cryptogamie
Mycologie
2023 • 44 • 2

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

Lidiane Alves dos SANTOS, André APTROOT, Maria Fernanda de SOUZA, Robert LÜCKING, Jorge GUZMÁN-GUILLERMO & Marcela Eugenia da Silva CÁCERES

art. 44 (2) — Published on 22 February 2023
www.cryptogamie.com/mycologie

PUBLICATIONS SCIENTIFIQUES
MUSÉUM NAL HIST NATURELLE

Couverture / *Cover*:
Extraits d'éléments de la Figure 5 / Extracts of the Figure 5

*Cryptogamie, Mycologie* est indexé dans / Cryptogamie, Mycologie *is indexed in*:
- Biological Abstracts
- Current Contents
- Science Citation Index
- Publications bibliographiques du CNRS (Pascal)

*Cryptogamie, Mycologie* est distribué en version électronique par / Cryptogamie, Mycologie *is distributed electronically by*:
- BioOne® (http://www.bioone.org/loi/crym)

***Cryptogamie, Mycologie*** est une revue en flux continu publiée par les Publications scientifiques du Muséum, Paris
**Cryptogamie, Mycologie** *is a fast track journal published by the Museum Science Press, Paris*

Les Publications scientifiques du Muséum publient aussi / *The Museum Science Press also publishes*: ***Adansonia***, ***Geodiversitas***, ***Zoosystema***, ***Anthropozoologica***, ***European Journal of Taxonomy***, ***Naturae***, ***Comptes Rendus Palevol***, ***Cryptogamie*** **sous-sections** ***Algologie***, ***Bryologie***.

Diffusion – Publications scientifiques Muséum national d'Histoire naturelle
CP 41 – 57 rue Cuvier F-75231 Paris cedex 05 (France)
Tél. : 33 (0)1 40 79 48 05 / Fax : 33 (0)1 40 79 38 40
diff.pub@mnhn.fr / http://sciencepress.mnhn.fr

ISSN (imprimé / *print*) : 0181-1584 / ISSN (électronique / *electronic*) : 1776-100

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

**Lidiane Alves dos SANTOS**
Universidade Federal de Pernambuco, Centro de Biociências, Departamento de Micologia, Avenida Professor Moraes Rego s/n, CEP 50670-420, Recife, Pernambuco (Brazil)

**André APTROOT**
**Maria Fernanda de SOUZA**
Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Avenida Costa e Silva s/n, Bairro Universitário, CEP 79070-900, Campo Grande, Mato Grosso do Sul (Brazil)

**Robert LÜCKING**
Botanischer Garten, Freie Universität Berlin, Königin-Luise-Straße 6-8, 14195 Berlin (Germany)

**Jorge GUZMÁN-GUILLERMO**
Facultad de Ciencias Biológicas y Agropecuarías, Universidad Veracruzana, Carretera Peñuela-Amatlán Kilómetro 177, 94500 Córdoba, Veracruz (Mexico)

**Marcela Eugenia da Silva CÁCERES**
Universidade Federal de Sergipe, Departamento de Biociências, Avenida Vereador Olimpio Grande s/n, Bairro Centro, CEP 49500-000, Itabaiana, Sergipe (Brazil)
mscaceres@hotmail.com (corresponding author)

Submitted on 21 February 2022 | Accepted on 29 November 2022 | Published on 22 February 2023

Santos L. A. dos, Aptroot A., Souza M. F., Lücking R., Guzmán-Guillermo J. & Cáceres M. E. da S. 2023. — Four new species of *Tephromela* M.Choisy (Ascomycota, Tephromelataceae), three containing lichexanthone, from Brazil and Mexico. *Cryptogamie, Mycologie* 44 (2): 11-25. https://doi.org/10.5252/cryptogamie-mycologie2023v44a2. http://cryptogamie.com/mycologie/44/2

**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 α-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 &

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.

