY567779
Micarea adnata	Norway	*H. L. Andersen*, -, 48, BG	Andersen & Ekman 2005	AY567751
M. adnata	France	*E. Sérusiaux*, 2014, s.n., LG DNA 3438		**KX459344**
M. alabastrites	Norway	*H. L. Andersen*, -, 17, BG	Andersen & Ekman 2005	AY567764
M. assimilata	Sweden	*Kanz & C. Printzen*, -, s.n., BG	Andersen & Ekman 2005	AY567739
M. botryoides	Norway	*H. L. Andersen*, -, 79b, BG	Andersen & Ekman 2005	AY567741
M. byssacea	Norway	*H. L. Andersen*, -, 34, BG (as *M. micrococca*)	Andersen & Ekman 2005	AY567749
M. byssacea	Estonia	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4781	Czarnota & Guzow-Krzemińska 2010	EF453670
M. byssacea	Estonia	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3956	Czarnota & Guzow-Krzemińska 2010	EF453690
M. byssacea	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4751	Czarnota & Guzow-Krzemińska 2010	EF453664
M. byssacea	Germany	*P. van den Boom*, 2014, 50037, hb. van den Boom, LG DNA 3495		**KX459345**
M. cinerea	Norway	*T. Tønsberg*, -, 28572, BG	Andersen & Ekman 2005	AY567763
M. clavopycnidiata	USA	*T. Tønsberg*, -, 27215, BG	Andersen & Ekman 2005	AY567747
M. coppinsii	Norway	*T. Tønsberg*, -, 26075, BG	Andersen & Ekman 2005	AY567761
M. deminuta	Not specified	*Z. Palice & Voriskova*, -, 6745, hb. Palice		AY756446
M. denigrata	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4593	Czarnota & Guzow-Krzemińska 2010	EF453681
M. denigrata	Netherlands	*A. M. Brand*, 2014, 63258, hb. Brand, LG DNA 3851		**KX459346**
M. denigrata	Germany	*E. Sérusiaux*, 2015, s.n., LG DNA 4381		**KX459347**
M. doliiformis	United Kingdom	*A. Orange*, 2006, s.n., A. Orange, LG, NMW	Sérusiaux *et al.* 2010	GU138666
M. doliiformis	Canary Isl., Tenerife	*P. van den Boom*, 2014, 52014, hb. van den Boom, LG DNA 4239		**KX459348**
M. elachista	Sweden	*Koffman*, -, 399, hb. Koffman	Andersen & Ekman 2005	AY567755
M. elachista	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 2986	Czarnota & Guzow-Krzemińska 2010	EF453680
M. erratica	Sweden	*U. Arup*, -, 99192, hb. Arup	Andersen & Ekman 2005	AY567737
M. eximia	Not specified	*Hermansson*,-, 8866b, UPS		AY756447
M. flagellispora	Australia, Tasmania	*G. Kantvilas*,-, 60/90, UPS		AY756448
M. hedlundii	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3895	Czarnota & Guzow-Krzemińska 2010	EF453672
M. hedlundii	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3915	Czarnota & Guzow-Krzemińska 2010	EF453667

TABLE 1 (*continued*).

Name	Country	Collector, year, collection number, herbarium and DNA sample number where appropriate	Publication	GenBank Acc. no.
Micarea hedlundii	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4589	Czarnota & Guzow-Krzemińska 2010	EF453677
M. herbarum sp. nov. Type	Netherlands	*P. & G. van den Boom*, 2015, 52575, hb. van den Boom, LG DNA 4236		**KX459349**
M. herbarum sp. nov.	Netherlands	*A. M. Brand*, 2014, 63193, hb. Brand, LG DNA 3852		**KX459350**
M. herbarum sp. nov.	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4634 (as *M. nowakii*)	Czarnota & Guzow-Krzemińska 2010	EF453692
M. cf. *herbarum*	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3464 (as *M. nowakii*)	Czarnota & Guzow-Krzemińska 2010	EF453665
M. incrassata	Not specified	*T. Tønsberg*, -, 17593 (BG)	Andersen & Ekman 2005	AY756449
M. lapillicola	Czech Republic	*C. Printzen*, -, s.n., BG	Andersen & Ekman 2005	AY567735
M. leprosula	Norway	*H. L. Andersen*, -, 35, BG	Andersen & Ekman 2005	AY567762
M. lignaria var. *lignaria*	Norway	*H. L. Andersen*, -, 18, BG	Andersen & Ekman 2005	AY567748
M. lignaria var. *lignaria*	France	*E. Sérusiaux*, 2014, s.n., LG DNA 3435		**KX459351**
M. lignaria var. *lignaria*	Romania	*E. Sérusiaux*, 2015, s.n., LG DNA 4375		**KX459352**
M. lithinella	Norway	*H. L. Andersen*, -, 80b, BG	Andersen & Ekman 2005	AY567734
M. lynceola	Czech Republic	*Z. Palice*, 1996, -, hb. Palice	Andersen & Ekman 2005	AY567738
M. meridionalis sp. nov. Type	Portugal	*P. van den Boom*, 2015, -, hb. van den Boom, LG DNA 4279		**KX459353**
M. meridionalis sp. nov.	Portugal	*P. van den Boom*, 2015, -, hb. van den Boom, LG DNA 4281		**KX459354**
M. meridionalis sp. nov.	Portugal	*P. van den Boom*, 2015, -, hb. van den Boom, LG DNA 4581		**KX459355**
M. marginata	Not specified	*S. Bayerová, J. Liska & Z. Palice*,-, 5159, hb. Palice		AY756451
M. melaena	Norway	*H. L. Andersen*, -, 25, BG	Andersen & Ekman 2005	AY567743
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3179	Czarnota & Guzow-Krzemińska 2010	EF453674
M. micrococca	Estonia	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4782	Czarnota & Guzow-Krzemińska 2010	EF453676
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4179	Czarnota & Guzow-Krzemińska 2010	EF453691
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3632	Czarnota & Guzow-Krzemińska 2010	EF453668
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4553	Czarnota & Guzow-Krzemińska 2010	EF453683
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4059	Czarnota & Guzow-Krzemińska 2010	EF453663
M. micrococca	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4456	Czarnota & Guzow-Krzemińska 2010	EF453662

