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Original Paper

Fungi in the litter of *Andreadoxa flava* and *Nectandra membranacea* in Southern Bahia

Thaiana Santos Oliveira[1,4], *Priscila Silva Miranda*[1,5], *Edna Dora Martins Newman Luz*[1,3], *Sabrina Braide Tartaglia*[2] & *José Luiz Bezerra*[1,6,7]

Abstract
In the present research were studied fungi on the litter of *Andreadoxa flava* and *Nectandra membranacea*, in a remaining area of the Atlantic Forest in Ilhéus, BA. The mycota of those plants had not been studied. Samples were obtained in three collections between October 2018 and July 2019, during which 20 leaves at different stages of decomposition were collected per plant species. The leaves were washed and stored in wet chambers. Fungal preparations were used for microscopic analysis and identification of species. A total of 25 genera and 32 fungal species were found, 26 in the litter of *A. flava* and 22 of *N. membranacea*. Sixteen genera were associated with both plants. *Microcallis* was identified for the first ever associated with *A. flava*, a species native to the Atlantic Forest, and the taxon *Thozetella falcata*, was found for the first time in Bahia in the same plant. This is the first report of *Parasympodiella lauri* on *N. membranacea* in Brazil. The mycota found was analyzed taxonomically and for its diversity. Further studies on the mycota associated with the two plants must be carried out, particularly for *A. flava* as it is nearly extinct in nature.
Key words: biodiversity, decomposing fungi, taxonomy, Ascomycota.

Resumo
Este trabalho contém fungos da serapilheira de *Andreadoxa flava* e *Nectandra membranacea* em uma área remanescente da Mata Atlântica e Ilhéus - BA, plantas ainda não estudadas quanto a sua micobiota. Foram realizadas três coletas, entre outubro de 2018 e julho de 2019, recolhendo-se em cada 20 folhas por espécie vegetal, em diferentes estágios de decomposição. As folhas foram lavadas e acondicionadas em câmaras úmidas. Foram feitas preparações para análise microscópica e identificação das espécies. Foram reconhecidos 25 gêneros e 32 espécies fúngicas, sendo 26 associadas à serapilheira *de A. flava* e 22 à de *N. membranacea*. Foram comuns às duas espécies arbóreas 16 gêneros. O táxon *Thozetella falcata* foi encontrado pela primeira vez na Bahia e foi feita a identificação inédita de *Microcallis* sp., ambos em serapilheira de *A. flava*, que é uma espécie nativa da Mata Atlântica e quase extinta em a natureza. Também é o primeiro registro para o Brasil de *Parasympodiella lauri* em *N. membranacea*. A micota encontrada foi analisada do ponto vista taxonômico e da sua diversidade. Mais estudos sobre a micota associada às duas plantas devem ser realizados principalmente em *A. flava,* por ser uma espécie em extinção.
Palavras-chave: biodiversidade, fungos decompositores, taxonomia, Ascomycota.

[1] Universidade Estadual De Santa Cruz - UESC, PPGPV, Ilhéus, BA, Brazil.
[2] Instituto Federal Baiano, Campus Uruçuca, Uruçuca, BA, Brazil. ORCID: <https://orcid.org/0000-0001-9259-3402>.
[3] Cacao Research Center, CEPEC/CEPLAC, Ilhéus, BA, Brazil. ORCID: <https://orcid.org/0000-0003-1295-3960>.
[4] ORCID: <https://orcid.org/0000-0002-3508-9012>
[5] ORCID: <https://orcid.org/0000-0001-6480-7805>
[6] ORCID: <https://orcid.org/0000-0002-7917-3400>
[7] Author for correspondence: jlulabezerra@hotmail.com

Introduction

Studying mycological diversity is essential to understand the survival of fungal species and their ecological role for the conservation of forestry resources (Cain 2010). The mycota present in leaf litter is extremely rich in species that play important roles in forest decomposition and nutrient cycling (Dix & Webster 1995; Poll *et al.* 2010; Voříšková & Baldrian 2013; Purahong *et al.* 2016). In Brazil, Ascomycota associated with decomposition of litter in the Atlantic Forest has been investigated, resulting in the discovery of new taxa and new taxonomic records (Forzza *et al.* 2010; Santa Izabel & Gusmão 2018). Many litter fungi are generalists that tend to be present in different types of litter, whereas others exhibit preference for certain hosts (Prakash *et al.* 2015; Santos *et al.* 2015).