**KEY WORDS**
Phylogeny,
ITS,
lichenized fungi,
cryptic species,
new combinations,
new species.

**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 α-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.

**MOTS CLÉS**
Phylogénie,
ITS,
champignons lichénisés,
espèces cryptiques,
combinaisons nouvelles,
espèces nouvelles.

## 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.Sprib. (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 α-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 handmade, 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 (λ365 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 |
|---|---|---|
| *Calvitimela aglaea* 1 (Sommerf.) Hafellner | *Spribille 38382* | KR303633 |
| *C. aglaea* 1 | *Spribille 38829* | KR303634 |
| *C. aglaea* 2 | Austria, *Hafellner 70358* | JN009718 |
| *C. aglaea* 2 | OL-160708 | KR303636 |
| *C. aglaea* 2 | OL-163597 | KR303637 |
| *C. aglaea* 3 | OL-160503 | KR303628 |
| *C. aglaea* 3 | OL-173831 | KR303630 |
| *C. aglaea* 3 | OL-160462 | KR303631 |
| *C. armeniaca* (DC.) Hafellner | OL-170675 | KR303638 |
| *C. armeniaca* | OL-170644 | KR303639 |
| *C. armeniaca* | Norway, OL-195741 | KY266899 |
| *C. aff. armeniaca* | Austria, *Hafellner 71304* | JN009709 |
| *C. cuprea* Haugan & Timdal | OL-179616 | KR303661 |
| *C. cuprea* | OL-173292 | KR303662 |
| *C. cuprea* | OL-179566 | NR153907 |
| *C. livida* Haugan & Timdal | OL-174237 | KR303654 |
| *C. livida* | UPSL-520818 | KR303656 |
| *C. livida* | Norway, OL-163835 | NR153906 |
| *C. melaleuca* (Sommerf.) R.Sant. | United States, *Spribille 27965-B* | JN009714 |
| *C. melaleuca* | OL-170490 | KR303643 |
| *C. melaleuca* | OL-159803 | KR303644 |
| *C. melaleuca* | Norway, OL-195711 | KY266838 |
| *C. melaleuca* | Norway, OL-195915 | KY266897 |
| *C.* aff. *melaleuca* | OL-141654 | KR303640 |
| *C.* aff. *melaleuca* | OL-163829 | KR303641 |
| *C.* aff. *melaleuca* | OL-89218 | KR303642 |
| *C. perlata* (Haugan & Timdal) R.Sant. | OL-163770 | KR303679 |
| *C. perlata* | OL-170830 | KR303651 |
| *C. perlata* | OL-141628 | KR303652 |
| *C. talayana* | OL-191705 | KR303664 |
| *C. talayana* | *Hermansson 14958* | KR303666 |
| *Mycoblastus affinis* (Schaer.) T.Schauer | China, *Goffinet 10030* | JN009721 |
| *M. affinis* | Canada, *Spribille & Wagner s.n.*, 2009 | JN009722 |
| *M. sanguinarioides* Kantvilas | Japan, *Ohmura 6740* | JN009723 |
| *M. sanguinarioides* | Japan, *Ohmura 5996* | JN009724 |
| *M. sanguinarius* (L.) Norman | Sweden, *Muggia s.n.*, TSB 38893 | JN009725 |
| *M. sanguinarius* | Russia, *Spribille 23583* | JN009726 |
| *Tephromela alectoronica* Kalb | Australia, *Elix 43472* | KF712252 |
| *T. arafurensis Rambold* | Australia, *Elix 37763* | KF730635 |
| *T. atra* (Huds.) Hafellner | Italy, TSB 37121 | EU558649 |
| *T. atra* | Greece, TSB 37922 | EU558686 |
| *T. atra* | Greece, TSB 37924 | EU558688 |
| *T. atra* | Greece, TSB 37930 | EU558690 |
| *T. atra* | Bulgaria, *Atanassanova 010402* (GZU) | KF730529 |
| *T. atra* | Finland, *H gnabba 2079* (H) | KF730534 |
| *T. atra* | Finland, *H gnabba 2076* (H) | KF730536 |
| *T. atra* | Finland, *H gnabba 2078* (H) | KF730537 |
| *T. atra* | France, Grube (GZU) | KF730539 |
| *T. atra* | France, Grube (GZU) | KF730543 |
| *T. atra* | Ireland, *Mayrhofer 19215* | KF730544 |
| *T. atra* | Italy, Muggia (GZU) | KF730557 |
| *T. atra* | Italy, Muggia (GZU) | KF730561 |
| *T. atra* | Italy, Muggia (GZU) | KF730566 |
| *T. atra* | Italy, Muggia (GZU) | KF730568 |
| *T. atra* | Spain, *Perez-Ortega 1356* (GZU) | KF730581 |
| *T. atra* | Spain, *Perez-Ortega 1481* (GZU) | KF730592 |
| *T. atra* | Spain, *Perez-Ortega 1484* (GZU) | KF730594 |
| *T. atra* | United Kingdom, *Perez-Ortega 1633* (GZU) | KF730625 |
| *T. atra* | United Kingdom, *Perez-Ortega 1629* (GZU) | KF730628 |
| *T. atra* | United Kingdom, *Perez-Ortega 1640* (GZU) | KF730632 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181173 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181175 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181182 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181188 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181190 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181193 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181194 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181198 |
| *T. atra* | Greece, L. Muggia & A. Rorher | KX181200 |

TABLE 1. — Continuation.