TABLE 1 (*continued*).

Name	Country	Collector, year, collection number, herbarium and DNA sample number where appropriate	Publication	GenBank Acc. no.
Micarea micrococca	Netherlands	*P. & B. van den Boom*, 2014, 50314, hb. van den Boom, LG DNA 3853		**KX459356**
M. micrococca	Netherlands	*P. & B. van den Boom*, 2014, 51244, hb. van den Boom, LG DNA 3855		**KX459357**
M. micrococca	Netherlands	*P. & B. van den Boom*, 2015, 52570, hb. van den Boom, LG DNA 4237		**KX459358**
M. misella	Norway	*H. L. Andersen*, -, 73, BG	Andersen & Ekman 2005	AY567752
M. misella	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4593	Czarnota & Guzow-Krzemińska 2010	EF453687
M. myriocarpa	Norway	*H. L. Andersen*, -, 37, BG	Andersen & Ekman 2005	AY567736
M. nitschkeana	Czech Republic	*C. Printzen*, -, s.n., BG	Andersen & Ekman 2005	AY567758
M. nitschkeana	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3306	Czarnota & Guzow-Krzemińska 2010	EF453685
M. nowakii	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4181	Czarnota & Guzow-Krzemińska 2010	EF453688
M. nowakii	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4688	Czarnota & Guzow-Krzemińska 2010	EF453689
M. nowakii	Romania	*E. Sérusiaux*, 2015, s.n., LG DNA 4380		**KX459359**
M. nowakii	Romania	*E. Sérusiaux*, 2015, s.n., LG DNA 4385		**KX459360**
M. paratropa	Norway	*H. L. Andersen*, -, 94, BG	Andersen & Ekman 2005	AY567740
M. peliocarpa	Norway	*H. L. Andersen*, -, 29, BG	Andersen & Ekman 2005	AY567760
M. peliocarpa	Netherlands	*P. van den Boom*, 2014, 51318, hb. van den Boom, LG DNA 3847		**KX459361**
M. prasina	USA	*T. Tønsberg*, -, 30856, BG	Czarnota & Guzow-Krzemińska 2010	AY756452
M. prasina	France	*E. Sérusiaux*, 2014, s.n., LG DNA 3437		**KX459362**
M. prasina	Belgium	*E. Sérusiaux*, 2014, s.n., LG DNA 3609		**KX459363**
M. prasina	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3913	Czarnota & Guzow-Krzemińska 2010	EF453675
M. prasina	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3914	Czarnota & Guzow-Krzemińska 2010	EF453669
M. prasina	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4319	Czarnota & Guzow-Krzemińska 2010	EF453679
M. prasina	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4489	Czarnota & Guzow-Krzemińska 2010	EF453678
M. prasinella	USA	*B. McCune*, -, 35337, BG	Andersen & Ekman 2005	AY567745
M. pycnidiophora	USA	*T. Tønsberg*, -, 30881, BG	Andersen & Ekman 2005	AY567754
M. pycnidiophora	Belgium	*E. Sérusiaux*, 2014, s.n., LG DNA 3498		**KX459364**

TABLE 1 (*continued*).