Aiming to analyze this ecological aspect along with carrying out taxonomy study, this research sought to select two plant species present in the Atlantic Forest in the southern portion of the Brazilian state of Bahia whose litters had not been previously studied. The choice was made for the tree species *Andreadoxa flava* Kallunki and *Nectandra membranacea* (Sw.) Griseb, found in a remaining area of the Atlantic Forest at the Cacao Research Center, in the municipality of Ilhéus, Bahia. *Andreadoxa flava* was described as a new species of the Rutaceae family by Kallunki (1998) and has a single specimen known in nature (Kallunki 1998; Pirani 2002), located close to two specimens of *N. membranacea*. The latter species belongs to the Lauraceae family and is well represented in the Atlantic Forest. Its common name is canela-amarela and it can be used in woodworking and landscaping (Rohwer 1993). As it is close to the specimen of *A. flava*, any differences in the fungal population in the litter of both plants could not be attributed to differences in location.

This study is the first to add to our understanding of the mycota found in the litter of two plants in the Atlantic Forest that had previously not been studied in this way.

Material and Methods

The material collection, as well as its processing and analysis, were carried out at the Cacao Research Center - CEPEC, an organ linked to the Executive Commission of the Plan of Cacao Farming - CEPLAC of the Ministry of Agriculture, Livestock, and Food Supply - MAPA. The area is located at in the municipality of Ilhéus, southern state of Bahia, Brazil, at 14°47'20"S and 39°02'58"W. According to Köppen and Geiger the climate is confirmed as Af. In Ilheus, the average temperature is 23.9 °C. Average annual rainfall of 1,325 mm.

The leaf litter samples of *A. flava* and *N. membranacea* were collected in an area of 200 m^2 near a cocoa plantation, located at Block H' of the Experimental Station Arnaldo Medeiros - ESARM within the facilities of CEPEC.

Three collections for each species were carried out between October 2018 and July 2019 (1st collection - October 2018, 2nd collection - March 2019, 3rd collection - July 2019). A square hollow frame measuring 50 cm × 50 cm (0.25 m^2) made with PVC pipe was placed in the litter around *A. flava* and *N. membranacea* and one leaf was randomly collected at a time at different stages of decomposition for a total of 20 leaves of each species per collection. The samples were stored in kraft paper bags and transported to the Laboratory of Fungal Diversity of CEPEC/CEPLAC.

After the collection, the samples were placed in previously perforated containers and washed in running water for 1 h in a way that the water did not fall onto the leaves directly, with a small accumulation in the recipient, so that the water flowed through the openings and washed away the impurities on the leaves. After that, the samples were stored in wet chambers made with plastic containers, where they were incubated for 48 h in accordance with the methodology described by Castañeda-Ruiz *et al.* (2006). The containers were opened every day for 15 minutes so as to renovate the air inside them. After 48 h, the material was observed in a stereo microscope and reviewed periodically for 30 days. The leaves that exhibited fungus signs were examined under a dissecting microscopy. The fungal structures removed from the leaves were mounted onto microscope slides with cover slips using a fine needle and then placed in permanent mounting medium (PVLG resin: polyvinyl alcohol-lactic acid-glycerol) (Morton *et al.* 1993) and were identified using specific bibliography.

After the taxa were classified, the indices of richness, frequency, and constance of the species found in the sample material were determined. Richness was defined as the total number of species found in the collection (Brower *et al.* 1998). The frequency of occurrence was calculated using the formula: $F = n \times 100/N$, where: n = number of samples in which a species was found; N = number of samples. The following frequency classes were determined: $F \leq 10\%$: Sporadic; $10 < F \leq 30\%$: Little frequent; $30 < F \leq 70\%$: Frequent; and $F > 70\%$: Very frequent (Dajoz 1983).

