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A new species of *Stigmatodiscus* (Ascomycota, Dothideomycetes, Stigmatodiscaceae) from Juan de Nova (Mozambique Channel, Scattered Islands, French Southern and Antarctic Lands)

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Abstract. *Stigmatodiscus touroultii* (Stigmatodiscaceae, Stigmatodiscales) is described and illustrated from corticated dead twigs of *Salvadora angustifolia* collected in Juan de Nova (Scattered Islands, Mozambique Channel). It is characterized by the irregularly shaped pruinose hymenial disc without distinct black marginal lips and a calcium oxalate crystal layer in the epithecium. Phylogenetic analyses of a multigene matrix containing a representative selection of *Dothideomycetes* from four genes (nucSSU-ITS-LSU rDNA, *RPB2*, *TEF1* and *TUB2*) revealed a highly supported placement within *Stigmatodiscus* as sister species to *Stigmatodiscus oculatus*. Micromorphology of the sexual and asexual morph matches the genus *Stigmatodiscus*. A key to all known species worldwide is provided.

Key words: Indian Ocean, Africa, Tropics, *Stigmatodiscales*, Mozambique Channel

Introduction

The genus *Stigmatodiscus* Voglmayr & Jaklitsch belongs to the family *Stigmatodiscaceae*, order *Stigmatodiscales* (*Dothideomycetes*) that has been recently described (Voglmayr et al. 2016). Up to now, it exhibited only a Central and Southern Europe distribution (Voglmayr et al. 2016, 2017; Voglmayr & Pintos Amengual 2018). Recently, field surveys focusing on corticolous lichen species were performed in 2019 on the Scattered Islands (French Southern and Antarctic Lands): Europa Island, Juan de Nova, Glorioso Islands, and Tromelin (Fig. 1), that are located in the Mozambique Channel and the Western Indian Ocean. These investigations contributed to filling the knowledge gap on lichens from this area, including the descriptions of several new species (Ferron et al. 2020; Poncet et al. 2021). The survey also resulted in collecting non-lichenized lignicolous ascomycetes, among which one could not be identified to species with the available literature, but revealed similarities to the genus *Stigmatodiscus*. This was confirmed by detailed morphological

and molecular phylogenetic investigations, resulting in the characterization of a new species.

Material and methods

Surveyed territories and sampling methodology

Juan de Nova constitutes, along with Europa Island, Glorioso Islands, Tromelin and Bassas da India the Scattered Islands, which is the fifth district of the French Southern and Antarctic Lands (TAAF). The Scattered Islands are oceanic sanctuaries of primitive nature and host a remarkable land and marine biological heritage, which has been mostly preserved due to the geographical isolation, and a historically very limited human occupation. Today, these territories are uninhabited, except for the military and scientists, and most of them benefit from protection status. Europa, Bassas da India, and Tromelin are protected by a prefectural decree which classifies them as a nature reserve since 1975, and the Glorioso Islands are classified as a national nature reserve since 10 June 2021. Juan de Nova benefits from no regulatory conservation status. Biodiversity collection in these territories is limited by remoteness and can only be done with the agreement of the French Southern and Antarctic Lands (TAAF) Administrative Authority. Regarding climate, according to Beck et al. (2018) Köppen-Geiger climate classification, Juan de Nova is ‘Aw’ (main climate: tropical savannah, precipitation: dry winter). Lichenized and non-lichenized