Name	Country	Collector, year, collection number, herbarium and DNA sample number where appropriate	Publication	GenBank Acc. no.
Micarea soralifera	Poland	*M. Kukwa*, 2014, 12722, UGDA	Guzow-Krzemińska *et al.* 2016	KT119884
M. soralifera	Poland	*M. Kukwa*, 2014, 12999, UGDA	Guzow-Krzemińska *et al.* 2016	KT119885
M. soralifera Type	Poland	*M. Kukwa* 13001 & *A. Łubek*, 2014, UGDA	Guzow-Krzemińska *et al.* 2016	KT119886
M. stipitata	USA	*S. Ekman*, -, s.n., BG	Andersen & Ekman 2005	AY567756
M. stipitata	Canary Is., Tenerife	*E. Sérusiaux*, 2014, s.n., LG DNA 3816		**KX459365**
M. subviridescens	U.K., Scotland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3599	Czarnota & Guzow-Krzemińska 2010	EF453666
M. synotheoides	Norway	*H. L. Andersen*, -, 47, BG	Andersen & Ekman 2005	AY567756
M. tomentosa	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3949	Czarnota & Guzow-Krzemińska 2010	EF453686
M. turfosa	Norway	*H. L. Andersen*, -, 59, BG	Andersen & Ekman 2005	AY567742
M. usneae	Portugal, Madeira	*P.& B. van den Boom*, 2012, 48057, BR	van den Boom & Ertz 2014	KF569511
M. viridileprosa	Netherlands	*P. & B. van den Boom*, 2013, 50066, hb. van den Boom, LG DNA 3493		**KX459366**
M. viridileprosa	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3436	Czarnota & Guzow-Krzemińska 2010	EF453671
M. viridileprosa	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 3869	Czarnota & Guzow-Krzemińska 2010	EF453673
M. viridileprosa	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4518	Czarnota & Guzow-Krzemińska 2010	EF453684
M. viridileprosa	Poland	*P. Czarnota & B. Guzow-Krzemińska*, -, GPN 4527	Czarnota & Guzow-Krzemińska 2010	EF453682
M. xanthonica	USA	*T. Tonsberg*, -, 25674, BG	Czarnota & Guzow-Krzemińska 2010	AY756454
Szczawinskia leucopoda	USA	*T. Tønsberg*, -, 30044, BG	Andersen & Ekman 2005	AY567746