Constance was calculated using the formula: $C = p \times 100/P$, where: p = number of excursions in which a fungal species was found; P = total number of excursions. The following consistency classification was used (Santos & Cavalcanti 1995): Accidental: $\leq 25\%$; Accessory: $25 < C \leq 50\%$; Constant: $> 50\%$.

Results and Discussion

Thirty-two species of Ascomycota were observed associated with litter of *A. flava* and *N. membranacea*, belonging to 25 genera, 17 families, and four classes (Sordariomycetes Dothideomycetes, Eurotiomycetes, and Orbiliomycetes), in addition to two *incertae sedis* genera (Tab. 1). The taxa are presented according to their families.

This study reports no new taxa. However, the first record of *Thozetella falcata* B.C. Paulus, Gadek & K.D. Hyde, was obtained for the state of Bahia, in leaf litter of *A. flava*, as well as the first report ever of *Microcallis* sp. in the litter of that plant. This biotrophic fungus likely completes its life cycle after the fall of leaves, in this case, of *A. flava.* Endophytic fungi may survive in those fragments of the host plant fallen onto the ground and start living as saprobes breaking down the litter (Korkama-Rajala *et al.* 2008; Voříšková & Baldrian 2013; Prakash *et al.* 2015). To that end, they undergo genetic alterations that allow them to turn into saprobes (Zuccaro *et al.* 2011).

The finding of *Parasympodiella lauri* Hern.-Restr., Gené & Guarro on *N. membranacea* corresponds to the first record of this taxon in Brazil.

The Sordariomycetes class was the most numerous, followed by Dothideomycetes. The Leotiomycetes class had no representatives, although the Rhytismataceae family of this class has been reported in the Atlantic Forest biome by Santos *et al.* (2019). Those classes of Ascomycota, the largest phylum of the Fungi kingdom, comprise species able to break down the lignocellulose present in the litter (Melo *et al.* 2018), which makes them very important in the decomposition of the plant material.

The Bionectriaceae and Stachybotryaceae families had the second best representation in the results, with three species found, followed by Chaetosphaeriaceae, Nectriaceae, Parasympodiellaceae, and Periconiaceae with two species (Tab. 1).

Virgatospora echinofibrosa Finley, *Pestalotiopsis* sp., and *Stachybotrys parvisporus* S. Hughes were considered very frequent according to the diversity indices calculated (Tab. 1). *Virgatospora echinofibrosa*, in addition to being present in the litter of the two species, was found in all three collections.

The two plant species had 16 taxa in common, whereas others were exclusive of either one of the species. The species in common to both plants were: *Cladosporium tenuissimum* Cooke, *Clonostachys rosea* Mussat, *Colletotrichum* sp., *Gyrothrix hughesii* Piroz, *Gyrothrix* sp., *Lasiodiplodia theobromae* Griffon & Maubl, *Memnoniella nilagirica* C.G. Lin, Yong Wang bis & K.D. Hyde, *Menisporopsis theobromae* S. Hughes, *Ophioceras* sp., *Parasympodiella laxa* Subram. & Vittal, *Thozetella falcata*, *Volutella* sp., and *Zygosporium cf. oscheoides* Mont. *Virgatospora echinofibrosa* Finley, *Pestalotiopsis* sp., and *Stachybotrys parvisporus* S. Hughes, besides being found in the litter of both trees, were found in all three collections.

The litter of *A. flava* contained 26 taxa, corresponding to an 81.25% richness, while the litter of *N. membranacea* contained 22 taxa, corresponding to a 65.63% richness. The richness observed in this study was comparable to that found in previous studies on the Atlantic Forest (Magalhães *et al.* 2011).

The distribution of the taxa by class of frequency showed the predominance of sporadic taxa in *N. membranacea*, whereas for *A. flava* the predominance was of little frequent and sporadic taxa. Only one taxon (*Memnoniella nilagirica*) was frequent (3.8%) in *A. flava*, which had 34.62% of sporadic taxa and 50% of little frequent taxa. In *N. membranacea*, 66.67% of the taxa were sporadic, 14.29% were little frequent, and 4.76% were frequent with one taxon (*Ophioceras* sp.). As for the frequency of taxa, similar results were obtained in other studies on areas of the Atlantic Forest in Bahia (Barbosa *et al.* 2009; Magalhães *et al.* 2011).

