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from Brazil and Mexico

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# Four new species of *Tephromela* M.Choisy (Ascomycota, Tephromelataceae), three containing lichexanthone, from Brazil and Mexico

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## ABSTRACT

Four new species of *Tephromela* M.Choisy are described here. Three are corticolous species from Brazil: *T. multireflexa* Aptroot & M.F.Souza, sp. nov., collected in the Chapada dos Guimarães, municipality of Cuiabá, a Cerrado region in the state of Mato Grosso; *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov., collected in Caraça, in Campo rupestre in Minas Gerais state; and *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov., collected in the Parque Nacional Vale do Catimbau, municipality of Buique, a Caatinga region in the state of Pernambuco, with additional specimens from Mato Grosso do Sul. All are somewhat similar to *T. atra* (Huds.) Hafellner in morphological and anatomical features but differ chiefly in secondary chemistry, as well as partially in ascospore dimensions and substrate ecology: lichexanthone in the apothecial margins and  $\alpha$ -collatolic acid in the medulla of the apothecial margin in *T. multireflexa* Aptroot & M.F.Souza, sp. nov.; lichexanthone in the thallus and the (unevenly thickened) apothecial margin in *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov.; and fatty acids in *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. Molecular data for *T. vinacea* L.A.Santos, Aptroot, Lücking &

**KEY WORDS**  
Phylogeny,  
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new species.

**MOTS CLÉS**  
Phylogénie,  
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espèces nouvelles.

M.Cáceres, sp. nov. and *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov., demonstrate that they are not closely related to *T. atra* but form part of a previously unrecognized clade apparently restricted to South America, several of them remaining undescribed. A further new, saxicolous species, *T. xanthonica* Guzmán-Guillermo, sp. nov., is described from Brazil and Mexico; it is similar to *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. and *T. velloziae* Kalb in producing lichexanthone in the thallus and apothecial margins, but differs in its substrate ecology and in having an areolate thallus. Two additional species described from Brazil are here combined into the genus: *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov. and *T. epichlorina* (Vain.) Aptroot & Lücking, comb. nov. A key is given for the 17 *Tephromela* species now known from Brazil.

## RÉSUMÉ

Quatre nouvelles espèces de *Tephromela* M.Choisy (Ascomycota, Tephromelataceae), trois contenant de la lichexanthone, du Brésil et du Mexique.

Quatre nouvelles espèces de *Tephromela* M.Choisy sont décrites ici. Trois sont des espèces corticoles du Brésil: *T. multireflexa* Aptroot & M.F.Souza, sp. nov., récoltée dans la Chapada dos Guimarães, municipalité de Cuiabá, une région du Cerrado dans l'état du Mato Grosso; *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov., collectée à Caraça, à Campo rupestre dans l'état de Minas Gerais; et *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov., collectée dans le Parque Nacional Vale do Catimbau, municipalité de Buique, une région de Caatinga dans l'état de Pernambuco, avec des spécimens supplémentaires du Mato Grosso do Sul. Toutes sont similaires à *T. atra* (Huds.) Hafellner en termes de caractéristiques morphologiques et anatomiques, mais diffèrent principalement par des métabolites secondaires, ainsi que partiellement par les dimensions des ascospores et l'écologie du substrat: lichexanthone dans les marges apothéciales et acide  $\alpha$ -collatolique dans la médulla de la marge apothéciale chez *T. multireflexa* Aptroot & M.F.Souza, sp. nov.; lichexanthone dans le thalle et la marge apothéciale (inégalement épaissie) chez *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov.; et des acides gras dans *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. Les données moléculaires pour *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. et *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov., démontrent qu'elles ne sont pas étroitement apparentées à *T. atra*, mais font partie d'un clade précédemment non reconnu apparemment limité à l'Amérique du Sud, plusieurs d'entre elles restant non décrites. Une autre espèce saxicole, *T. xanthonica* Guzmán-Guillermo, sp. nov., est nouvellement décrite du Brésil et du Mexique; elle est similaire à *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. et *T. velloziae* Kalb dans la production de lichexanthone dans le thalle et les marges apothéciales, mais diffère par son écologie de substrat et par son thalle aréolé. Deux espèces supplémentaires décrites du Brésil sont ici combinées dans le genre: *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov. et *T. epichlorina* (Vain.) Aptroot & Lücking, comb. nov. Une clé est fournie pour les 17 espèces de *Tephromela* actuellement connues du Brésil.

## INTRODUCTION

The genus *Tephromela* M.Choisy was proposed by Choisy (1929) to accommodate the species known as *Lecanora atra* (Huds.) Ach., characterized by a dark purple hymenium and straight conidia. The genus name was largely neglected until Hafellner (Kalb 1983) proposed the formal combination *Tephromela atra* (Huds.) Hafellner and subsequently proposed the new family Tephromelataceae Hafellner for it, based on characteristics of the ascus type (Hafellner 1984). He included in the family all species with large, lecanorine apothecia with purple hymenium and hypothecium and presumably with *Bacidia*-type asci. Currently, the family comprises four genera: *Calvitimela* Hafellner, *Mycoblastus* Norman, *Tephromela*, and *Violella* T.Spr. (Spribille *et al.* 2011; Lücking *et al.* 2017).

Within Tephromelataceae, *Tephromela* is characterized by (*Lecidella*-)*Biatora*-type asci with weakly amyloid walls, ascospores with simple walls, produced in numbers of eight

per ascus, and stout paraphyses with thin anastomoses (Spribille *et al.* 2011; Kantvilas 2015). In these characters, it largely agrees with *Calvitimela*, which differs chiefly in the *Lecanora*-type asci (Spribille *et al.* 2011). *Tephromela* has a cosmopolitan distribution (<https://www.gbif.org/species/2606691>), its species being found on various substrata in different habitats and exhibiting a high level of fine-scaled morphological and chemical variation (Muggia *et al.* 2014; Cestaro *et al.* 2016).

Most species of *Tephromela* are recognized through morphological and/or chemical characters (Fryday 2011; Elix 2012, 2013; Kantvilas 2015; Kantvilas & Elix 2017). However, global sampling has revealed many problems in defining species based only on phenotype, particularly in the *T. atra* morphodeme, as showed by Muggia *et al.* (2014). Material fitting the general morphology of *T. atra*, i.e., with a whitish thallus, rather large apothecia with white margins and black, glossy disc, and purple hymenium, has traditionally been identified with this name, regardless its habitat and

substrate ecology, anatomical characters, such as ascospore size, and secondary chemistry. However, the combination of molecular phylogeny with ecology, morphology and secondary metabolites has been shown to be an effective tool in the definition of species within *Tephromela* (Cestaro *et al.* 2016; Fryday 2019), characterizing *T. atra* s.str. as a saxicolous lichen producing alectoronic and  $\alpha$ -collatolic acid.

Here we describe three new, corticolous species of *Tephromela* from Brazil and one new saxicolous species from Mexico and Brazil. While agreeing with *T. atra* in general morphology, they differ in secondary chemistry, three of them producing lichexanthone and one fatty acids in addition to alectoronic acid and atranorin. Molecular data obtained for two of the new species demonstrate that they are not closely related to *T. atra*, but belong to a separate clade of taxa apparently restricted to South America.

## MATERIAL AND METHODS

### SAMPLING

*Tephromela* species were collected over the past few years by the authors, during field expeditions to several areas in Brazil and Mexico. In general, as the thalli tend to be quite large, each collection consists of (part of) a thallus. Material was air dried and preserved in paper packets.

### MORPHOLOGICAL AND CHEMICAL METHODS

Morphological examination was performed with an Olympus SZX7 dissecting microscope and images were taken with a Nikon Coolpix 995 camera. Anatomical sections were hand-made, mounted and studied in water, 5% KOH (K) and/or Lugol's iodine (1% IKI) after pre-treatment with KOH (IKI). Microscopic photographs were prepared using an Olympus BX50 compound microscope with Nomarski interference contrast and a Nikon Coolpix 995 camera.

The specimens were tested with UV light ( $\lambda$ 365 nm), and spot reactions of KOH (K), paraphenylenediamine (Pd) and calcium hypochlorite (C). Thin layer chromatography followed White & James (1985), Huneck & Yoshimura (1996), and Orange *et al.* (2001).