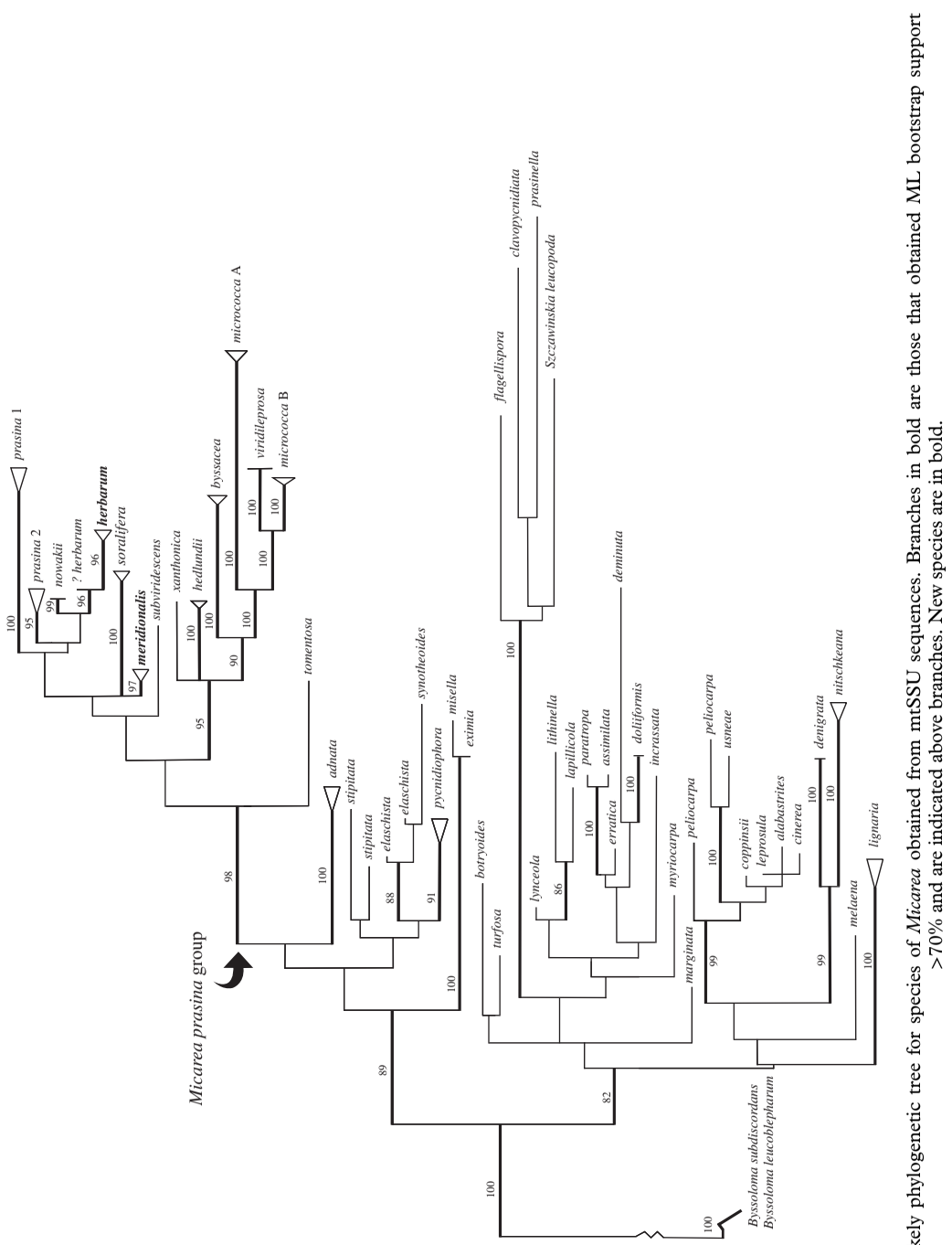

FIG. 1. Most-likely phylogenetic tree for species of *Micarea* obtained from mtSSU sequences. Branches in bold are those that obtained ML bootstrap support >70% and are indicated above branches. New species are in bold.

sequences for *M. stipitata* from Tenerife (Canary Islands) which differ by 22 substitutions from the sequence from the USA retrieved from GenBank (Andersen & Ekman 2005). Thus, our limited sampling illuminates the rather poorly known diversity within *Micarea*.

Morphological, anatomical and chemical results are included in the description of both new species.

Taxonomy

Micarea herbarum Brand, Coppins, Sérus. & van den Boom sp. nov.

MycoBank No.: MB 811051

Species inconspicuous, with a very thin thallus comprised of small greenish, flattened or slightly convex areoles, and often covered by a thin film of gelatinous green algal cells. Apothecia abundant, dark brown to black, 0·15–0·25 mm diam., immarginate. Ascospores ellipsoid, 6·5–9·7 × 2·0–2·6 µm, (0–)1-septate. Pigment Sedifolia-grey, K+ violet. Mesopycnidia often abundant; mesoconidia shortly bacilliform, 3·8–6·1 × 1·0–1·2(–1·3) µm. No secondary compounds detected.

Type: the Netherlands, Noord-Brabant, S of Oirschot, S rim of Oirschotse Heide, *Pinus-Quercus* forest with many fallen trunks of *Quercus*, on wood of fallen trunk, TDN grid ref. 51.33.42, 22 January 2015, *P. & B. van den Boom* 52575 (LG—holotype; hb. v.d. Boom–isotype).

(Fig. 2A–D)

Thallus very thin, consisting of small greenish, flattened or slightly convex areoles, less than 0·1 mm diam., with crystals, often partly coated by a thin gelatinous film of green algal cells. *Photobiont* micareoid, 6–8 µm diam., thin-walled, clustered in compact masses.

Apothecia abundant, subglobose, immarginate, 0·15 to 0·25 mm diam., dark brown to black. *Hymenium c.* 30–40 µm high; *epihymenium* with dark green-brown (K+ violet) spots; *paraphyses* sparse, branched, *c.* 1·2–1·4 µm wide. *Hypothecium* hyaline. *Asci* 20–28 × 7–12 µm. *Ascospores* ellipsoid, 6·5–9·7 × 2·0–2·6 µm, (0–)1-septate.

Mesopycnidia often abundant, *c.* 40–80 µm, top dark greenish grey (K+ violet), formed inside a thallus granule, or outside of the lichenized thallus in the gelatinous matrix of free algal cells. *Mesoconidia* shortly bacilliform, rarely obovoid, 3·8–6·1 × 1·0–1·2 (–1·3) µm, non-septate.

Chemistry. No chemical compounds; pigment Sedifolia-grey (Meyer & Printzen 2000), K+ violet.

Etymology. The epithet chosen for this species refers to a quite unusual habitat (decaying herbs) on which it has been found several times.

Habitat and distribution. On soft and decaying wood, on standing dead trunks, on dead and wet stems of herbaceous plants, or directly on soil. In the Netherlands, accompanying species on decaying wood include *Absconditella* sp., *Micarea micrococca* and *Placynthiella dasaea*, and on soil *Absconditella fossarum* and *Thelocarpon lichenicola*. It is distributed throughout the Netherlands, and has been detected in one collection from Poland, filed under the recently described *M. nowakii* (Czarnota 2007). In the latter collection, it grows on decorticated wood with *M. denigrata* and *M. misella*. The species is very inconspicuous, and can be easily overlooked because it is frequently covered with gelatinous algal cells. No doubt it will be found in other European countries.

Notes. In the monograph and revisions of the genus in Europe (Coppins 1983, 2009; Czarnota 2007), this new species would key out as *Micarea denigrata* if no chemical test for gyrophoric acid is performed. Indeed, it looks like a small or depauperate *M. denigrata*, a common and highly polymorphic species, but clearly differs by the lack of gyrophoric acid and the shorter mesoconidia in *M. denigrata* (3·0–4·2(–5·0) × 1·4–1·8(–2·0) µm, *fide* Czarnota 2007). We interpret the conidia produced by our new species as mesoconidia as they originate in rather large pycnidia and they are regularly bacilliform (rarely obovoid) in shape, and thus akin to the mesoconidia produced by *M. denigrata*. No validly published epithet reduced into synonymy with this species in Coppins (1983) can be assigned to the new species. *Micarea misella* might also be confused with this species and differs mainly by its simple ascospores.