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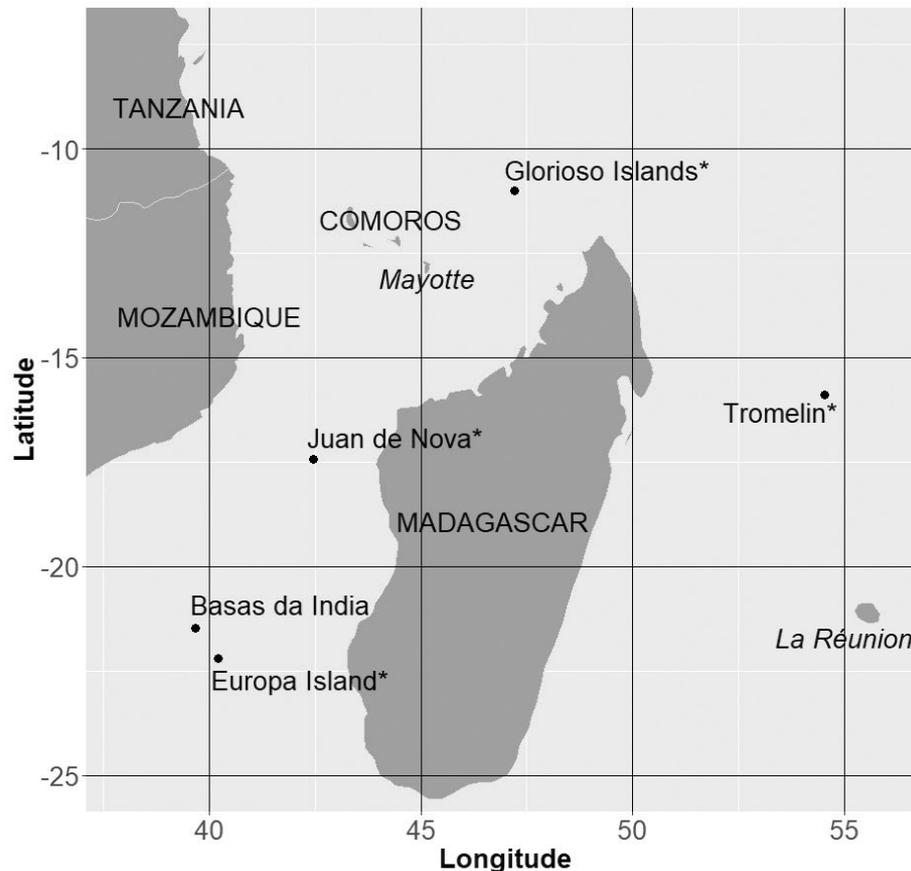


Figure 1. Map of surveyed locations. Territories in lower case not in italics correspond to the Scattered Islands, only those marked with an asterisk (*) have been surveyed; territories in lower case in italics correspond to other French overseas territories; countries are marked in upper case.

species were surveyed in 2019 within the frame of the RECOFFIE Project (‘Renforcement des Connaissances sur la Flore et la Fonge des Iles Eparses’) in four of the five territories constituting the Scattered Islands (Fig. 1). Samples were stored dry in paper envelopes, and associated with collection number, ecological information (phorophytes, when applicable, were identified), date, and location obtained from a field GPS device.

Species identification and description

Identification and descriptive work were performed using a Zeiss Stemi SV8 stereomicroscope and a Leitz Orthoplan compound microscope with phase contrast, connected to a Sony E3CMOS camera sensor. Sections were mounted in tap water, from which all measurements were taken. Ascospore measurements indicate the minimum and maximum values (*n* indicates the number of ascospores measured), and the value in parentheses indicates an exceptionally lower or higher value, which was only observed once among the measured ascospores. In all other criteria, values in parentheses indicate exceptional values outside the minimum and maximum range measured. Chemical spot reactions have been tested on the structures present in the fungus. They are abbreviated as K (10% KOH), I (iodine), and/or N (50% HNO₃). A “-” indicates lack of reaction and “+” indicates a positive reaction followed by information on the reaction.

PCR and sequencing

As the ascospores were no longer viable upon examination, no pure cultures could be obtained for DNA extraction. Therefore, a direct PCR approach was used for sequencing the ITS-LSU rDNA gene. For this, thin sections of apothecia were made using a sterile razor blade, which were directly added to 10 µl of KAPA2G Robust PCR mix (Kapa Biosystems, Cape Town) containing the primers ITS5 (White et al. 1990) and LR5 (Vilgalys & Hester 1990). Prior to PCR, the PCR mix containing the sections was incubated at 80°C for 10 min. The following PCR protocol was applied: 2 min initial denaturation at 95°C, followed by 40 cycles of 10 sec denaturation at 95°C, 15 sec annealing at 55°C, 1 min 30 sec elongation at 72°C, and a final elongation step of 2 min at 72°C. PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) and the PCR primers and the primers ITS4 (White et al. 1990), LR2R-A (Voglmayr et al. 2012) and LR3 (Vilgalys & Hester 1990). Sequencing was performed on an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems).

Table 1. Isolates and GenBank accession numbers of sequences used in the phylogenetic analyses. **Sequences in bold** were generated during the present study.