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

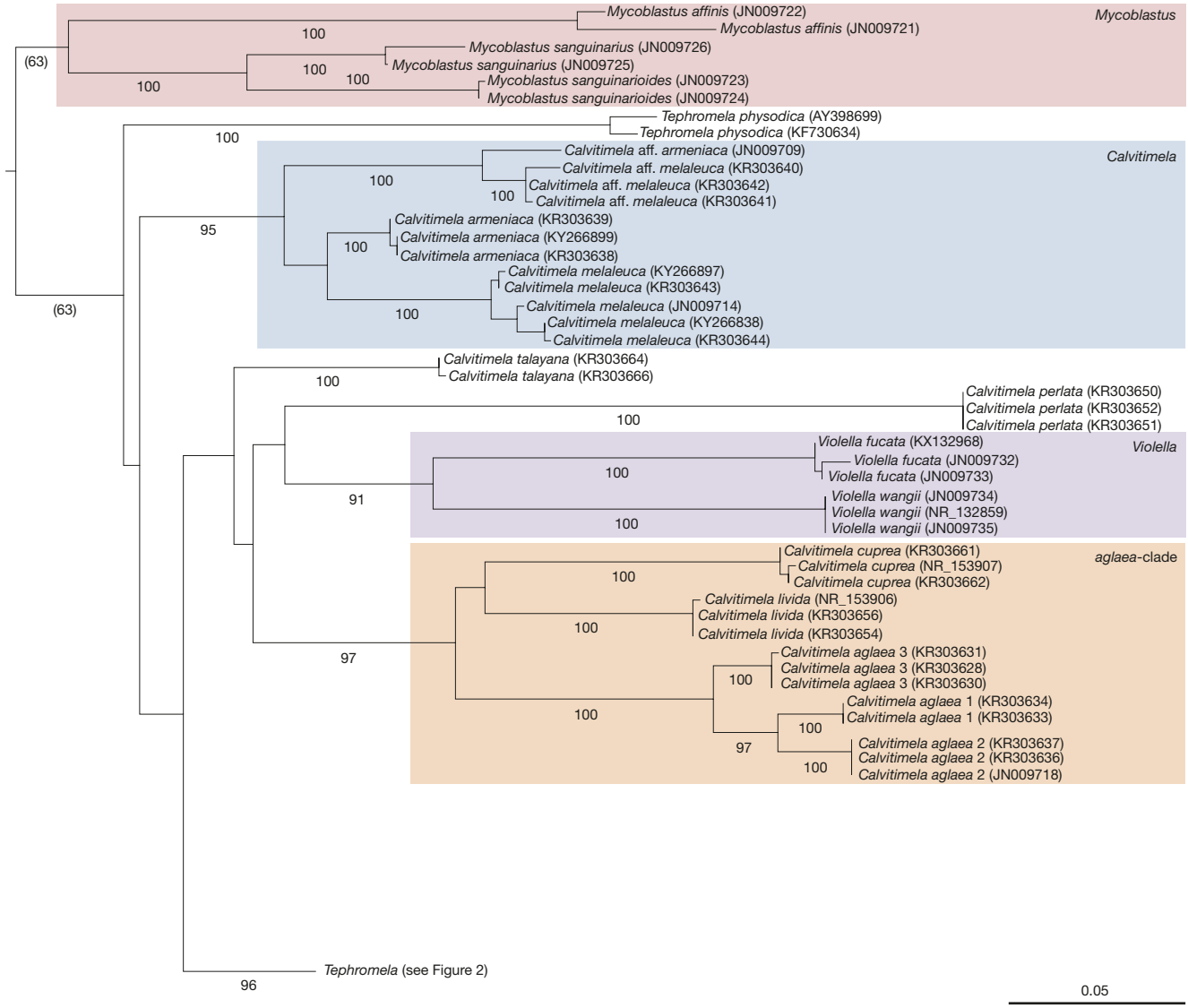


FIG. 1. — Phylogenetic relationship of *Tephromela* M.Choisy based on maximum likelihood analysis of ITS rDNA sequences. Bootstrap support values are reported below branches. GenBank accession numbers are given in Table 1.