Regarding the constance of the fungi in the plant species collected, accidental fungi prevailed in *N. membranacea* while accessory fungi prevailed in *A. flava*. *Andreadoxa flava* exhibited 34.61% accidental taxa, 50% accessory taxa, and 15.38% constant fungi, namely: *Memnoniella nilagirica*,

Table 1 – Fungal species found in litter of *Andreadoxa flava* and *Nectandra membranacea* in the Atlantic Forest in the southern state of Bahia, Brazil, along with the diversity indices obtained. (Sp = Sporadic; LF = Little frequent; Fr = Frequent; VF = Very frequent; Ac = Accidental; As = Accessory; C = Constant).

	Indexes			
	Andreadoxa flava		*Nectandra membranacea*	
	Frequency	**Constancy**	**Frequency**	**Constancy**
Ascomycota, genera incertae sedis				
Cryptophiale sp.	Sp	Ac	-	-
Gyrothrix hughesii Piroz.	LF	As	Sp	Ac
Gyrothrix sp.	Sp	Ac	Sp	Ac
Beltraniaceae				
Beltraniella fertilis Heredia, R.M. Arias, M. Reyes & R.F. Castañeda	-	-	Sp	Ac
Bionectriaceae				
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	Sp	Ac	Sp	Ac
Clonostachys sp.	-	-	Sp	Ac
Hyalocylindrophora cf. *rosea* (Petch) Réblová & W. Gams	LF	As	-	-
Virgatospora echinofibrosa S. Hughes	VF	C	VF	C
Botryosphaeriaceae				
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	LF	As	LF	As
Chaetosphaeriaceae				
Dinemasporium sp.	-	-	Sp	Ac
Menisporopsis theobromae S. Hughes	LF	As	LF	As
Thozetella falcata B.C. Paulus, Gadek & K.D. Hyde	LF	As	Sp	Ac
Chaetothyriaceae				
Microcallis sp.	LF	As	-	-
Cladosporiaceae				
Cladosporium tenuissimum Cooke	LF	As	Sp	Ac
Glomerellaceae				
Colletotrichum sp.	LF	As	Sp	Ac
Nectriaceae				
Volutella minima Höhn	LF	As	-	-
Volutella sp.	Sp	Ac	Sp	Ac
Ophioceraceae				
Ophioceras leptosporum (SH Iqbal) J. Walker	LF	As	-	-
Ophioceras sp.	LF	As	Fr	C
Orbiliaceae				
Arthrobotrys sp.	LF	As	-	-
Parasympodiellaceae				
Parasympodiella lauri Hern.-Restr., Gené & Guarro, sp. nov.	-	-	Sp	Ac
Parasympodiella laxa (Subram. & Vittal) Ponnappa	Sp	Ac	Sp	Ac

	Indexes			
	Andreadoxa flava		*Nectandra membranacea*	
	Frequency	Constancy	Frequency	Constancy
Pestalotiopsidaceae				
Pestalotiopsis sp.	VF	C	VF	C
Periconiaceae				
Periconia byssoides Pers.	Sp	Ac	-	-
Periconia sp.	Sp	Ac	-	-
Sporidesmiacae				
Sporidesmium tropicale M.B. Ellis	-	-	Sp	Ac
Stachybotryaceae				
Digitiseta multidigitata Decock & Gordillo	-	-	Sp	Ac
Digitiseta setiramosa (RF Castañeda) Gordillo & Decock	LF	As	-	-
Memnoniella nilagirica (Subram.) CG Lin, Yong Wang bis and KD Hyde	Fr	C	Sp	Ac
Stachybotrys parvisporus S. Hughes	VF	C	VF	C
Sordariaceae				
Sordaria sp.	Sp	Ac	-	-
Zygosporiaceae				
Zygosporium cf. *oscheoides*	Sp	Ac	LF	As

Pestalotiopsis sp., *Stachybotrys parvisporus*, and *Virgatospora echinofibrosa* present in all three collections. The *N. membranacea* plant exhibited 66.6% accidental, 14.29% accessory, and 19.05% taxa: *Ophioceras* sp., *Pestalotiopsis* sp., *S. parvisporus*, and *V. echinofibrosa*. Santos *et al.* (2015), when studying three plant species [*Inga thibaudiana* DC, *Myrcia splendens* DC, and *Pera glabrata* (Schott) Poepp. *ex* Baill] in Una, BA, Brazil, observed predominance of constant or accessory taxa in all three plants. However, 53.8% of the taxa in *P. glabrata* fell into the accidental category.