### MOLECULAR METHODS

Sequences of the ITS fungal barcoding locus were generated for four specimens. Apothecial fragments were transferred to a 1.5 ml reaction tube and stored at -20°C. Subsequently, genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega), following the leaf extraction protocol. The primers used were ITS1f (Gardes & Bruns 1993) and ITS4a (Larena *et al.* 1999) for amplification of the targeted locus. For PCR reactions, we used the REDExtract-N-Amp Plant PCR Sigma-Aldrich (St. Louis, Missouri, United States) following the manufacturer's protocol. PCR was performed as in Zhao *et al.* (2016). Successfully amplified products were purified using kit Wizard® SV Gel and PCR Clean-Up (Promega) and sequenced by the genetics laboratory at the Universidade Federal de Pernambuco.

## PHYLOGENETIC ANALYSES

The newly generated sequences were added to a set of ITS sequences downloaded from GenBank, representing all of Tephromelataceae and all available sequences of *Tephromela*, for a total of 140 terminals (Table 1). Sequences were first assembled and edited manually using BioEdit 7.2.0 (Hall 1999) and then aligned using MAFFT 7 (Katoh *et al.* 2009; Katoh 2013), and the alignment was subsequently inspected manually. The alignment (Appendix 1) had a length of 612 bases and only few columns showed minor degrees of alignment ambiguity, hence these were not removed. Maximum likelihood analyses were performed using RAxML v8.1.11 (Stamatakis 2006), with GTRGamma and 1000 bootstrap replicates.

## RESULTS AND DISCUSSION

### PHYLOGENY

The ITS-based tree of Tephromelataceae separated the core genera on moderately (*Mycoblastus*: 63%) to strongly supported branches (*Calvitimela* s.str.: 95%; *Violella*: 91%; *Tephromela*: 96%); however, the *C. aglaea* clade (strongly supported at 91%) fell outside *Calvitimela* s.str. (Fig. 1), reflecting the topology of the three-marker set analyzed by Spribille *et al.* (2011). Three further species, not included in the study by Spribille *et al.* (2011), also fell outside these genera, namely *C. perlata* (Haugan & Timdal) R. Sant., *C. talayana* (Haugan & Timdal) M.P. Andreev, and *Tephromela physodica* Kalb (Fig. 1). This suggests that the genus-level classification in the family is not yet settled.

The ITS marker did not result in a supported backbone for *Tephromela*, although the clade including *T. atra* s.str., as part of a larger complex, was well-supported with 95% (Fig. 2). *Tephromela alectoronica* Kalb from Australia was recovered as supported sister (70%) to all other sequenced species, and *T. pertusarioides* (Degel.) Hafellner & Cl.Roux as supported sister (70%) to the *T. atra* complex (Fig. 2). All other species represent early emerging clades within the genus, with a partly supported, partly unsupported backbone, including eight lineages identified as *T. atra* but obviously not representing that species. Of these, one is from Europe (aff. *atra* 1), five are from Australia or mostly New Zealand (aff. *atra* 2-6), and two are from Peru (aff. *atra* 7-8; Table 1).

All sequenced Brazilian material fell in a strongly supported clade (97%) containing also an undescribed species (aff. *atra* 8) from lowland Peru (Fig. 2). The clade was well-supported throughout, suggesting a structured diversification in lowland South America. The corticolous habit and the presence of lichexanthone or other substances support the notion that the Brazilian specimens do not represent *Tephromela atra* and they are therefore described as new species below. *Tephromela vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov., is thereby characterized by the presence of alectoronic acid, atranorin and fatty acids. Fatty acids were first reported as unusual substances for the genus in Muggia *et al.* (2014). Subsequently, Cestaro *et al.* (2016) used them as one of the criteria to describe *T. pacifica* Björk & Muggia. Despite the chemical similarity, the Brazilian specimen was positioned in a clade distant from the latter. *Tephromela obesimarginata* L.A.Santos, Aptroot &

TABLE 1. — Specimens of *Calvitimela* Hafellner, *Mycoblastus* Norman, *Violella* T.Sprib. and *Tephromela* M.Choisy used in this study. New sequences are indicated in **boldface**.

Species	Voucher	ITS accession number
<i>Calvitimela aglaea</i> 1 (Sommerf.) Hafellner	<i>Spribille</i> 38382	KR303633
<i>C. aglaea</i> 1	<i>Spribille</i> 38829	KR303634
<i>C. aglaea</i> 2	Austria, <i>Hafellner</i> 70358	JN009718
<i>C. aglaea</i> 2	OL-160708	KR303636
<i>C. aglaea</i> 2	OL-163597	KR303637
<i>C. aglaea</i> 3	OL-160503	KR303628
<i>C. aglaea</i> 3	OL-173831	KR303630
<i>C. aglaea</i> 3	OL-160462	KR303631
<i>C. armeniaca</i> (DC.) Hafellner	OL-170675	KR303638
<i>C. armeniaca</i>	OL-170644	KR303639
<i>C. armeniaca</i>	Norway, OL-195741	KY266899
<i>C. aff. armeniaca</i>	Austria, <i>Hafellner</i> 71304	JN009709
<i>C. cuprea</i> Haugan & Timdal	OL-179616	KR303661
<i>C. cuprea</i>	OL-173292	KR303662
<i>C. cuprea</i>	OL-179566	NR153907
<i>C. livida</i> Haugan & Timdal	OL-174237	KR303654
<i>C. livida</i>	UPSL-520818	KR303656
<i>C. livida</i>	Norway, OL-163835	NR153906
<i>C. melaleuca</i> (Sommerf.) R.Sant.	United States, <i>Spribille</i> 27965-B	JN009714
<i>C. melaleuca</i>	OL-170490	KR303643
<i>C. melaleuca</i>	OL-159803	KR303644
<i>C. melaleuca</i>	Norway, OL-195711	KY266838
<i>C. melaleuca</i>	Norway, OL-195915	KY266897
<i>C. aff. melaleuca</i>	OL-141654	KR303640
<i>C. aff. melaleuca</i>	OL-163829	KR303641
<i>C. aff. melaleuca</i>	OL-89218	KR303642
<i>C. perlata</i> (Haugan & Timdal) R.Sant.	OL-163770	KR303679
<i>C. perlata</i>	OL-170830	KR303651
<i>C. perlata</i>	OL-141628	KR303652
<i>C. talayana</i>	OL-191705	KR303664
<i>C. talayana</i>	<i>Hermansson</i> 14958	KR303666
<i>Mycoblastus affinis</i> (Schaer.) T.Schauer	China, <i>Goffinet</i> 10030	JN009721
<i>M. affinis</i>	Canada, <i>Spribille</i> & <i>Wagner s.n.</i> , 2009	JN009722
<i>M. sanguinarioides</i> Kantvilas	Japan, <i>Ohmura</i> 6740	JN009723
<i>M. sanguinarioides</i>	Japan, <i>Ohmura</i> 5996	JN009724
<i>M. sanguinarius</i> (L.) Norman	Sweden, <i>Muggia s.n.</i> , TSB 38893	JN009725
<i>M. sanguinarius</i>	Russia, <i>Spribille</i> 23583	JN009726
<i>Tephromela alectoronica</i> Kalb	Australia, <i>Elix</i> 43472	KF712252
<i>T. arafurensis</i> Rambold	Australia, <i>Elix</i> 37763	KF730635
<i>T. atra</i> (Huds.) Hafellner	Italy, TSB 37121	EU558649
<i>T. atra</i>	Greece, TSB 37922	EU558686
<i>T. atra</i>	Greece, TSB 37924	EU558688
<i>T. atra</i>	Greece, TSB 37930	EU558690
<i>T. atra</i>	Bulgaria, <i>Atanassanova</i> 010402 (GZU)	KF730529
<i>T. atra</i>	Finland, <i>H gnabba</i> 2079 (H)	KF730534
<i>T. atra</i>	Finland, <i>H gnabba</i> 2076 (H)	KF730536
<i>T. atra</i>	Finland, <i>H gnabba</i> 2078 (H)	KF730537
<i>T. atra</i>	France, Grube (GZU)	KF730539
<i>T. atra</i>	France, Grube (GZU)	KF730543
<i>T. atra</i>	Ireland, <i>Mayrhofer</i> 19215	KF730544
<i>T. atra</i>	Italy, Muggia (GZU)	KF730557
<i>T. atra</i>	Italy, Muggia (GZU)	KF730561
<i>T. atra</i>	Italy, Muggia (GZU)	KF730566
<i>T. atra</i>	Italy, Muggia (GZU)	KF730568
<i>T. atra</i>	Spain, <i>Perez-Ortega</i> 1356 (GZU)	KF730581
<i>T. atra</i>	Spain, <i>Perez-Ortega</i> 1481 (GZU)	KF730592
<i>T. atra</i>	Spain, <i>Perez-Ortega</i> 1484 (GZU)	KF730594
<i>T. atra</i>	United Kingdom, <i>Perez-Ortega</i> 1633 (GZU)	KF730625
<i>T. atra</i>	United Kingdom, <i>Perez-Ortega</i> 1629 (GZU)	KF730628
<i>T. atra</i>	United Kingdom, <i>Perez-Ortega</i> 1640 (GZU)	KF730632
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181173
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181175
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181182
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181188
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181190
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181193
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181194
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181198
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181200

TABLE 1. — Continuation.