Taxon	Origin	Host	Voucher	Type ¹	Isolate	GenBank accession numbers ²				
						SSU	ITS-LSU	RPB2	TEFI	TUB2
<i>Anisomeridium ubitanum</i>	Fiji	–	Lumbsch 19845j	–	MPN94	GU327682	GU327709 ³	–	JN887421	–
<i>Dyfalomyces rhizophorae</i>	Hawaii, Oahu	–	–	–	JK 5456A	GU479766	GU479799 ³	–	GU479860	–
<i>Megalotremis verrucosa</i>	Colombia	–	Luecking 26316	–	MPN104	JN887383	GU327718 ³	–	JN887426	–
<i>Palawanita thailandense</i>	Thailand	<i>Dypsis lutescens</i>	MFLU 16-1872	H	MFLUCC 14-1121	KY086495	KY086493 ³	KY086496	–	–
<i>Stigmatodiscus enigmaticus</i>	Austria, Vienna	<i>Acer campestre</i>	WU-MYC 0035913	–	L84	–	KU234114	KU234127	MH756082	KU234146
<i>S. enigmaticus</i>	Austria, Vienna	<i>Acer monspessulanum</i>	WU-MYC 0035914	H	L69 = CBS 132036	KU234130	KU234108	KU234121	MH756078	KU234140
<i>S. enigmaticus</i>	Croatia, Istria	<i>Carpinus orientalis</i>	WU-MYC 0035915	–	L68	–	KU234107	KU234120	MH756077	KU234139
<i>S. enigmaticus</i>	Croatia, Istria	<i>Carpinus orientalis</i>	WU-MYC 0035916	–	L71 = CBS 131997	–	KU234109	KU234122	–	KU234141
<i>S. enigmaticus</i>	Czech Republic, Morava	<i>Acer monspessulanum</i>	WU-MYC 0035917	–	L64	KU234129	KU234106	KU234119	–	KU234138
<i>S. enigmaticus</i>	France, Alpes-de-Haute-Provence	<i>Acer monspessulanum</i>	WU-MYC 0035918	–	L76 = CBS 132037	–	KU234111	KU234124	–	KU234143
<i>S. enigmaticus</i>	France, Var	<i>Acer monspessulanum</i>	WU-MYC 0035919	–	L75	–	KU234110	KU234123	MH756079	KU234142
<i>S. enigmaticus</i>	Greece, Crete	<i>Acer sempervirens</i>	WU-MYC 0035911	–	L82	–	KU234112	KU234125	MH756080	KU234144
<i>S. enigmaticus</i>	Greece, Crete	<i>Acer sempervirens</i>	WU-MYC 0035912	–	L83	KU234131	KU234113	KU234126	MH756081	KU234145
<i>S. enigmaticus</i>	Italy, Lazio	<i>Acer campestre</i>	WU-MYC 0035920	–	L122	–	KU234104	KU234118	–	KU234137
<i>S. labiatus</i>	Spain, Mallorca	<i>Quercus</i> sp.	WU-MYC 0039973	H	AP6516 = CBS 144700	MH756065	MH756065	MH756074	MH756083	MH756089
<i>S. labiatus</i>	Spain, Mallorca	<i>Quercus coccifera</i>	WU-MYC 0039980	–	AP141216	–	MH756066	–	–	–
<i>S. ocellatus</i>	Spain, Mallorca	<i>Populus canadensis</i>	WU-MYC 0039975	–	AP10816	MH756067	MH756067	MH756075	MH756084	–
<i>S. ocellatus</i>	Spain, Mallorca	<i>Cistus albidus</i>	WU-MYC 0039976	–	AP231016B	–	MH756068	–	MH756085	–
<i>S. ocellatus</i>	Spain, Mallorca	<i>Olea europaea</i>	WU-MYC 0039974	H	AP161116 = CBS 144701	–	MH756069	–	MH756086	MH756090
<i>S. ocellatus</i>	Spain, Mallorca	<i>Pistacia lentiscus</i>	WU-MYC 0039977	–	AP171116	–	MH756070	–	MH756087	MH756091
<i>S. ocellatus</i>	Spain, Mallorca	<i>Globularia atypum</i>	WU-MYC 0039978	–	AP311216	–	MH756071	–	MH756088	MH756092
<i>S. ocellatus</i>	Spain, Mallorca	<i>Globularia atypum</i>	WU-MYC 0039978	–	AP311216A	–	MH756072	–	–	–
<i>S. pinicola</i>	Spain, Mallorca	<i>Pinus halepensis</i>	WU-MYC 0039979	H	AP21916B = CBS 144702	MH756073	MH756073	MH756076	–	MH756093
<i>S. pruni</i>	Austria, Niederösterreich	<i>Prunus spinosa</i>	WU-MYC 0035945	H	L167 = CBS 142598	KX611110	KX611110	KX611109	KX611111	MH756094
<i>S. tamaricis</i>	Austria, Vienna	<i>Tamarix tetrandra</i>	WU-MYC 0035906	H	L114 = CBS 136919	KU234128	KU234101	KU234116	KU234133	KU234135
<i>S. tamaricis</i>	France, Bourgogne	<i>Tamarix gallica</i>	WU-MYC 0035908	–	L113 = CBS 136918	–	KU234100	KU234115	KU234132	KU234134
<i>S. tamaricis</i>	Italy, Lazio	<i>Tamarix</i> sp.	WU-MYC 0035910	–	L124	–	KU234102	KU234117	–	KU234136
<i>S. touroultii</i>	France, Juan de Nova	<i>Salvadora angustifolia</i>	WU-MYC 0040049	I	–	–	OM311170	–	–	–