Given the close proximity of the trees in the woods where the collections were conducted common species would be expected on both hosts; however, several fungi showed preference for the litter of one tree or the other. Regarding the specificity of the host, Polishook *et al.* (1996) raised the hypothesis that some fungi considered host-specific may be found in other plant species with similar chemical composition, texture, and structure. Santos *et al.* (2015) stated that some microfungi reported as specific to certain hosts are later found in others. Factors such as nutrient availability, water content, pH, and anatomical peculiarities of the substrates may influence mycelial growth and impact the fungi occurrence (Pinruan *et al.* 2007; Voříšková & Baldrian 2013). Fungi that are restricted to a single plant may evolve into generalist endophytic or saprobes in multiple plants, resulting in a reduction in fungal diversity (Govinda Rajulu *et al.* 2014).

Andreadoxa flava had 26 identified individuals, ten only in genus and 15 at the species level, being the following exclusive to this host: *Arthrobotrys* sp., *Cryptophiale* sp., *Digitiseta setiramosa* (R.F. Castañeda) Gordillo & Decock, *Hyalocylindrophora* cf. *rosea* (Petch) Réblová & W. Gams, *Microcallis* sp., *Ophioceras leptosporum* (S.H. Iqbal) J. Walker, *Periconia byssoides* Pers, *Periconia* sp., *Sordaria* sp., and *Volutella minima* Höhn.

In *N. membranacea*, there were 22 individuals, eight of which were identified only up to the genus and 15 up to the species level, with the following fungi belonging only to this plant species: *Beltraniella fertilis* Heredia, R.M. Arias, M. Reyes & R.F. Castañeda, *Clonostachys* sp., *Digitiseta multidigitate* Decock & Gordillo, *Dinemasporium* sp., *Parasympodiella lauri* Hern.-Restr., Gené & Guarro, and *Sporidesmium tropicale* M.B. Ellis.

A great part of the fungal species found in this study was reported in other researches in the Atlantic Forest (Costa & Gusmão 2015, 2017; Grandi 2004; Grandi & Gusmão 2002; Grandi & Silva 2006; Marques *et al.* 2008; Magalhães *et al.* 2011, 2014a,b; Santos *et al.* 2015, 2016, 2017).

Several genera of Ascomycota studied in the litter of palm trees native to the Amazon (Monteiro *et al.* 2019) belonging to the families Beltraniaceae, Chaetosphaeriaceae, Nectriaceae, and Xylariaceae were found in this study, which shows the amplitude of the geographic distribution and the range of hosts. *Beltraniopsis rhombispora* Matsush and *Hemibeltrania decorosa* R.F. Castañeda & W.B. Kendr were found before from leaf litter of other trees of the Atlantic Forest in Bahia (Santos *et al*. 2016).

Part of the species found in this study is cosmopolitan and has been reported in other continents (Polishook *et al.* 1996; Prakash *et al.* 2015; Parungao *et al.* 2002). Barbosa *et al*. (2007) observed that the number of taxa increased with the onset of rainfall, correlating with the findings in this study, which showed in *A. flava* a greater number of taxa in the third collection (rainy season) compared to the previous two.

New occurrences

Microcallis sp. Fig. 1d-e

Pelliculous mycelium formed by branched, dark-brown hyphae. Small, superficial, sub-globose, setose, ostiolate perithecial ascomata. Bitunicate, clavate, octosporic asci. Hyaline, uniseptate ellipsoid ascospores. Measurements not taken.