Species	Voucher	ITS accession number
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181210
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181211
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181213
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181215
<i>T. atra</i>	Greece, L. Muggia & A. Rorher	KX181216
<i>T. atra</i> var. <i>calcarea</i> (Jatta) Clauzade & Roux	Italy, TSB 37936	EU558605
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37937	EU558606
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37940	EU558609
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37942	EU558614
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37943	EU558615
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37456	EU558659
<i>T. atra</i> var. <i>calcarea</i>	Italy, TSB 37461	EU558660
<i>T. atra</i> var. <i>calcarea</i>	Greece, TSB 37909	EU558679
<i>T. atra</i> var. <i>calcarea</i>	Greece, TSB 37910	EU558680
<i>T. atra</i> var. <i>calcarea</i>	Greece, TSB 37912	EU558681
<i>T. atra</i> var. <i>torulosa</i> (Flörke ex Flot.) Hafellner	Italy, TSB 38697	EU558618
<i>T. atra</i> var. <i>torulosa</i>	Italy, TSB 37946	EU558645
<i>T. atra</i> var. <i>torulosa</i>	Italy, TSB 37125	EU558651
<i>T. atra</i> var. <i>torulosa</i>	Italy, TSB 37472	EU558664
<i>T. atra</i> var. <i>torulosa</i>	United Kingdom, E:DNA:EDNA09-02098	FR799294
<i>T. aff. atra</i> 1	<i>U. Arup</i> L97376 (Arup priv. herb.)	AY541279
<i>T. aff. atra</i> 1	Austria, Muggia & Hafellner (TSB)	KF712208
<i>T. aff. atra</i> 1	Austria, Muggia & Hafellner (TSB)	KF712209
<i>T. aff. atra</i> 2	New Zealand, Muggia (GZU)	KF712237
<i>T. aff. atra</i> 2	New Zealand, Muggia (GZU)	KF712239
<i>T. aff. atra</i> 2	New Zealand, Muggia (GZU)	KF712242
<i>T. aff. atra</i> 3	New Zealand, Muggia (GZU)	KF712233
<i>T. aff. atra</i> 3	New Zealand, Muggia (GZU)	KF712235
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712256
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712257
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712258
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712260
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712261
<i>T. aff. atra</i> 4	New Zealand, Muggia (GZU)	KF712263
<i>T. aff. atra</i> 5	New Zealand, <i>Wedin M. 9018</i> (S)	KF712255
<i>T. aff. atra</i> 6	Australia, <i>Elix 39844</i>	KF712251
<i>T. aff. atra</i> 6	New Zealand, Muggia (GZU)	KF712262
<i>T. aff. atra</i> 7	Peru, <i>Perez-Ortega 1470</i>	KF712245
<i>T. aff. atra</i> 7	Peru, <i>Perez-Ortega 1469</i>	KF712246
<i>T. aff. atra</i> 8	Peru, <i>Perez-Ortega 1453</i>	KF712248
<i>T. aff. atra</i> 8	Peru, <i>Perez-Ortega 1454</i>	KF712249
<i>T. atrocaesia</i> (Nyl. ex Cromb.) Fryday	Chile, <i>Perez-Ortega 1194</i> (GZU)	KF712201
<i>T. atrocaesia</i>	Chile, <i>Perez-Ortega 1199</i> (GZU)	KF712202
<i>T. aff. atrocaesia</i>	Peru, <i>Perez-Ortega 1455</i> (GZU)	KF712203
<i>T. aff. atrocaesia</i>	Peru, <i>Perez-Ortega 1457</i> (GZU)	KF712204
<i>T. aff. atrocaesia</i>	Peru, <i>Perez-Ortega 1458</i> (GZU)	KF712205
<i>T. follmannii</i> Pérez-Vargas, Hern.-Padr. & Elix	Spain, Tafner R. (GZU)	KF730616
<i>T. follmannii</i>	Spain, Tafner R. (GZU)	KF730617
<i>T. grumosa</i> (Pers.) Hafellner & Cl.Roux	Italy, TSB 38686	EU558625
<i>T. grumosa</i>	Italy, TSB 38687	EU558626
<i>T. grumosa</i>	Italy, TSB 37081	EU558637
<i>T. nashii</i>	AFTOL-ID 780	HQ650606
<i>T. nashii</i>	AFTOL-ID 1328	HQ650607
<i>T. nashii</i>	United States, <i>Wetmore 73995</i> (MSC0074769)	KF712200
<b><i>T. obesimarginata</i> sp. nov.</b>	<b>L.A. Santos &amp; A. Aptroot (ISE 52321)</b>	<b>OP881898</b>
<i>T. pacifica</i> Björk & Muggia	United States, <i>Bjork 22123</i>	KF712216
<i>T. pacifica</i>	United States, <i>T. Tonsberg 43077</i> (BG)	KX181230
<i>T. pacifica</i>	United States, <i>T. Tonsberg 43077</i> (BG)	KX181231
<i>T. pertusarioides</i> (Degel.) Hafellner & Cl.Roux	Russia, <i>Spribille 31797</i>	JN009730
<i>T. pertusarioides</i>	Russia, <i>Yakovchenko T127</i>	KF712244
<i>T. physodica</i> Kalb	Austria, <i>J. Hafellner 53025</i> (GZU)	AY398699
<i>T. physodica</i>	Australia, <i>Elix 38159</i>	KF730634
<b><i>T. vinacea</i> sp. nov.</b>	<b>Brazil, L.A. Santos s.n. (ISE 46534)</b>	<b>OP881896</b>
<b><i>T. vinacea</i> sp. nov.</b>	<b>Brazil, L.A. Santos s.n. (ISE 54172)</b>	<b>OP881895</b>
<b><i>T. aff. vinacea</i></b>	<b>Brazil, Aptroot &amp; M.F. Souza (CGMS 81999)</b>	<b>OP881897</b>
<i>Violella fucata</i> (Stirt.) T.Sprib.	Germany, <i>Spribille 32112</i>	JN009732
<i>V. fucata</i>	Slovenia, <i>Spribille 30276</i>	JN009733
<i>V. fucata</i>	Switzerland, BC-109-4 (WSL)	KX132968
<i>V. wangii</i> T.Sprib. & Goffinet	China, <i>Goffinet 10029</i>	JN009734
<i>V. wangii</i>	China, <i>Goffinet 10033</i>	JN009735
<i>V. wangii</i>	China, <i>Goffinet 10029</i> (KUN)	NR132859