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 α-collatolic acid in the medulla.

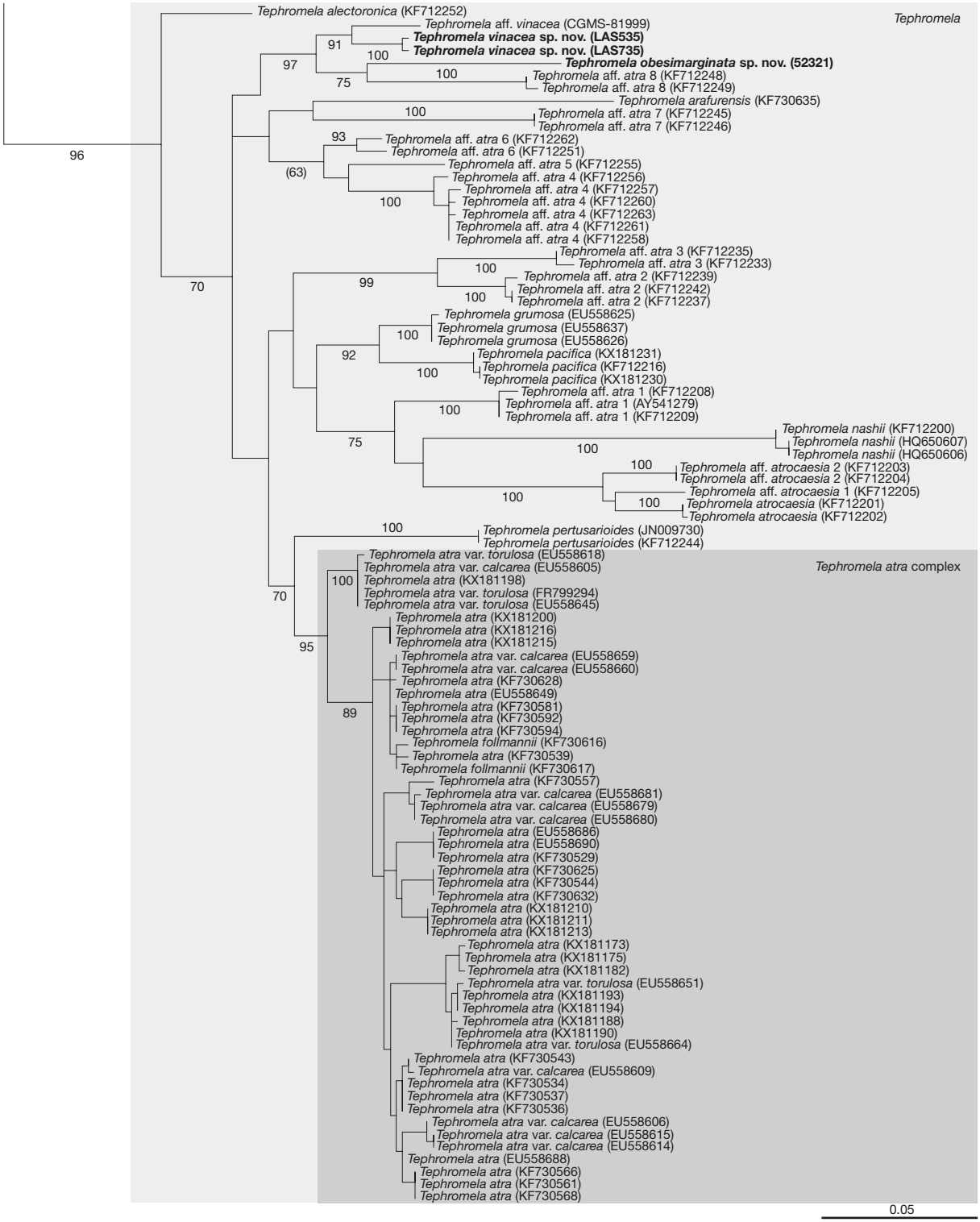


FIG. 2. — Phylogenetic relationship of *Tephromela* M.Choisy based on maximum likelihood analysis of ITS rDNA sequences. Bootstrap support values are reported below branches. The new *Tephromela* species are indicated in **boldface**. GenBank accession numbers are given in Table 1.

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 α-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 α-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 α-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-. Alectoronic acid, atranorin and an unknown hydrophobic substance detected by TLC. This unidentified fatty acid has an RF value of 18 in solvent C.