Material examined: municipality of Ilhéus, CEPLAC, on decomposing leaves of *Andreadoxa flava*, 14°45'24.7"S, 39°14'22.9"W, 26.X.2018, *T.S. Oliveira*; 5.VII.2019, *T.S. Oliveira*. CEPEC- FUNGI 2650 and 2651.

The species was found in Ecuador, Argentina (Catania & Romero 2011), India (Müller & Bose 1959) and Brazil (Batista *et al.* 1966).

There are nine species reported for this genus (Index Fungorum 2020) which are biotrophic and occur in tropical regions (Petrak & Ciferri 1932; Hansford 1957; Bose & Müller 1965). The records for South America are from Ecuador, Brazil, and Argentina (Catania & Romero 2011). This genus had never been reported in leaf litter.

Parasympodiella lauri Hern. -Restr., Gené & Guarro, in Hernández-Restrepo, Gené, Castañeda-Ruiz, Mena-Portales, Crous & Guarro, Stud. Mycol. 86:87(2017). Fig. 1a-c

Mycelium constituted by pale-brown, smooth, septate, branched hyphae. Conidiophores solitary macronematose, mononematose, upright, medium-brown, smooth, sub-cylindrical, straight, non-branched, septate. Conidiogenous cells terminal or intercalary integrated, undetermined, proliferating sympodially, smooth, pale. Conidia septate, cylindrical, obtuse apex, truncate base, hyaline, catenulate, dry. Measurements not taken.

Material examined: municipality of Ilhéus, CEPLAC, on decomposing leaves of *Nectandra membranacea*, 14°45'25"S, 39°14'22"W, 12.III.2019, *T.S. Oliveira*, CEPEC- FUNGI 2657.

The species was found in Spain (Hernández-Restrepo *et al.* 2017); Brazil in the present study.

This is the first record of *N. membranacea* in Brazil. The material was collected a single time in this work and is considered scarce. Described originally in *Laurus* sp. (Lauraceae) in Spain (Hernández-Restrepo *et al*. 2017).

Similar to the genus *Sympodiella*, however, these have small conidiophores (up to 280 µm) with terminal or sub-terminal conidiogenous cells and conidial chains with up to six conidia (Kendrick 1958), whereas *Parasympodiella* has larger conidiophores (up to 700 µm), the conidiogenous cells are along the conidiophore in uneven intervals and the conidia are produced in chains that seem to extend indefinitely. *Parasympodiella lauri* is morphologically similar to *P. elongata* Crous, M.J. Wingf. & W.B. Kendr and *P. eucalypti* Cheew. & Crous as it has cylindrical conidia, (0–)1(–2) septo (Cheewangkoon *et al.* 2009).

There are records of nine species of this genus, colonizing leaves and branches of conifers and dicotyledon plants (Crous *et al.* 1995; Cheewangkoon *et al.* 2009; Seifert *et al.* 2011).

Thozetella falcata B.C. Paulus, Gadek and K.D. Hyde, Micologia 96(5):1078(2004). Fig. 1f

Sporodochium with cylindrical base, 113 × 135 µm, brown. Conidia lunate, hyaline, 10-15 × 2 µm, with setula at the end, measuring 4–5 µm in length, recurved. Microawns in S or L shape, smooth to slightly verrucose, hyaline, 37–58 × 2 µm, basal part sometimes with lumen.

Material examined: municipality of Ilhéus, CEPLAC, on decomposing leaves of *Andreadoxa flava*, 14°45'24.7"S, 39°14'22.9"W, 26.X.2018, *T.S. Oliveira*; 5.VII.2019, *T.S. Oliveira*. CEPEC- FUNGI 2646.

The species was found in Australia (Paulus *et al.* 2004), Brazil (Silva & Grandi 2013).

First report for Bahia. Only a single colony of this species was found in this work in leaf litter of

A. flava forming a mucilaginous mass comprising conidia and microawns, which are sterile cells with unknown function and peculiar to the genus, used to differentiate the species (Barron 1968; Pirozynski & Hodges Junior 1973; Castañeda-Ruiz 1984; Allegrucci *et al.* 2004; Jeewon *et al.* 2009).