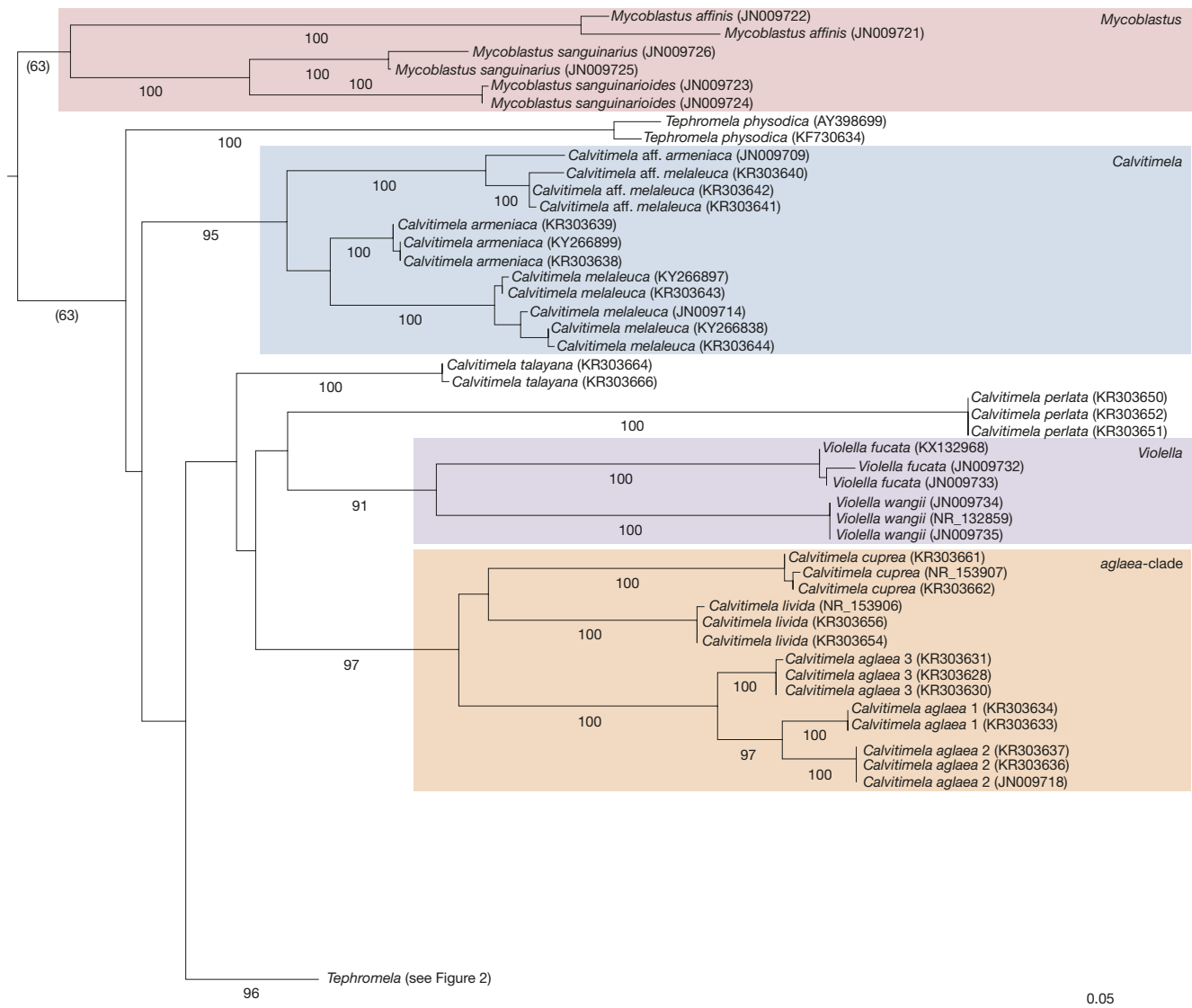


FIG. 1. — Phylogenetic relationship of *Tephromela* M.Choisy based on maximum likelihood analysis of ITS rDNA sequences. Bootstrap support values are reported below branches. GenBank accession numbers are given in Table 1.

*M. Cáceres*, sp. nov., is chemically characterized by the production of lichexanthone. Lichexanthone was reported for the first time in the genus from *T. velloziae* by Kalb (1984). That species has not yet been sequenced, but *T. obesimarginata* L.A.Santos, Aptroot & M. Cáceres, sp. nov., has distinctly longer ascospores and thicker apothecial margins and also differs in substrate (see below). Notably, the lineage identified as *T. aff. vinacea*, with a single accession from Brazil, is conspecific with other Brazilian material which Kalb (2008) had originally assigned to *T. alectoronica*, a new species described by him based on type material from Australia. The accession representing the early diverging lineage within *Tephromela* originates from Australia (Muggia *et al.* 2014) and appears to represent that taxon, although Muggia *et al.* (2014) reported methyl-alectoronic acid for that material. The Brazilian material is apparently something else and not closely related to the Australian taxon. Our data thus corroborate that ITS provides good resolution for species delimitation in

the genus *Tephromela* (and Tephromelataceae as a whole), and the limited data thus far available indicate that tropical material from South America, but also material from Australasia, represents numerous previously unrecognized lineages.

NEW SPECIES

Family TEPHROMELATACEAE Hafellner  
Genus *Tephromela* M.Choisy

*Tephromela multireflexa*  
Aptroot & M.F.Souza, sp. nov.  
(Fig. 3A, B)

Similar to *Tephromela atra*, but corticolous and differing in the apothecium thalline margin containing lichexanthone in the cortex and  $\alpha$ -collatolic acid in the medulla.



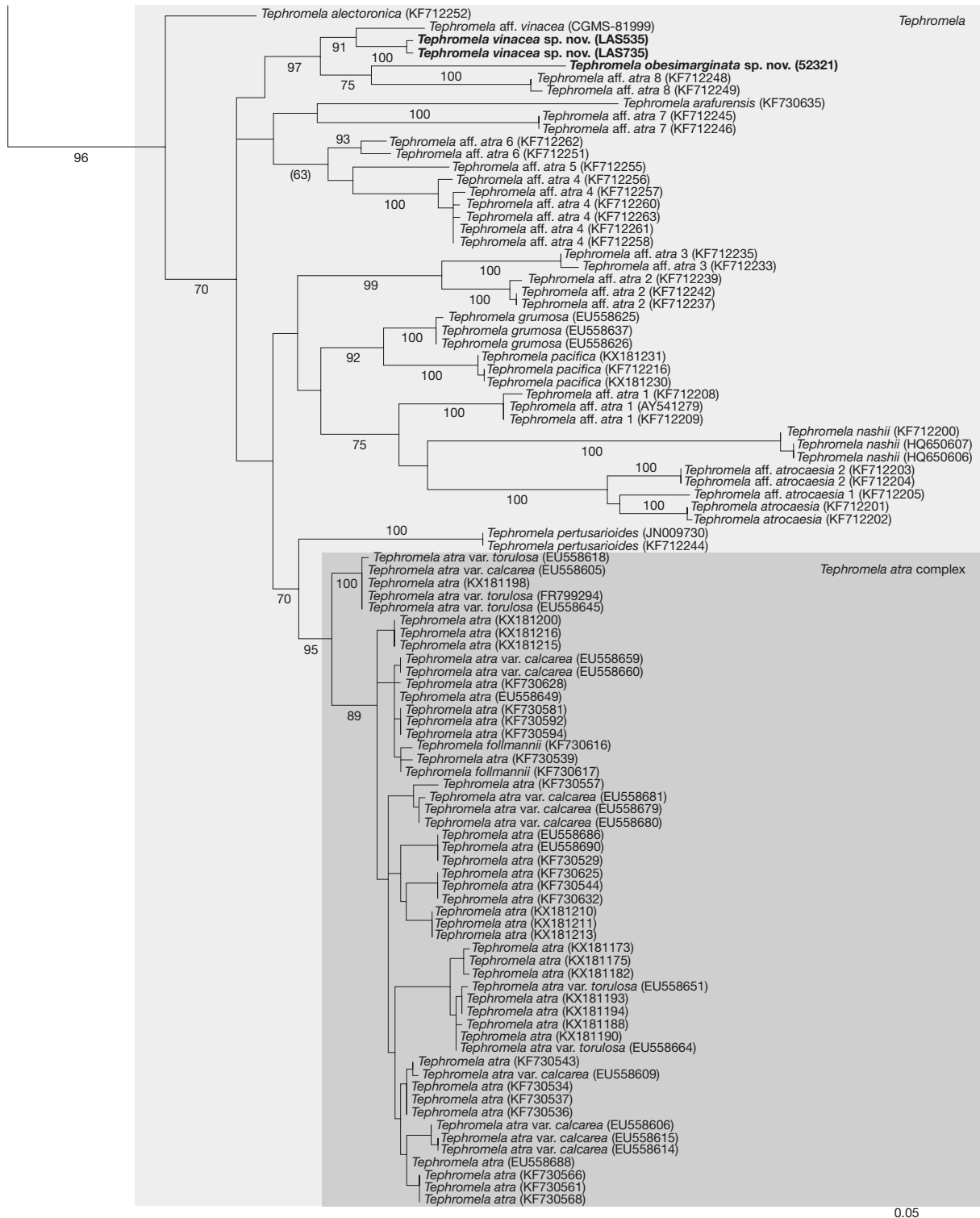


FIG. 2. — Phylogenetic relationship of *Tephromela* M.Choisy based on maximum likelihood analysis of ITS rDNA sequences. Bootstrap support values are reported below branches. The new *Tephromela* species are indicated in boldface. GenBank accession numbers are given in Table 1.