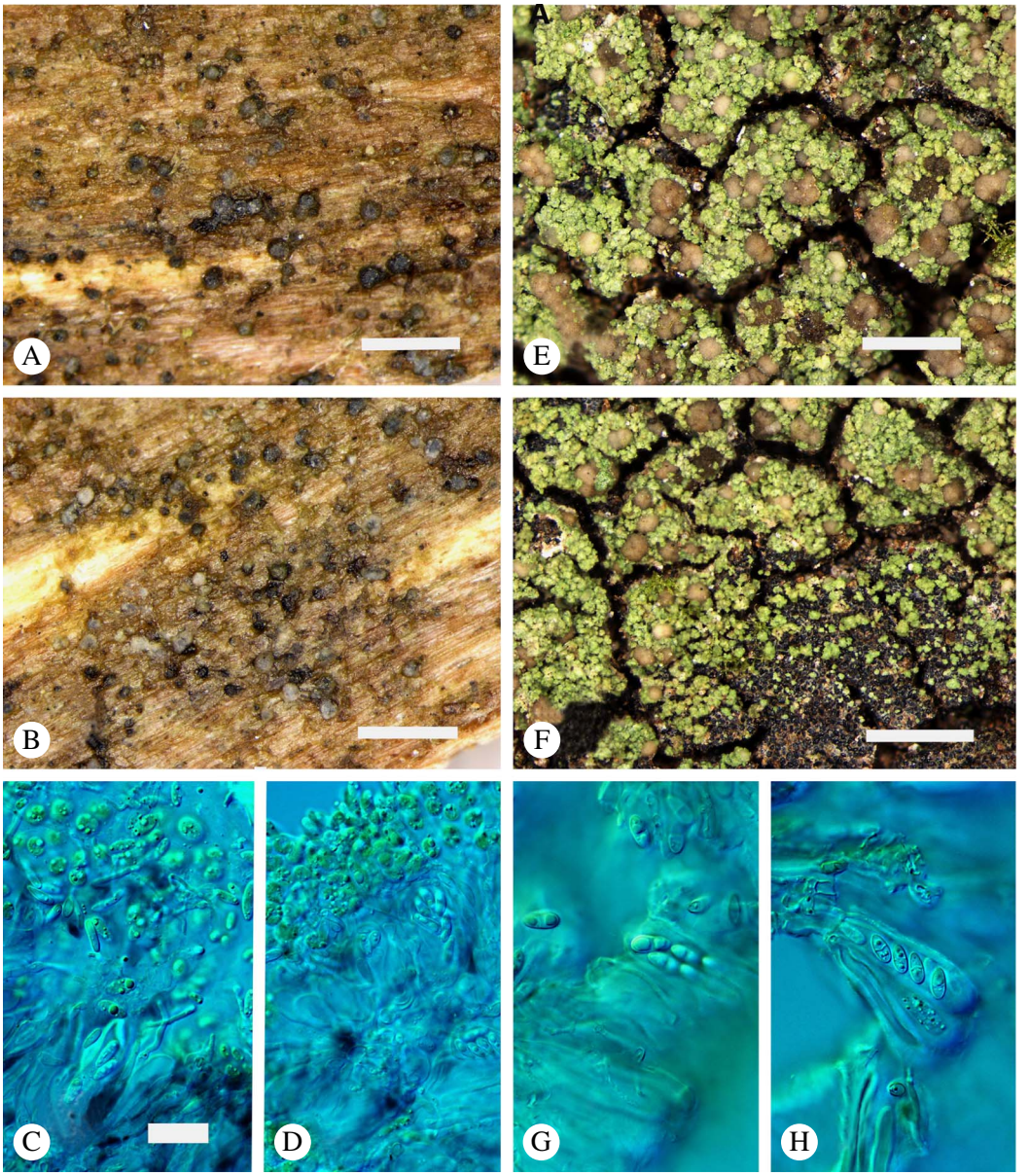

FIG. 2. *Micarea herbarum* and *M. meridionalis*. A–D, *Micarea herbarum* (holotype); A & B, habitus; C & D, ascospores with green algal cells growing over the hymenium. E–H, *Micarea meridionalis* (holotype); E & F, habitus; G & H, ascospores. Scales: A, B, E & F = 0·5 mm; C, D, G & H = 20 µm. In colour online.