The findings of this study are a preliminary contribution to the knowledge on the fungi present in leaf litter of *A. flava* and *N. membranacea*, since the mycota in the litter of the two hosts has not been thoroughly studied. All of taxa described here are being reported for the first time for the two tree species which have never been studied for decomposing litter fungi. It is highly important to point out that *A. flava* is a virtually extinct species in nature of which only a single individual is known worldwide. The results obtained contribute to broadening the interest in new collections of litter fungi of plants in the Atlantic Forest.

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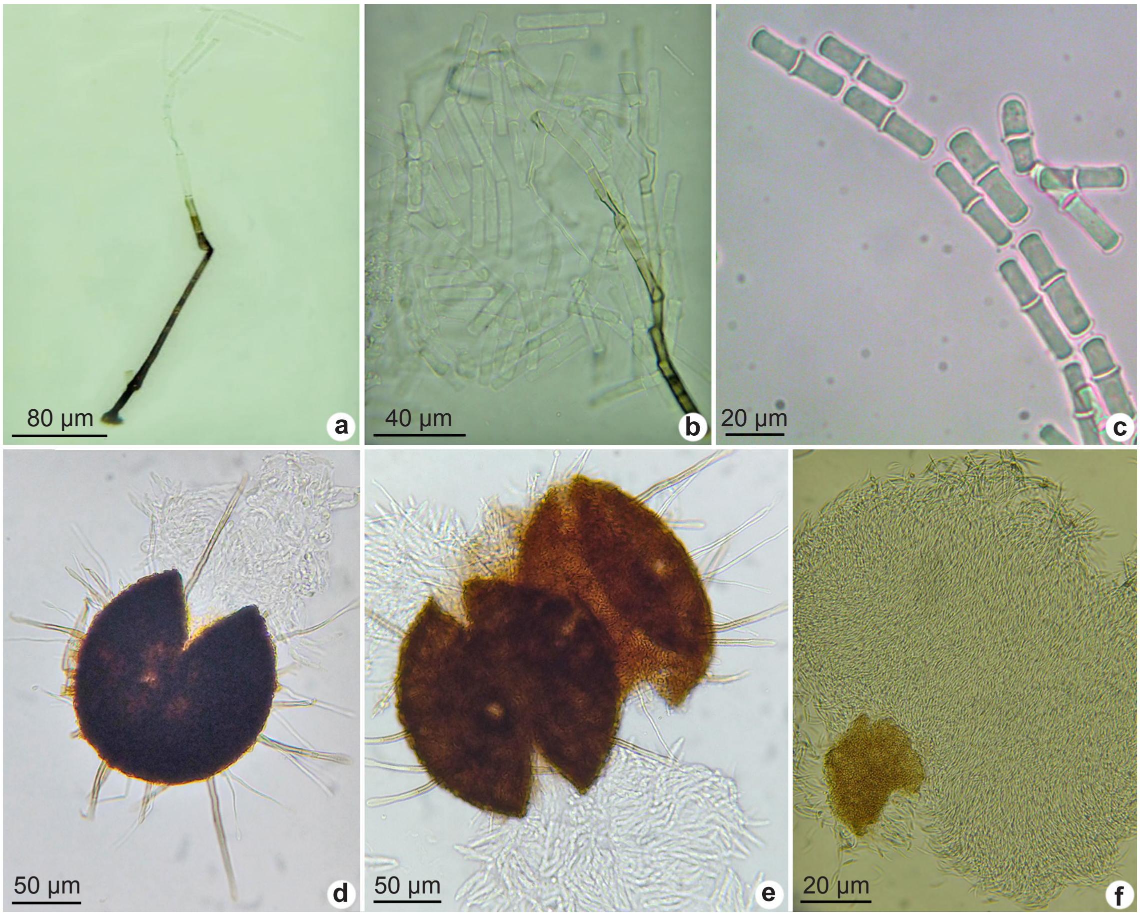

Figure 1 – a-b. *Parasympodiella lauri* – a. conidiophore and conidia; b. articulate conidia. c-d. *Microcallis* sp. – c. squashed perithecia and asci scattered in the field; d. setose perithecium with extrusion of asci. e. *Thozetella falcata* – sporodochium.

Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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