HOLOTYPE. — Brazil. Mato Grosso, Cuiabá, Chapada dos Guimarães, Pousada do Parque private area, alt. 700 m, 15°26'50"S, 55°49'50"W, on bark, 12-19.IX.2020, *A. Aptroot & M.F. Souza 81950* (holo-, CGMS; iso-, ABL).

ETYMOLOGY. — The name refers to the two different UV-reflectant colours.

CHEMISTRY. — Thallus UV-, C-, P-, K-, apothecium margin UV+ yellow, apothecium medulla UV+ bluish-green. With lichexanthone (apothecial margins) and  $\alpha$ -collatolic acid detected by TLC.

ECOLOGY AND DISTRIBUTION. — On exposed tree branch in Cerrado forest; known only from the type collection from Brazil.

MYCOBANK. — MB 846314.

DESCRIPTION

Thallus crustose, mineral to greenish-grey, rimose to areolate, minutely verrucose, somewhat glossy, *c.* 0.3 mm thick, areoles 0.05–0.2 mm in diam. Prothallus absent. Photobiont an unicellular green alga, arranged in one layer below the cortex. Apothecia present, sessile, roundish to lobate, homogeneously distributed on the thallus. Disk black, 0.5–2.5 mm in diam., flat to slightly convex, epruinose. Thalline margin concolorous with the thallus, glossy, *c.* 0.3 mm wide, raised above the level of the disk, minutely crenate. Proper exciple thickest at the base, becoming thinner at the edges, hyaline. Exciple with crystals that after treatment with K partly dissolve. Hymenium *c.* 90–180 µm thick, blue, colour in K unchanged. Subhymenium 50–70 µm thick, dark blue, in K dark purple. Epihymenium dark purple, pigment in K dissolving leaving the blue of the hymenium. Paraphyses simple, septate, 2–3 µm wide, with 4–6 µm wide hyaline apical cells, surrounded by lilac pigmented gel. Hypothecium 0–80 µm thick, pale yellow, intensifying orange yellow in K. Asci slightly clavate, 70–55 µm tall, 8-spored. Ascospores simple, hyaline, ellipsoid, 14–16 × 5–6.5 µm, wall 1 µm thick. Pycnidia not observed.

DISCUSSION

The substance  $\alpha$ -collatolic acid is commonly occurring in the genus *Tephromela*, while lichexanthone is so far only reported from *T. velloziae*. In that species, and in two further species described below, lichexanthone occurs in the thallus and the apothecium margins, not only in the thallus. The new species is the first in the genus that combines both lichexanthone and  $\alpha$ -collatolic acid.

*Tephromela obesimarginata*

L.A.Santos, Aptroot & M.Cáceres, sp. nov.  
(Figs 3C, D; 4A, B)

Similar to *Tephromela velloziae* but corticolous and differing in the unevenly thickened thalline apothecium margin and the larger ascospores.

HOLOTYPE. — **Brazil**. Minas Gerais, Catas Altas, Caraça, alt. 1200–1400 m, 20°06'S, 43°29'W, on tree bark, 17–25.V.2021, *L.A. Santos & A. Aptroot s.n.* (holo-, ISE[ISE52321]; iso-, CGMS).

ADDITIONAL SPECIMENS EXAMINED. — **Brazil**. Minas Gerais, Catas Altas, Caraça, alt. 1200–1400 m, 20°06'S, 43°29'W, on tree bark, 17–25.V.2021, *L.A. Santos & A. Aptroot 52143, 52312, 52309* (ISE, CGMS).

ETYMOLOGY. — The species is named after the irregularly thickened, plump, thalline apothecium margin.

CHEMISTRY. — Thallus UV+ yellow, C-, P-, K-, apothecium margin UV+ yellow. With lichexanthone.

ECOLOGY AND DISTRIBUTION. — On exposed trees in Campo rupestre vegetation in SE Brazil; known only from Brazil.

MYCOBANK. — MB 846315.

GENBANK. — OP881898.

DESCRIPTION

Thallus crustose, whitish to pale ochraceous-grey, rimose to areolate, verrucose, somewhat glossy, *c.* 0.1 mm thick. Prothallus absent. Photobiont an unicellular green alga, arranged in one layer below the cortex, algal layer *c.* 50 µm high. Apothecia always present, sessile, roundish, homogeneously distributed on the thallus, with unevenly crenate, irregularly thickened margin. Disk black, 0.7–2 mm in diam., flat to somewhat concave or saddle-shaped, epruinose. Thalline margin concolorous with the thallus, *c.* 0.2–0.6 mm wide, raised above the level of the disk (100–150 µm). Proper exciple thickest at the base, becoming thinner at the edges, pale to intense yellow-brown, intensifying yellow in K. Exciple with crystals that after treatment with K partly or completely dissolve. Hymenium *c.* 100–150 µm thick, violet, K+ greenish-grey. Epihymenium violet, K+ greenish-grey. Paraphyses simple, septate, 2–3 µm wide, with 3–5 µm wide hyaline apical cells, surrounded by violet pigmented gel. Hypothecium 100–150 µm thick, violet, K+ greenish-grey, with a narrow golden-brown band below the hymenium (25–50 µm thick). Asci slightly clavate, 50–75 µm tall, 8-spored. Ascospores simple, hyaline, ellipsoid, 14–16 × 6.5–7.5 µm. Pycnidia black. Conidia not observed.

DISCUSSION

The new species somewhat resembles *Tephromela velloziae*, but differs in the irregularly thickened apothecium margin, the larger ascospores (14–16 × 6.5–7.5 µm vs 9–12 × 5–7 µm), and the corticolous habit.

*Tephromela vinacea*

L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov.  
(Figs 3E, F; 4C, D)

Similar to *Tephromela atra* but corticolous and differing in the presence of a fatty acid, and in the wine-red pigment in the upper hymenium and epihymenium.

HOLOTYPE. — **Brazil**. Pernambuco, Buíque, Parque Nacional Vale do Catimbau, alt. 745 m, 8°30'41"S, 37°16'37"W, on bark of tree, 13–15.VIII.2017, *L.A. Santos s.n.* (holo-, ISE[ISE46534]).

ADDITIONAL SPECIMENS EXAMINED. — **Brazil**. Pernambuco, Buíque, Parque Nacional Vale do Catimbau, alt. 745 m, 8°29'49"S, 37°18'02"W, on bark of tree, 13–15.VIII.2017, *L.A. Santos s.n.* (ISE[ISE54172]); Mato Grosso do Sul, Fazenda Marambaia, Bonito, alt. 650 m, 20°58'S, 56°42'W, on wood, 30.X.2018, *A. Aptroot 77088* (ABL, CGMS); Fazenda Santa Fé, Jardim, alt. 650 m, 21°32'S, 56°45'W, on bark of tree, 02.XI.2018, *A. Aptroot 77478* (ABL, CGMS); Bonito, outskirts, near tower, alt. 475 m, 21°07'44"S, 56°30'41"W, on bark of tree, 09.XI.2018, *A. Aptroot 78049* (CGMS).

ETYMOLOGY. — The species is named after the wine-red pigmented gel that surrounds the paraphyses.

CHEMISTRY. — Apothecia and medulla UV+ greenish white, C-, P-, K-. Alecoronic acid, atranorin and an unknown hydrophobic substance detected by TLC. This unidentified fatty acid has an RF value of 18 in solvent C.