In addition, *M. denigrata* and *M. misella* are resolved outside the *M. prasina* clade in which *M. herbarum* is nested with strong support. Phylogenetic inferences from mtSSU sequences position *M. herbarum* in an unsupported clade within the strongly supported *M. prasina* group, together with both lineages referred to as *M. prasina* and *M. nowakii*, a species described from Poland (Czarnota 2007). *M. nowakii* was segregated from *M. denigrata*

and *M. misella* based on the production of micareic acid (vs gyrophoric acid or no substances for the other two, respectively). Compared with *M. herbarum*, *M. nowakii* has slightly smaller ascospores (0(–1)-septate, 6·0–8·0(–8·5) × 2·0–3·0(–3·2) µm *fide* Czarnota 2007) and shorter and wider mesoconidia (3·5–4·0 × 1·5–1·8 µm *fide* Czarnota 2007).

The position of the two accessions of *M. herbarum* in our phylogenetic tree points to a relationship between *M. herbarum* and *M. nowakii*, or even that *M. herbarum* cannot be distinguished from *M. nowakii*. Among the mtSSU sequences published by Czarnota & Guzow-Krzemińska (2010) for *M. nowakii*, one accession (Poland, *P. Czarnota* 4634, GPN) is resolved with strong support as closely related to *M. herbarum*. We found that this collection represents *M. herbarum* (no micareic acid detected by TLC); a further collection (Poland, *P. Czarnota* 3464, GPN) might represent either a further species in that group, or a variant of *M. herbarum*. In fact, this collection does not have apothecia but only pycnidia; no crystals of any lichen substance could be detected by microcrystallization and the phylogenetic tree resolved it as sister with all accessions referred to *M. herbarum*.

Micarea melanobola (Nyl.) Coppins is a species so far known only from the type collection in Finland, collected in 1866 (Coppins 1983). Although it has been reduced into synonymy with *M. prasina* by Czarnota (2007), we suspect this species to be close to *M. nowakii* and thus to *M. herbarum*. Indeed, *M. melanobola* has paraphyses with "apices thickened with greenish (K+ violet) pigment and up to 1·7 µm wide overtopping the tops of asci" (Coppins 1983), a typical feature that seems to be quite similar to the paraphyses of *M. nowakii* described with "apices, thickened to 2–2·5 µm and dull olive pigment", said to react K+ violet (legend to fig. 46 in Czarnota 2007). The chemistry of the type of *M. melanobola* could not be studied for lack of material (Coppins 1983). The genuine identity of the type material of *M. melanobola* should therefore be re-evaluated.

It is worth mentioning that *M. herbarum* is the first species within the *prasina* group that does not produce any secondary compounds other than the pigment present in apothecia.

The biology of *M. herbarum* sp. nov. is remarkable, as it very often grows over or within a gelatinous film of green algal cells. Hyphae connected with the apothecia and pycnidia penetrate into this layer. Furthermore, these unknown algae can penetrate into the subhymenium of the *Micarea* apothecia.

Specimens examined. **The Netherlands:** *Groningen*: 1·8 km N van Sellingen, E bank of Ruiten Aa, N of bridge near Rijsdam, 52°57·8'N, 7°8·6'E, open area, loamy soil recently scraped, 1999, *M. Brand* 39768 (hb. Brand). *Overijssel*: Hardenberg, Rheezerveen, Klimberg, 52°34·8'N, 6°32·9'E, dead wood in forest, 2010, *M. Brand* 61133 (hb. Brand). *Noord-Holland*: Santpoort, Duin en Kruidberg, tank ditch between Argus and Westerveld, 52°26·6'N, 4°37·4'E, open forest in dune area, wood of *Populus alba* branches, 2014, *M. Brand* 63193 (hb. Brand). *Zuid-Holland*: Zoetermeer, Sprinterpad, N of Westerpark, 52°3·6'N, 4°26·7'E, rotting wood of dead *Populus* trunks, 2010, *M. Brand* 60950 (hb. Brand). *Gelderland*: Vierhouten, Elspeetsche Heide, W of bicycle trail, 52°18·6'N, 5°48·5'E, rotting wood in heathland, 1999, *M. Brand* 39206 (hb. Brand); Heerde, Sprengen, 52°23·9'N, 6°0·2'E, wood of bridge in forest, 1973, *M. Brand* 3254 (hb. Brand); Garderen, Speulderbos, 14 m E of Dodenweg, 52°14·65'N, 5°41·53'E, dead wood of fallen *Fagus* in forest, 1998, *M. Brand* 37726 (hb. Brand). *Zeeland*: Tholen, recently reclaimed area between Slaakdam and Haaftenpolder, S side, 51°36·3'N, 4°10·8'E, twigs in open grass vegetation, 1983, *M. Brand* 33842 (hb. Brand). *Noord-Brabant*: E of Oirschot, Oirschotse Heide, N side of secondary road to Oirschot, *Pinus* forest and edge of *Calluna* heathland with some *Quercus robur* trees, 51°30'N, 5°21'E, wood of fallen trunk, 2014, *P. & B. van den Boom* 52533 (hb. v.d. Boom). *Limburg*: Weert, Kolenhofweg, 1 km NE of Mildert, 51°14·6'N, 5°48·3'E, rotting wood of trunk in young *Pinus* plantation, 2000, *M. Brand* 41201 (hb. Brand); Swalmen, 2 km NE, Boschheide, 51°14·4'N, 6°3·7'E, recently partly scraped *Calluna* heathland, 1992, *M. Brand* 29016 (hb. Brand); E of Wanssum, NE of Wellerlooi, De Hamert, open *Pinus* forest with *Calluna* heathland, a few scattered *Pinus strobus* trees and fallen trunks, 51°32·7'N, 6°08·6'E, dead wood of fallen *P. strobus*, 2015, *P. & B. van den Boom* 53197 (hb. v.d. Boom).—**Poland:** Pojezierze Lubuskie Lakeland, S of Motski Village, 52°14'42"N, 15°22'14"E, on wooden fence in well-lit place near the edge of pine forest, 2005, *P. Czarnota* 4634 (GPN).