¹ H – holotype, I – isotype² Sources of GenBank sequences: Nelsen et al. (2009, 2011), Suetrong et al. (2009), Mapook et al. (2016, 2017), Voglmayr et al. (2016, 2017), Voglmayr and Pintos Amengual (2018)³ only LSU available

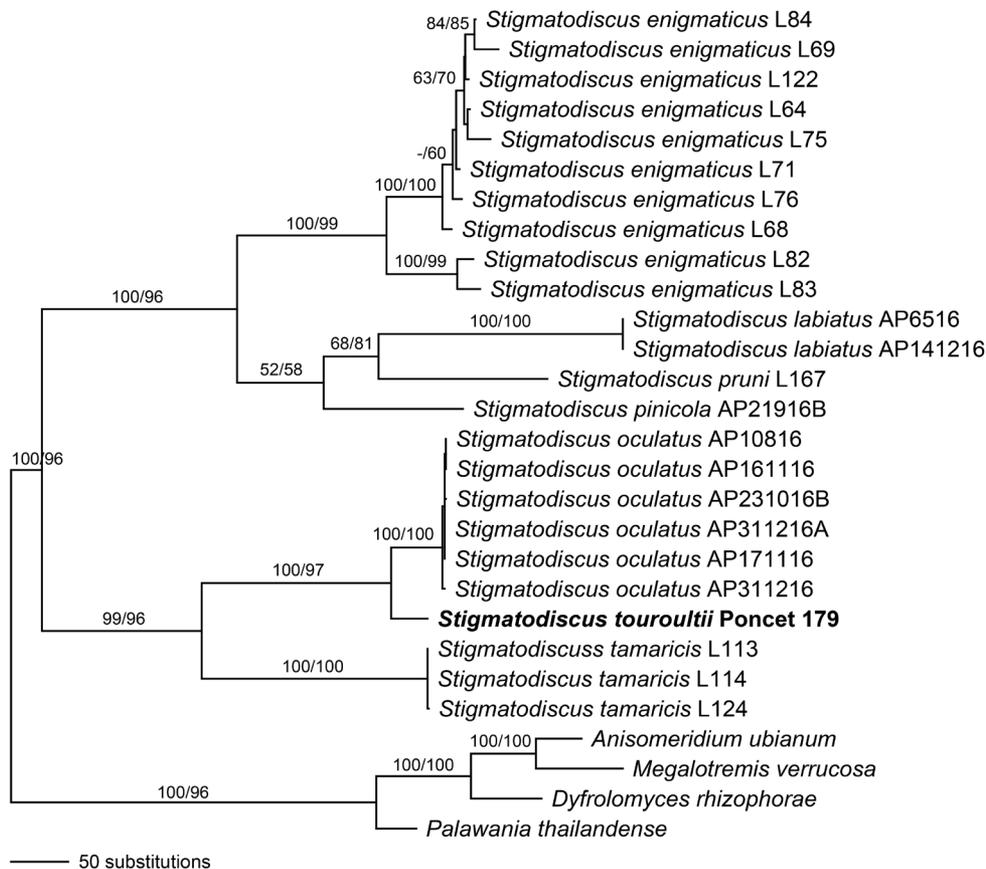


Figure 2. Phylogram showing one of 27 MP trees 2,441 steps long obtained from an MP analysis of the combined multigene matrix of nucSSU-ITS-LSU rDNA, *RPB2*, *TEF1* and *TUB2* from *Stigmatodiscus*. MP and ML bootstrap values above 50% are given at first and second position, respectively, above the branches. The newly described *S. touroultii* is formatted in bold.

Phylogenetic analyses

To reveal the phylogenetic position of the *Stigmatodiscus* from Juan de Nova, a matrix of aligned nucleotide sequences from the four different phylogenetic markers (SSU-ITS-LSU, *RPB2*, *TEF1* and *TUB2*) was produced. GenBank sequences of four taxa (*Anisomeridium ubianum* and *Megalotremis verrucosa* from *Monoblastiales*, *Dyfrolomyces rhizophorae* from *Dyfrolomycetales* and *Palawania thailandense* from *Palawaniaceae*) were added as outgroups according to Voglmayr and Pintos Amengual (2018). Sequences were aligned with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft) and subsequently checked and refined using BioEdit v. 7.0.9.0 (Hall 1999). The combined sequence matrix contained 6,727 nucleotide positions (1,601 from SSU, 1,687 from ITS-LSU, 1,167 from *RPB2*, 1,417 from *TEF1*, 855 from *TUB2*). GenBank accession numbers of the sequences included in the phylogenetic analyses are given in Table 1.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2014) as implemented in raxml-GUI 2.0 (Edler et al. 2021) using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1,000 bootstrap replicates. The matrix was partitioned for the individual gene regions and substitution model parameters were calculated separately for them.