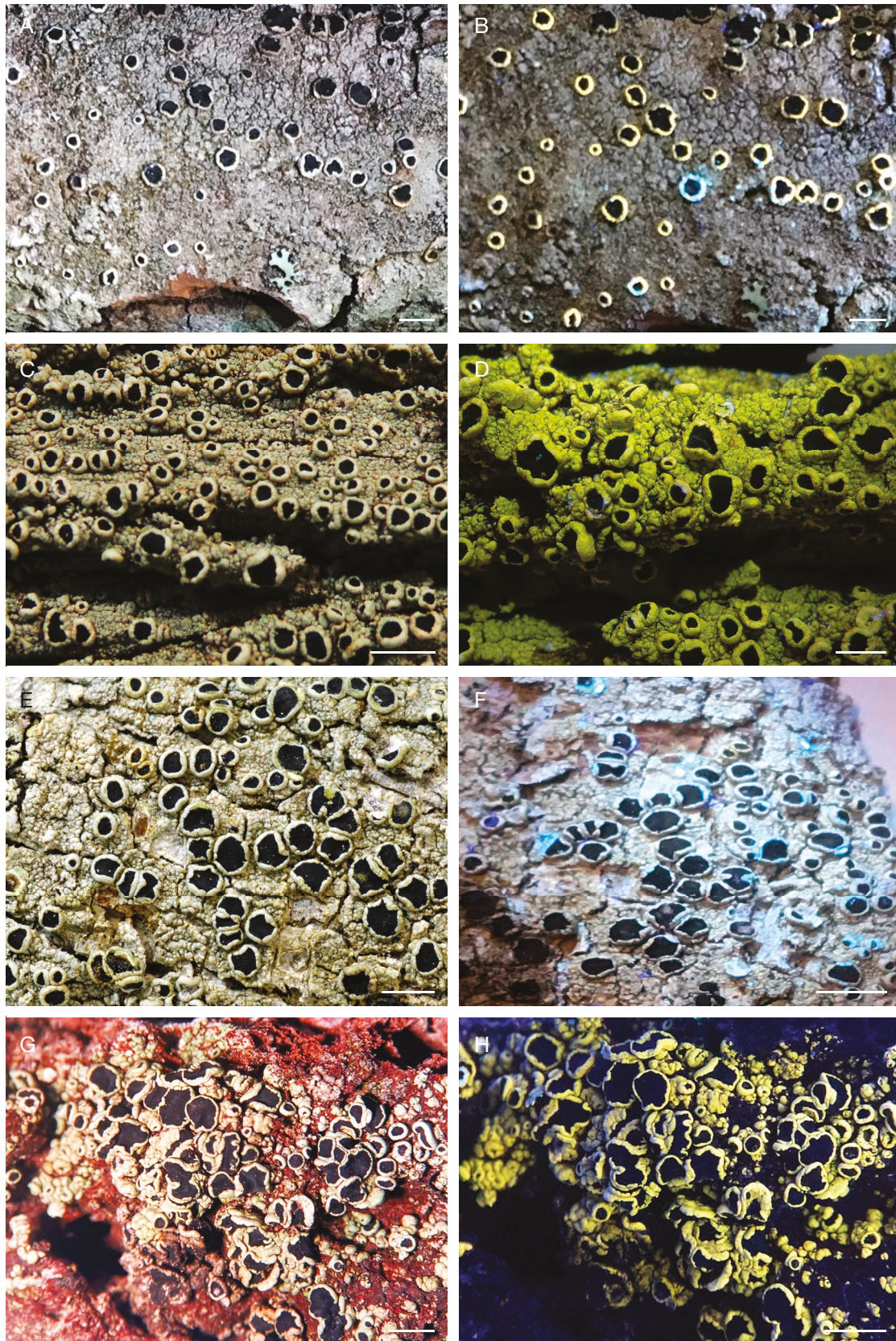


FIG. 3. — Habitus and UV reaction: **A, B**, *Tephromela multireflexa* Aptroot & M.F.Souza, sp. nov. (holotype), apothecial margins UV+ yellow and UV+ bluish green; **C, D**, *Tephromela obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. (holotype), thallus and apothecium margin UV+ yellow; **E, F**, *Tephromela vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. (holotype), medulla UV+ greenish white; **G, H**, *Tephromela xanthonica* Guzmán-Guillermo, sp. nov. (holotype), thallus and apothecium margin UV+ yellow. Scale bars: 2 mm.

ECOLOGY AND DISTRIBUTION. — On exposed tree branch in Caatinga vegetation in NE Brazil and on trees and especially wooden poles in Cerrado in S Brazil; known only from Brazil, but probably occurring in adjacent countries.

MYCOBANK. — MB 846316.

GENBANK. — OP881896.

#### DESCRIPTION

Thallus crustose, whitish to pale ochraceous-grey, rimose to areolate, minutely verrucose, somewhat glossy, *c.* 0.1 mm thick. Prothallus black to greyish-white or absent. Photobiont an unicellular green alga, arranged in one layer below the cortex, algal layer 37–70 µm high. Apothecia always present, sessile, roundish, homogeneously distributed on the thallus, with somewhat unevenly crenate, sometimes lobate margin. Disk black, 0.5–2 mm in diam., flat to slightly convex, epruinose. Thalline margin concolorous with the thallus, *c.* 0.3 mm wide, raised above the level of the disk (100–125 µm). Proper exciple thickest at the base, becoming thinner at the edges, pale to intense yellow-brown, intensifying yellow in K. Exciple with crystals that after treatment with K partly or completely dissolve. Hymenium *c.* 63–100 µm thick, pale wine red. Epithymenium dark wine red, K+ intensifying wine red. Paraphyses simple, septate, 2–3 µm wide, with 4–6 µm wide hyaline apical cells, surrounded by wine red pigmented gel. Subhymenium 37.5–50 µm thick, brown. Hypothecium 62–87.5 µm thick, golden brown. Asci slightly clavate, 40–55 µm tall, 8-spored. Ascospores simple, hyaline, ellipsoid, 10–13 × 5–8 µm. Pycnidia black. Conidia rod-shape, 12.5–15 × 1 µm.

#### DISCUSSION

The new species superficially resembles *Tephromela atra*, but differs in secondary chemistry and is not closely related to the latter phylogenetically (Fig. 2). Fatty acids were first reported for the genus in Muggia *et al.* (2014). At the time, the authors did not describe the material as a new taxon and questioned the application of chemical characters in the delimitation of species within the genus. Subsequently, based on the presence of fatty acid, Cestaro *et al.* (2016) described *T. pacifica*. Kantvilas (2015) and Kantvilas & Elix (2017) also recently used chemical patterns to describe new species with unique chemistry. The metabolite found in our species is a fatty acid, as reported by Cestaro *et al.* 2016, but has a chemical standard, with a RF value of 18 in solvent C, different from that found by Cestaro *et al.* (2016) with a RF value of 50.

Most similar to the new species are *T. alectoronica*, described from Australia but in the protologue also reported from Brazil, and *T. rhizophorae* Kalb, described from Brazil. Both lack fatty acids but contain traces of physodalic acid instead. The ascospores of *T. rhizophorae* are distinctly smaller (8–10 × 5–6 µm) whereas those of *T. alectoronica* are only slightly larger (12–17 × 6–9 µm). Thus, without co-chromatography, *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. and *T. alectoronica* are difficult to distinguish. The specimen clustering as sister to *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov., and here named *T. aff. vinacea*

(Fig. 2) is of interest in this respect. It presents a UV+ greenish white fluorescence, although alectoronic acid was not clearly detectable with TLC. It was collected at one of the localities where Kalb (2008) originally reported material identified as *T. alectoronica* (Chapada dos Guimarães, Mato Grosso). Thus, Kalb's material might be conspecific with this sequenced specimen, which would imply that the Brazilian material of *T. alectoronica* is not that species but is represented by the clade here named *T. aff. vinacea*, quite distant from the Australian *T. alectoronica* (Fig. 2). However, given that the underlying specimen is rather small and the chemistry could not be determined with certainty, we refrain from any formal conclusions in this case.

#### *Tephromela xanthonica*

Guzmán-Guillermo, sp. nov.

(Figs 3G, H; 4E, F)

Similar to *Tephromela velloziae*, differing in its saxicolous ecology and its areolate thallus.

HOLOTYPE. — Mexico. Veracruz, Municipality of Las Vigas de Ramírez, locality of Volcancillo, alt. 2300 m, 19°37'00.5"N, 97°04'01.0"W, on volcanic rock, IX.2020, *Cárdenas-Mendoza s.n.* (holo-, XALU[XALU1413]; para-, XALU[XALU1414, XALU1415, XALU1416, XALU1417]).

ADDITIONAL SPECIMENS EXAMINED. — Brazil. Minas Gerais, Catas Altas, Caraça, alt. 1200–1400 m, 20°06'S, 43°29'W, on sandstone, 17–25.V.2021, L.A. Santos & Aptroot 52016, 52057 (ISE, CGMS).

ETYMOLOGY. — The name refers to its chemistry.

CHEMISTRY. — Thallus UV+ yellow, C-, P-, K-, apothecium margin UV+ yellow. With lichexanthone.

ECOLOGY AND DISTRIBUTION. — On exposed volcanic rock associated with *Pinus* forest in Volcancillo, Veracruz, and on sandstone in Campo rupestre in Caraça, Minas Gerais, Brazil; known from Brazil and Mexico.

MYCOBANK. — MB 846317.