Micarea meridionalis van den Boom, Brand, Coppins & Sérus. sp. nov.

MycoBank No.: MB 811050

Thallus areolate, areoles subglobose to irregularly flattened, 40–100 µm diam., pale to medium greenish,

or greenish grey to pale brownish; apothecia abundant, 0·10–0·30(–0·35) mm diam., pale to dark brownish grey; ascospores ellipsoid, 1(–3)-septate, 8·0–9·4 × 3·4–4·0 µm; mesopycnidia often present, 50–70 µm diam.; mesoconidia 5·8–6·7 × 1·0–1·2 µm, bacilliform to fusiform. Thallus and apothecia with micareic acid.

Type: Portugal, Alentejo, ESE of Odemira, *c.* 4 km E of Santa Clara-a-Velha, near storage lake (W side), *Pinus* forest, 37°30·90'N, 8°26·66'W, 150 m, 16 February 2015, on *Pinus*, *P.* & *B. van den Boom* 52904 (LG—holotype; hb. Brand, hb. v.d. Boom—isotypes).

(Fig. 2E–H)

Thallus ± effuse, up to 4 cm wide, appearing granular, with ecorticate areoles, 40–100 µm diam., subglobose to irregularly flattened, coalescing and forming a continuous crust or scattered patches; upper surface usually smooth, pale to medium greenish, or greenish grey to brownish grey to pale brown, matt to slightly shiny, up to 20 µm thick, mostly thinly coated by gelatinous algae, fine crystals present; rarely forming soredioid structures consisting of loose goniocysts 10–14 µm in diam. *Photobiont* micareoid, algal cells globose, 4–6 µm diam., thin-walled, clustered in compact masses.

Apothecia 0·10–0·30(–0·35) mm diam., abundant, scattered, sometimes tuberculate, immersed to semi-immersed in thallus granules and thus seemingly marginate, because of thallus parts present on the edge of apothecium, eventually becoming immarginate; *disc* beige or pale to dark brownish grey, never black, slightly to moderately convex, a few crystals sometimes seen in apothecium section. *Hymenium* hyaline, 40–50 µm high, with spots pale greyish brown, K+ violet, N+ reddish; *excipulum* in young apothecia hardly distinct, up to 10 µm wide; *paraphyses* abundantly branched, *c.* 1·2 µm wide, tips not or sometimes slightly widened, up to 1·5 µm, not pigmented; *epithecium* olive-greenish, K+ violet, N+ reddish; *hypothecium* hyaline. *Asci* slightly clavate, 35–40 × 8–10 µm, 8-spored. *Ascospores* ellipsoid, 1(–3)-septate, 8·0–9·4 × 3·4–4·0 µm.

Macropycnidia very rare, *c.* 50 µm diam., hyaline but top greyish; *macroconidia* 15–17 × 1·1–1·3 µm, 1-septate, slightly curved. *Mesopycnidia* often present, immersed, 50–70 µm diam., hyaline; *mesoconidia* bacilliform to fusiform, 5·8–6·7 × 1·0–1·2 µm.

Chemistry. Thallus and apothecia K−, C−, KC−, P−; micareic acid detected by TLC in thallus and apothecia; pigment Sedifolia-grey (Meyer & Printzen 2000), K+ violet.

Etymology. The epithet chosen for this species refers to its southern distribution in Europe.

Habitat and distribution. *Micarea meridionalis* is known from several localities in western Portugal, in lowland and maritime areas, in the Alentejo, Estremadura and Lisboa provinces, between 39°40'N and 37°30'N. In these localities it is a corticolous species, in ruderal and even dusty environments including parklands and roadsides; it has been found on indigenous or planted tree species such as *Acacia longifolia*, *Eucalyptus*, *Pinus*, *Morus*, *Nerium oleander* and *Thuja*. On *Acacia longifolia*, accompanying species include *Arthonia pruinata*, *Cliostomum griffithii*, *Coenogonium tavaresiana*, *Hyperphyscia adglutinata*, *Lecania naegelii* and *Lecanora lividocinerea*. *Diploicia canescens*, *Waynea stoechadeana*, *Candelariella reflexa* and *Physconia grisea* are further associated species found growing together. Two further collections have also been found from Calabria/Italy: here the species grows on *Pinus* trees at sea level in unknown ecological conditions. Further north it is known from the Botanical Garden of Rome in the city centre on *Acanthosyris spinescens* and *Quercus pubescens*, and within the urban area of Rome on *Quercus suber* in a small remnant of semi-natural forest.