Maximum parsimony (MP) analyses were performed with PAUP v. 4.0a169 (Swofford 2002) using 1,000 replicates of heuristic search with random addition of sequences

and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to NO. Bootstrap analysis with 1,000 replicates was performed in the same way, but using 5 rounds of random sequence addition and subsequent TBR branch swapping during each bootstrap replicate. Bootstrap support below 70% was considered low, between 70–90% medium/moderate and above 90% high.

Results

Molecular phylogeny

For the *Stigmatodiscus* specimen from Juan de Nova, only the ITS-LSU rDNA could be obtained. The parsimony analyses revealed 27 MP trees 2,441 steps long, one of which is shown as phylogram in Fig. 2. The tree backbone of the 27 MP trees was identical, except for minor differences within *S. enigmaticus*. The best tree revealed by RAxML ($-\ln = 21297.9387$) was fully compatible with the MP strict consensus tree. In both MP and ML analyses, the genus *Stigmatodiscus* was highly supported. In the phylogenetic analyses, the *Stigmatodiscus* specimen from Juan de Nova was revealed to represent a distinct species described as *S. touroultii* below, with a sister group relationship to *S. oculatus* receiving maximum (MP) or high (97% ML) support.

Taxonomy

Stigmatodiscus touroultii R. Poncet & Voglmayr, sp. nov.
(Figs 3–4)

Mycobank MB 843510

Diagnosis: The species is morphologically similar to *Stigmatodiscus oculatus* Voglmayr & Pintos, but differs in the irregularly shaped pruinose hymenial disc without distinct black marginal lips and a calcium oxalate crystal layer in the epithecium.

Type: Juan de Nova, S 17°03'40.9589", E 42°43'49.8947", 3 m a.s.l., lignicolous on dead twigs of *Salvadora angustifolia* Turill, leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 14 April 2019, Poncet 179 (PC0784917 – holotype; WU-MYC 0040049 – isotype).

Description. Ascomata numerous, evenly distributed, apothecioid, variable in shape, elongate-sublirelliform or angulose-subrounded, simple, unbranched, sometimes

slightly crenulated, embedded in cortex of dead twigs, initially covered by bark, emerging through irregular cracks, (0.3–)0.4–0.8(–1.1) mm in the longest length, hymenial disc exposed, flat, black to greyish due to a calcium oxalate crystals layer covering the disc, surrounded by a thin black margin visible and persistent excipulum. Excipulum of prosoplectenchymatous cells, brownish, 40–100 µm wide laterally, K+/- olivaceous, N+ slight reddish tinge, continuous below the hypothecium. Hymenium mostly hyaline, brownish-olivaceous in the upper part, 125–140 µm high, I–, K/I–. Paraphyses cellular, simple (sometimes furcate or geniculate at the apex), 2.5–3 µm wide, swollen at their apices up to 4 µm. Epithecium brownish-olivaceous, with a calcium oxalate crystals layer visible in polarized light (not totally disappearing in K). Hypothecium brownish-yellowish, 50–65 µm high, I–, K/I–. Asci subglobose to short-clavate, bitunicate,