#### DESCRIPTION

Thallus crustose, mineral to greenish-grey, rimose to verrucose, somewhat glossy, *c.* 0.3 mm thick, areoles 0.5–1 mm in diam. Prothallus absent. Photobiont an unicellular green alga, arranged in one layer below the cortex. Apothecia present, sessile, roundish to lobate when old, numerous on the center of the thallus. Disk black, 0.5–2.5 mm in diam., flat to slightly convex, epruinose. Thalline margin concolorous with the thallus, glossy, 0.15–0.18 mm wide, raised above the level of the disk, minutely crenate, with motes of white pruina when young. Proper exciple thickest at the base (50–60 µm), becoming thinner at the edges, intense yellow-brown. Hymenium *c.* 100–130 µm thick, pigment reddish purple, K+ purple. Subhymenium 80–90 µm thick, purple pigmented, dark purple in K. Epithymenium reddish-purple, pigment K+ purple. Paraphyses simple, septate, surrounded by dark red pigmented gel. Hypothecium 0–60 µm thick,

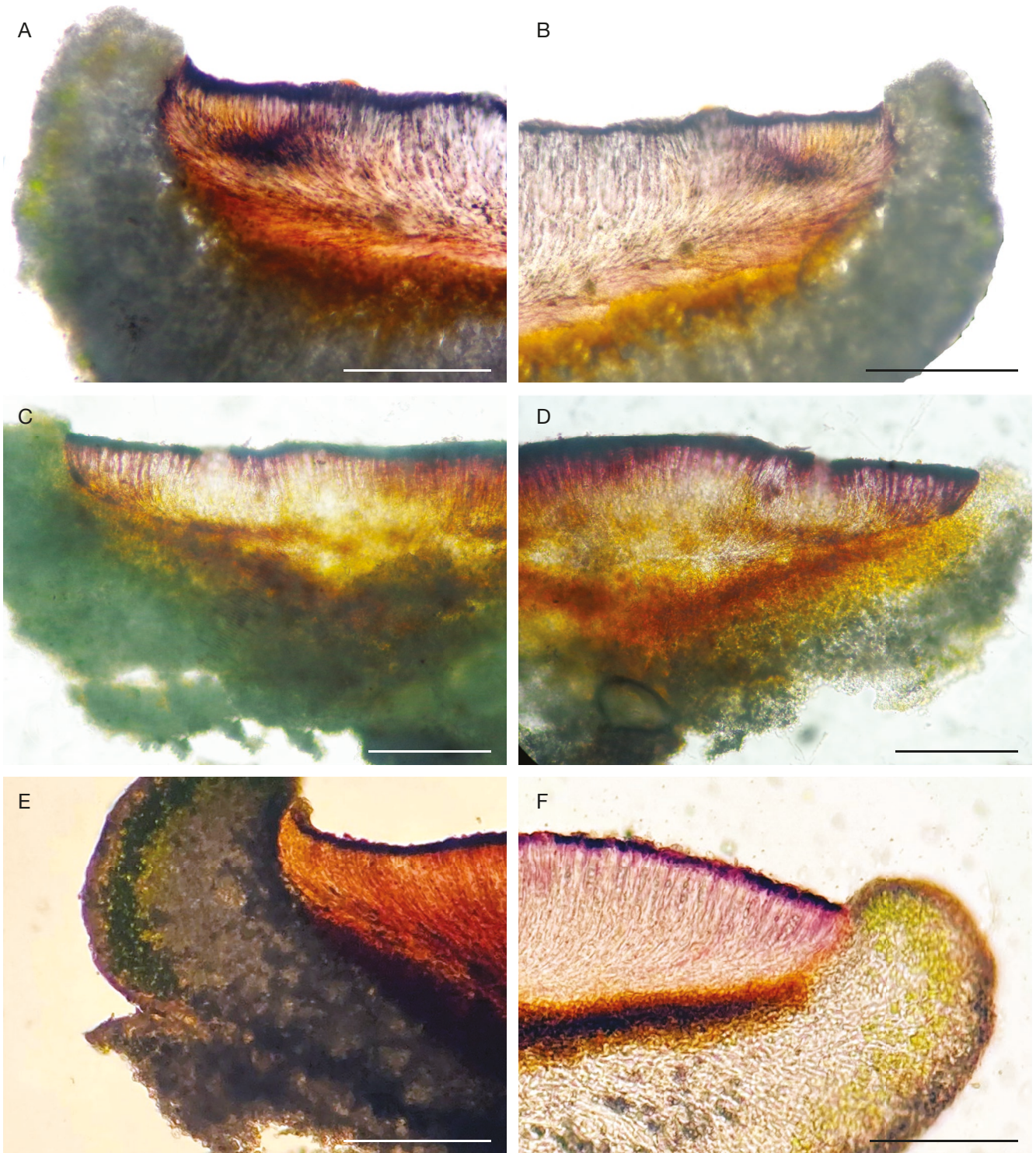


FIG. 4. — Section ascocata: **A, B**, *Tephromela obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. (holotype), before and after KOH treatment; **C, D**, *Tephromela vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. (holotype), before and after KOH treatment; **E, F**, *Tephromela xanthonica* Guzmán-Guillermo, sp. nov. (holotype), before and after KOH treatment. Scale bars: 100  $\mu$ m.

with similar colorations as the hymenium. Asci slightly clavate, 65-55  $\mu$ m tall, 8-spored. Ascospores simple, hyaline, ellipsoid, 9-13  $\times$  5-6  $\mu$ m, wall 1-2  $\mu$ m thick. Pycnidia not observed.

#### DISCUSSION

*Tephromela velloziae* was described as containing lichexanthone and it also agrees in ascospore size (Kalb 1984). However, the isotypes *Kalb 349* (B[B600157687, B600093712]) depos-

ited in Herbarium Berolinense (digital material consulted in BiNHum 2021) and mentioned in the original description (Kalb 1984), differ morphologically and in ecology from the new species. They have a verrucose thallus without areoles, in contrast to the areolate thallus of *T. xanthonica* Guzmán-Guillermo, sp. nov. Also, *T. velloziae* grows on dead stems of a semi-woody herb in semi-arid Caatinga vegetation, whereas *T. xanthonica* Guzmán-Guillermo, sp. nov., is a saxicolous species, found growing on sandstone and on volcanic rock between species of *Cladonia*, *Stereocaulon*, *Rhizocarpon*, and other unidentified crustose lichens, in temperate to tropical montane ecosystem, including *Pinus* forest at 2250–2300 m in Mexico and campos rupestres at 1200–1400 m in Brazil.

#### NEW COMBINATIONS

*Tephromela carassensis*  
(Vain.) Aptroot & Lücking, comb. nov.  
(Fig. 5A, B)

*Lecanora carassensis* Vain., *Acta Societatis pro Fauna et Flora Fennica* 7 (1): 91 (Vainio 1890).

HOLOTYPE. — **Brazil**. Minas Gerais, Catas Altas, Caraça, alt. 1400–1500 m, on moss on sandstone, 1885, E. Vainio distributed in Lichenes Brasilinses Exsiccati 1572 (holo-, TUR[TUR-VAIN 5217]).

ADDITIONAL SPECIMEN EXAMINED. — **Brazil**. Minas Gerais, Catas Altas, Caraça, on sandstone, 1997, *Aptroot 40719* (ABL, SP).

CHEMISTRY. — Thallus UV+ yellow, C-, P-, K-, apothecium margin UV+ yellow. With lichexanthone.

ECOLOGY AND DISTRIBUTION. — On mosses on sandstone in Campo rupestre in Caraça, Minas Gerais, Brazil; known only from Brazil.

MYCOBANK. — MB 846318.

#### DESCRIPTION

Thallus crustose, mineral to greenish-grey, rimose to verrucose, somewhat glossy, *c.* 0.3 mm thick, areoles 0.5–1 mm in diam. Prothallus absent. Apothecia present, sessile, roundish to lobate when old, numerous on the center of the thallus. Disk black, 0.5–1.5 mm in diam., flat, epruinose. Thalline margin concolorous with the thallus, internally with small crystals, glossy, 0.15–0.18 mm wide, raised above the level of the disk, crenate. Hymenium *c.* 100 µm thick, mottled bluish grey and pale brown. Subhymenium *c.* 200 µm thick, dark reddish brown. Epihymenium bluish grey, *c.* 60 µm thick, pigment unchanged in K. Asci slightly clavate, 8-spored. Ascospores simple, hyaline, ellipsoid, 9–12 × 5–6 µm, wall 1–2 µm thick. Pycnidia not observed.