Notes. *Micarea meridionalis* is resolved within the *M. prasina* clade with strong support but its relationships within it are unclear. It is easily distinguished by its granular thallus and the production of micareic acid. The only other *Micarea* species that produce micareic acid are *M. nowakii*, *M. prasina* s. str. and *M. soralifera* (Czarnota 2007; Czarnota & Guzow-Krzemińska 2010; Guzow-Krzemińska *et al.* 2016). In addition to the typical granular thallus, *M. meridionalis* can be distinguished from *M. nowakii* by its longer mesoconidia (3·5–4·0 × 1·5–1·8 µm for *M. nowakii*, *fide* Czarnota (2007)) and from *M. prasina* s. str. by its shorter

ascospores ((6–)8–12(–14) × (2·5–)3·0–4·0 (–5·5) µm for *M. prasina* s. str., *fide* Czarnota (2007)). *Micarea soralifera* has similar granular areoles to *M. meridionalis*, but is distinguished by its distinct, mostly discrete soralia. Furthermore, the ecology of *M. meridionalis* is much more ruderal and eutrophic than *M. nowakii*, *M. prasina* s. str. and *M. soralifera*, all of which prefer more acidic and nutrient-poor substrata.

No validly published epithet reduced into synonymy with *M. prasina* in the monograph and revisions of the genus in Europe (Coppins 1983, 2009; Czarnota 2007) can be assigned to the new species.

Specimens examined. **Portugal:** *Estremadura*: E of Caldas da Rainha, road to Santa Catarina, between Cabeça Alta and Portela, *Pinus* forest along road, on *Pinus*, 2001, *P. & B. van den Boom* 27727, 27731, 27736 (hb. v.d. Boom). *Beira*: Nazaré, NE of town, *Pinus* forest, on *Pinus*, 2003, *M. Brand* 49616 (hb. Brand). *Setubal*: Serra da Arrábida, 2 km SW of Aldeia, near gate of former chapel, on old *Morus*, 2003, *M. Brand* 49832 (hb. Brand); *ibid.*, on *Nerium oleander*, *M. Brand* 49839 (hb. Brand). *Alentejo*: NE of Cercal, road N261 from Alvalade to São Domingo, mature *Quercus suber* trees along field, on *Q. suber*, 37°55·91'N, 8°27·93'W, 2015, *P. & B. van den Boom* 53059 (hb. v.d. Boom); WNW of Cercal, N of Vila Nova de Milfontes, Praia da Ilha, coastal outcrops with shrubs, including *Acacia longifolia*, on *Acacia*, 37°49·79'N, 8°47·48'W, 2015, *P. & B. van den Boom* 52966 (hb. v.d. Boom); ENE of Cercal, *c.* 1 km E of the city, along road N262, rows of mature *Eucalyptus globulus* trees, on *Eucalyptus*, 37°48·36'N, 8°38·32'W, 2015, *P. & B. van den Boom* 53089 (hb. v.d. Boom); WNW of Odemira, E of Almograve, Longueira, roadside *Acacia longifolia* shrubs, on *Acacia*, 37°39·41'N, 8°46·17'W, 2015, *P. & B. van den Boom* 52664 (hb. v.d. Boom); WNW of Odemira, Cabo Sardão, *Acacia longifolia* shrubs in coastal area, near lighthouse, on *Acacia*, 37°35·95'N, 8°48·95'W, 2015, *P. & B. van den Boom* 52690 (hb. v.d. Boom); SW of Odemira, Boavista dos Pinheiros, botanical garden 'Parque das Águas', mixed trees, including *Salix* and *Mimosaceae*, on a stump, 37°34·97'N, 8°39·62'W, 2015, *P. & B. van den Boom* 52920 (hb. v.d. Boom); SW of Odemira, just S of Zambujeira, 37°31·18'N, 8°47·11'W, coastal dune area with *Acacia longifolia*, on *Acacia*, 2015, *P. & B. van den Boom* 53032 (hb. v.d. Boom). *Lisboa*: Sintra, park, on trunk of *Thuja*, 2015, *P. & B. van den Boom* 53940 (hb. v.d. Boom).—**Italy:** *Calabria*: Nicotera, on *Pinus* bark, 2000, *D. Puntillo* (E). *Lazio*: Rome, Orto Botanico di Roma, alt. 50 m, on *Acanthosyris spinescens*, 1999, *S. Ravera* 3228 (RO); *ibid.*, alt. 60 m, on *Quercus pubescens*, 1999, *G. Brezzi* [*Ravera* 3229] (RO); Rome, Parco di Monte Mario, alt. 137 m, on *Quercus suber*, 2000, *G. Brezzi* [*Ravera* 3231] (RO).

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