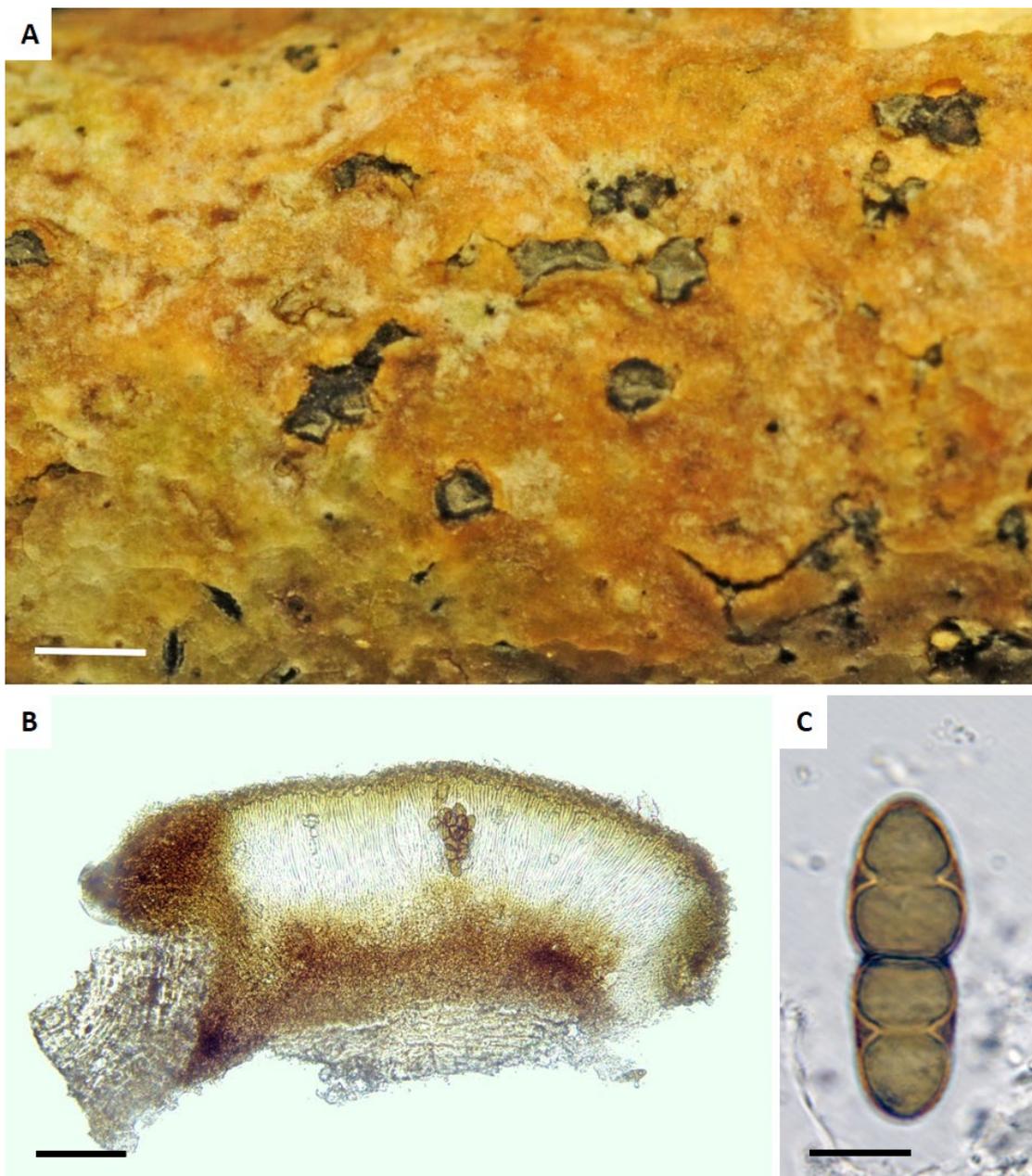


Figure 3. *Stigmatodiscus touroultii*, holotype, PC0784917 (Poncet 179). A – habitus; B – ascomata in vertical section in water; C – ascospore in water. Scales: A = 1 mm; B = 100 µm; C = 10 µm.