#### DISCUSSION

This species was described in *Lecanora* but on account of the anatomical and chemical characters belongs in *Tephromela*, as also suggested by an annotation label by Klaus Kalb. It agrees in many aspects with *T. epichlorina* (Vain.) Aptroot & Lücking, comb. nov. (see below), and both were described from the same locality, but there are notably differences in the pigmentation patterns of the hymenium, epihymenium,

and subhymenium. Both species differ from *T. xanthonica* Guzmán-Guillermo, sp. nov. also largely in apothecial pigment patterns (see key below).

*Tephromela epichlorina*  
(Vain.) Aptroot & Lücking, comb. nov.  
(Fig. 5C, D)

*Lecanora epichlorina* Vain., *Acta Societatis pro Fauna et Flora Fennica* 7 (1): 91 (Vainio 1890).

HOLOTYPE. — **Brazil**. Minas Gerais, Catas Altas, Caraça, alt. 1400–1500 m, on moss on sandstone, 1885, E. Vainio distributed in Lichenes Brasilinses Exsiccati 164 (holo-, TUR[TUR-VAIN 5645]).

ADDITIONAL SPECIMEN EXAMINED. — **Brazil**. Minas Gerais, Catas Altas, Caraça, on sandstone, 1997, *Aptroot 41012* (ABL, SP).

CHEMISTRY. — Thallus UV+ yellow, C-, P-, K-, apothecium margin UV+ yellow. With lichexanthone.

ECOLOGY AND DISTRIBUTION. — On sandstone in Campo rupestre in Caraça, Minas Gerais, Brazil; known only from Brazil.

MYCOBANK. — MB 846230.

#### DESCRIPTION

Thallus crustose, mineral to greenish-grey, rimose to verrucose, somewhat glossy, *c.* 0.3 mm thick, areoles 0.5–1 mm in diam. Prothallus absent. Apothecia present, sessile, roundish to lobate when old, numerous on the center of the thallus. Disk black, 0.5–1.5 mm in diam., flat, epruinose. Thalline margin concolorous with the thallus, glossy, 0.15–0.2 mm wide, raised above the level of the disk, crenate. Hymenium *c.* 100–150 µm thick, hyaline. Subhymenium *c.* 200 µm thick, dark orange brown. Epihymenium dark blue to black, *c.* 60 µm thick, pigment unchanged in K. Asci slightly clavate, 8-spored. Ascospores simple, hyaline, ellipsoid, 9–15 × 5–6 µm, wall 1–2 µm thick. Pycnidia not observed.

#### DISCUSSION

This species was described in *Lecanora* but on account of the anatomical and chemical characters belongs in *Tephromela*, as also suggested by an annotation label by Klaus Kalb. For differences with *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov. and *T. xanthonica* Guzmán-Guillermo, sp. nov., see Discussion under *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov. and in the key below.

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KEY TO SPECIES OF *TEPHROMELA* M. CHOISY IN BRAZIL

The Brazilian material keying out here possibly does not represent *T. alectoronica* s.str. but perhaps *T.* aff. *vinacea* in our phylogenetic tree.

1. Saxicolous or muscicolous on rock or soil or lichenicolous on saxicolous *Lecanora* ..... 2  
— Corticolous or on *Vellozia* stems or lichenicolous on corticolous *Dirinaria* ..... 10
2. Lichenicolous on saxicolous *Lecanora* ..... *T. campestricola* (Nyl.) Rambold & Triebel  
— Saxicolous or muscicolous on rock or on soil ..... 3
3. Apothecia with constricted base; medulla UV+ greenish-white (alectoronic and  $\alpha$ -collatolic acids) or medulla UV+ yellow (lichexanthone) ..... 4  
— Apothecia margin not distinguishable from raised thallus parts (apothecia seemingly emarginate or immersed) ..... 7
4. Medulla UV+ greenish-white (alectoronic and  $\alpha$ -collatolic acid) ..... *T. atra* (Huds.) Hafellner  
— Medulla UV-negative; thallus and apothecium margin UV+ yellow (lichexanthone) ..... 5
5. Hymenium, epihymenium and subhymenium all equally reddish purple .....  
..... *T. xanthonica* Guzmán-Guillermo, sp. nov.  
— Subhymenium reddish; epihymenium bluish; hymenium hyaline or mottled bluish and brownish ..... 6
6. Subhymenium reddish brown; epihymenium and hymenium mottled bluish and brownish .....  
..... *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov.  
— Subhymenium dark orangish brown; epihymenium dark blue to black; hymenium hyaline .....  
..... *T. epichlorina* (Vain.) Aptroot & Lücking, comb. nov.
7. Apothecia (sub)convex, almost without thalline margin; thallus with black hypothallus visible between the areoles ..... 9  
— Apothecia more or less flat, in raised thallus warts; thallus continuous to rimose ..... 7
8. Ascospores 10-14  $\times$  6-8  $\mu$ m; medulla UV+ bluish-green ( $\alpha$ -collatolic acid) .....  
..... *T. buelliana* (Müll. Arg.) Kalb  
— Ascospores 10-11  $\times$  6-7  $\mu$ m; medulla UV- (colensoic acid) .....  
..... *T. colensoica* Rambold & Knoph
9. Ascospores 7-10  $\times$  3.5-4.5  $\mu$ m; medulla UV+ white (loxodellonic and glomelliferic acids) .....  
..... *T. matogrossensis* Kalb & Elix  
— Ascospores 10-12  $\times$  8-9  $\mu$ m; medulla UV+ greenish-white (alectoronic and physodic acids) .....  
..... *T. immersa* Kalb & Elix
10. Lichenicolous on corticolous *Dirinaria* ..... *T. cerasina* (Müll. Arg.) Rambold & Triebel  
— Corticolous ..... 11
11. Thallus UV-, but apothecial margins may be UV+ yellow; medulla UV- or UV+ greenish-white or UV+ bluish-green ..... 12  
— Thallus UV+ yellow (lichexanthone); ascospores 9-16  $\times$  5-7.5  $\mu$ m ..... 16
12. Medulla UV- (no substances in medulla); ascospores long ellipsoid, 10-12  $\times$  3-4  $\mu$ m .....  
..... *T. americana* (Fée) Kalb  
— Medulla UV+ greenish-white or UV+ bluish-green; ascospores ellipsoid, at least 5  $\mu$ m wide ..... 13
13. Apothecial margin UV+ yellow (lichexanthone), medulla UV+ bluish green ( $\alpha$ -collatolic acid); fatty acids absent; ascospores 14-16  $\times$  5-6.5  $\mu$ m ..... *T. multireflexa* Aptroot & M.F.Souza, sp. nov.  
— Apothecial margin UV- or (medulla) UV+ greenish-white (alectoronic and/or physodic acids); fatty acids (TLC) present or absent ..... 14
14. Ascospores 8-10  $\times$  5-6  $\mu$ m; in mangroves ..... *T. rhizophorae* Kalb  
— Ascospores 10-17  $\times$  5-9  $\mu$ m; in inland ecosystems ..... 15
15. Fatty acids present (TLC); ascospores 10-13  $\times$  5-8  $\mu$ m .....  
..... *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov.  
— Fatty acids absent (TLC); ascospores 12-17  $\times$  6-9  $\mu$ m ..... *T. alectoronica* Kalb s.lat.
16. On *Vellozia* stems; ascospores 9-12  $\times$  5-7  $\mu$ m ..... *T. velloziae* Kalb  
— On tree bark; ascospores 14-16  $\times$  6.5-7.5  $\mu$ m .....  
..... *T. obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov.



Fig. 5. — Habitus and UV reaction: **A, B**, *Tephromela carassensis* (Vain.) Aptroot & Lücking, comb. nov. (type material of *Lecanora carassensis* Vainio), thallus and apothecium margin UV+ yellow; **C, D**, *Tephromela epichlorina* (Vain) Aptroot & Lücking, comb. nov. (lectotype material of *Lecanora epichlorina* Vainio), thallus and apothecium margin UV+ yellow. Scale bars: 2 mm.

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## APPENDIX

APPENDIX 1. — ITS alignment of the 140 sequences of *Tephromela* M.Choisy, *Calvitimela* Hafellner, *Mycoblastus* Norman and *Violella* T.Sprib. used in this study: [https://doi.org/10.5852/cryptogamie-mycologie2023v44a2\\_s1](https://doi.org/10.5852/cryptogamie-mycologie2023v44a2_s1)