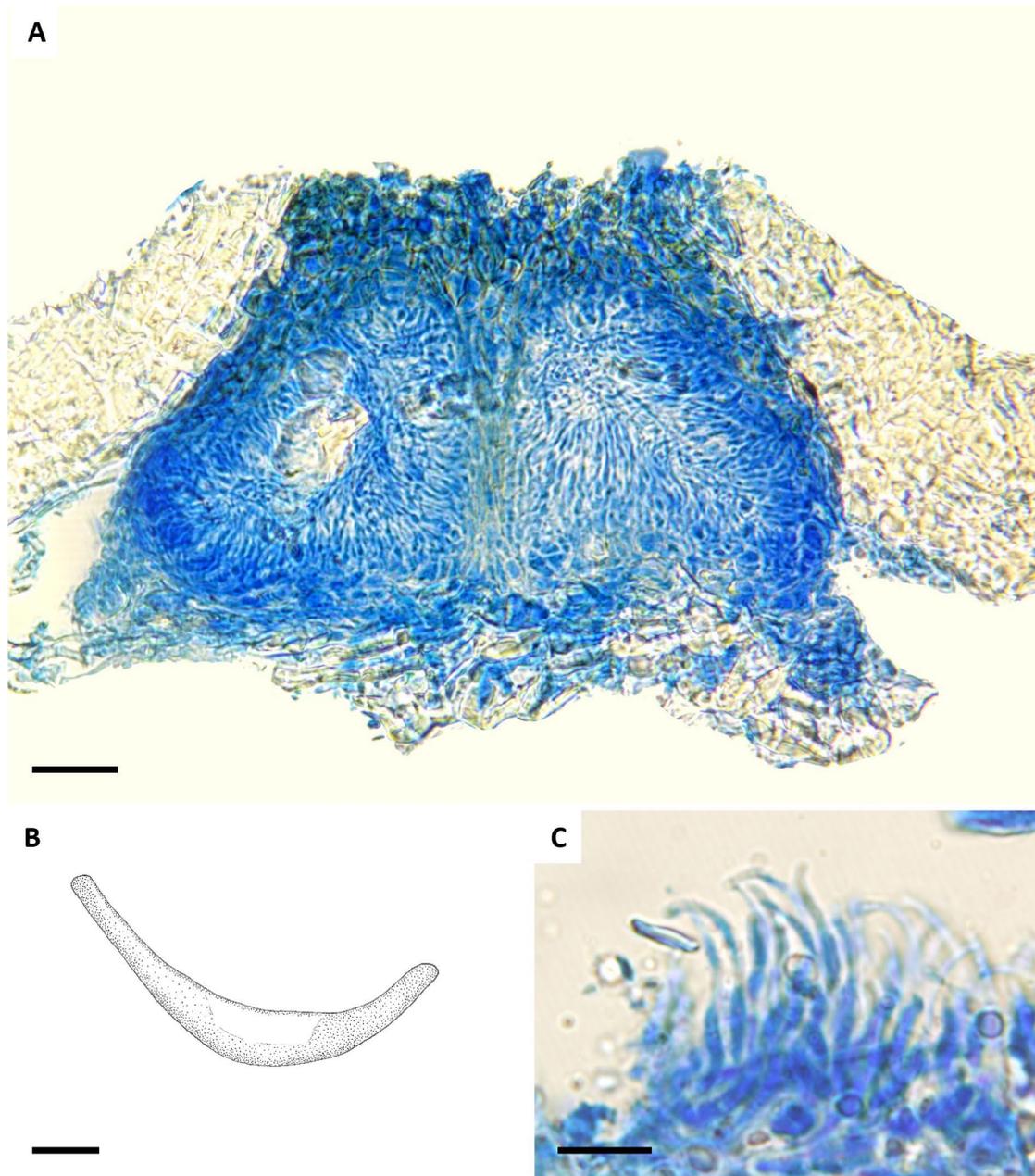


Figure 4. *Stigmatodiscus touroultii*, holotype, PC0784917 (Poncet 179). A – two pycnidia in section in cotton blue; B – conidia in water (drawing A.-H. Paradis); C – conidiogenous cells in cotton blue. Scales: A = 25 μm ; B = 2 μm ; C = 10 μm .

fissitunicate, apically with a wide ocular chamber, I–, K/I–, 8-spored, 65–80 \times 35–40 μm (n = 5). Ascospores hyaline at first in the asci but brown at maturity before discharge, wall distinctly verrucose, I–, first 1-septate (upper cell often slightly larger), developing 2 additional distosepta and becoming 3-septate with age, ellipsoid to sole-shaped, straight, constricted at the septum (at least at first septum), 30–35 \times 11.5–13.8 μm (n = 20), thick gelatinous sheath present when young. Pycnidia present, associated with ascomata, immersed, bilocular, of circular to irregular shape, opening in irregular black cracks, 170–200 μm diam., wall thin, of prosoplectenchymatous cells laterally and of paraplectenchymatous cells in upper parts, hyaline below and brownish-olivaceous in upper parts. Ostiole dark brown. Conidiogenous cells phialidic, cylindrical, (7–)8–10(–12) \times (0.8–)1–1.5 μm (n = 10). Conidia falcate, hyaline, 9–13 \times 1.2–1.4 μm (n = 20).

Distribution and ecology. Coastal lignicolous species only known from Juan de Nova, growing on dead twigs of *Salvadora angustifolia*.

Etymology. The species is dedicated to the French forest engineer and entomologist Julien Touroult, who dedicates his life to improving knowledge of insects (mostly Coleoptera) of mainland France and overseas territories. Touroult leads projects and produces expertise to support public policies on biodiversity knowledge and conservation.

Notes. *Stigmatodiscus touroultii* shares three-septate, brown ascospores with *S. enigmaticus*, *S. oculatus* and *S. pinicola*. However, the ascospores of *S. enigmaticus* and *S. pinicola* are distinctly longer (> 40 μm) than those of *S. touroultii* (< 35 μm). Ascospore sizes of *S. oculatus* (25.5–33 \times 9.5–12.5 μm) overlap with those of

S. touroultii (30–35 × 11.5–13.8 µm; however, *S. oculatus* markedly differs from *S. touroultii* by hysteriform ascomata with prominent black marginal lips.

Key to the species of *Stigmatodiscus* (modified from Voglmayr and Pintos Amengual 2018)

- 1 Ascospores at maturity with a primary septum, only very rarely developing two additional distosepta, brown; ascomata distinctly hysteriform 2
 - 2 Ascospores at maturity with a primary septum and two additional distosepta, hyaline or brown; ascomata apothecioid or hysteriform 3
- 2(1) Ascospores (26.5–)29–32.5(–34.5) × (10.8–)11.5–12.7(–13.8) µm; on *Prunus spinosa*. *S. pruni*
 - 2 Ascospores (34.5–)38–43(–47.5) × (13.8–)15.5–17.5(–19.3) µm; on Mediterranean *Quercus* spp. *S. labiatus*
- 3(1) Mature ascospores in vital asci hyaline to light brown, becoming dark brown after ejection, (33.5–)40–45(–49) × (12.8–)14.3–16.5(–17.7) µm; ascomata apothecioid, circular; paraphyses tips covered by an olivaceous, emerald to deep blue amorphous incrustation; on *Tamarix* spp. *S. tamaricis*
 - 2 Mature ascospores brown; paraphyses tips covered by a dark brown amorphous incrustation; on other hosts 4
- 4(3) Ascospores shorter than 40 µm, ascomata hysteriform or irregularly apothecioid. 5
 - 2 Ascospores longer than 40 µm, ascomata mostly circular, apothecioid 6
- 5(4) Ascomata hysteriform, with distinct black marginal lips; ascospores (25.5–)27.5–31(–33) × (9.5–)10.5–12.0(–12.5) µm; polyphagous in the Mediterranean area *S. oculatus*
 - 2 Ascomata irregularly apothecioid, with a brownish excipulum not forming distinct black marginal lips; ascospores 30–35 × 11.5–13.8 µm; on *Salvadorea* in East Africa *S. touroultii*
- 6(4) Ascomata 0.4–1.5 mm diam, surrounded by irregular bark flaps; ascospores (46–)54–64 (–73) × (16.5–)20.0–24.3(–32.5) µm; on *Acer* spp., *Carpinus orientalis*. *S. enigmaticus*
 - 2 Ascomata 0.2–0.4(–0.6) mm diam, not surrounded by bark flaps; ascospores (40.5–)43.5–50(–52.5) × (13.5–)14.5–16.8(–18.0); on *Pinus halepensis* *S. pinicola*

Discussion

The recently described genus *Stigmatodiscus* is well-characterized by erumpent apothecial ascomata often with blackish margins, a distinct darker epithecium, more or less saccate bitunicate asci with a large ocular chamber and large, 3-eudistoseptate, brown verruculose ascospores with a large gel sheath that are remarkably similar to the unrelated genera *Stigmatomassaria* or *Asteromassaria* (Voglmayr et al. 2016; Voglmayr & Pintos Amengual 2018). Where known, the associated anamorphs are pycnidial with phialidic conidiogenous cells bearing falcate to semicircular

hyaline conidia. Ecologically, all species are corticolous on recently dead branches of various shrubs and trees. Phylogenetically, the genus *Stigmatodiscus* occupies an isolated position within *Dothideomycetes*, and the genus is therefore classified within the monotypic family and order *Stigmatodiscaceae* and *Stigmatodiscales*, respectively (Voglmayr et al. 2016; Voglmayr & Pintos Amengual 2018).

To date, 6 *Stigmatodiscus* species are known, all of which were recently described from Central and Southern Europe (Voglmayr et al. 2016, 2017; Voglmayr & Pintos Amengual 2018). Although some species like *S. enigmaticus* and *S. tamaricis* appear to be rather common and widespread on their hosts in suitable habitats, it is remarkable that they have remained unnoticed until their recent description. So far, the genus is only known from Europe, and the current description of *S. touroultii* from Juan de Nova extends its distribution range to East Africa. This indicates that the genus *Stigmatodiscus* may be much more widespread than currently perceived and additional new species may await description.

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Permits

Collecting of the lichen species was authorized in the Scattered Islands according to the permit delivered by C. Geoffroy, General Secretary of French Southern and

Antarctic Lands and district head of the Scattered Island. The RECOFFIE (CBN-CPIE Mascarin, MBG, UMS 2006 PatriNat (OFB – CNRS – MNHN)) project was authorized by order n°2019-40 of April 1, 2019.

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