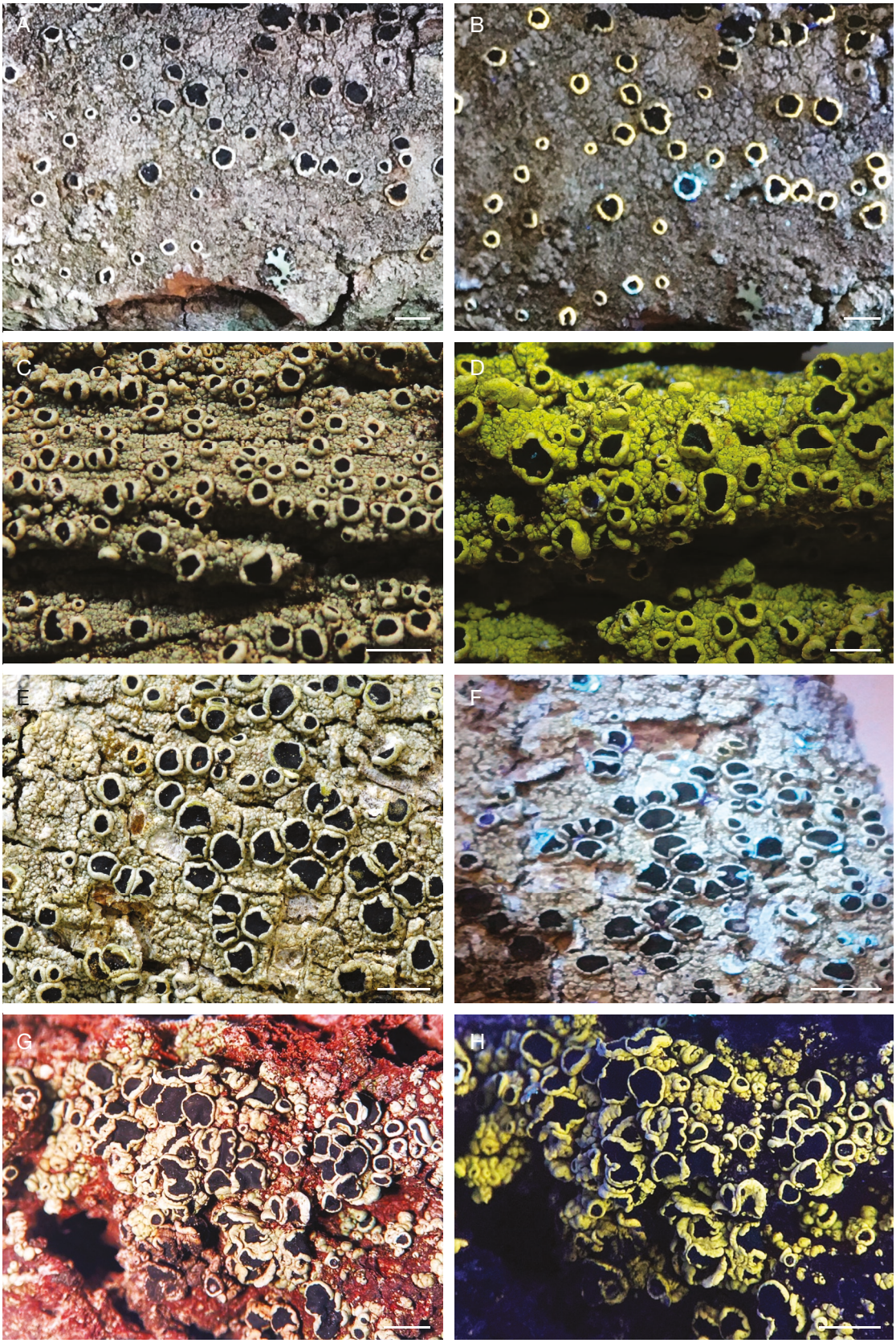


FIG. 3. — Habitus and UV reaction: **A**, **B**, *Tephromela multireflexa* Aptroot & M.F.Souza, sp. nov. (holotype), apothecial margins UV+ yellow and UV+ bluish green; **C**, **D**, *Tephromela obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. (holotype), thallus and apothecium margin UV+ yellow; **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. Epihymenium 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. Epihymenium reddish-purple, pigment K+ purple. Paraphyses simple, septate, surrounded by dark red pigmented gel. Hypothecium 0-60 µm thick,

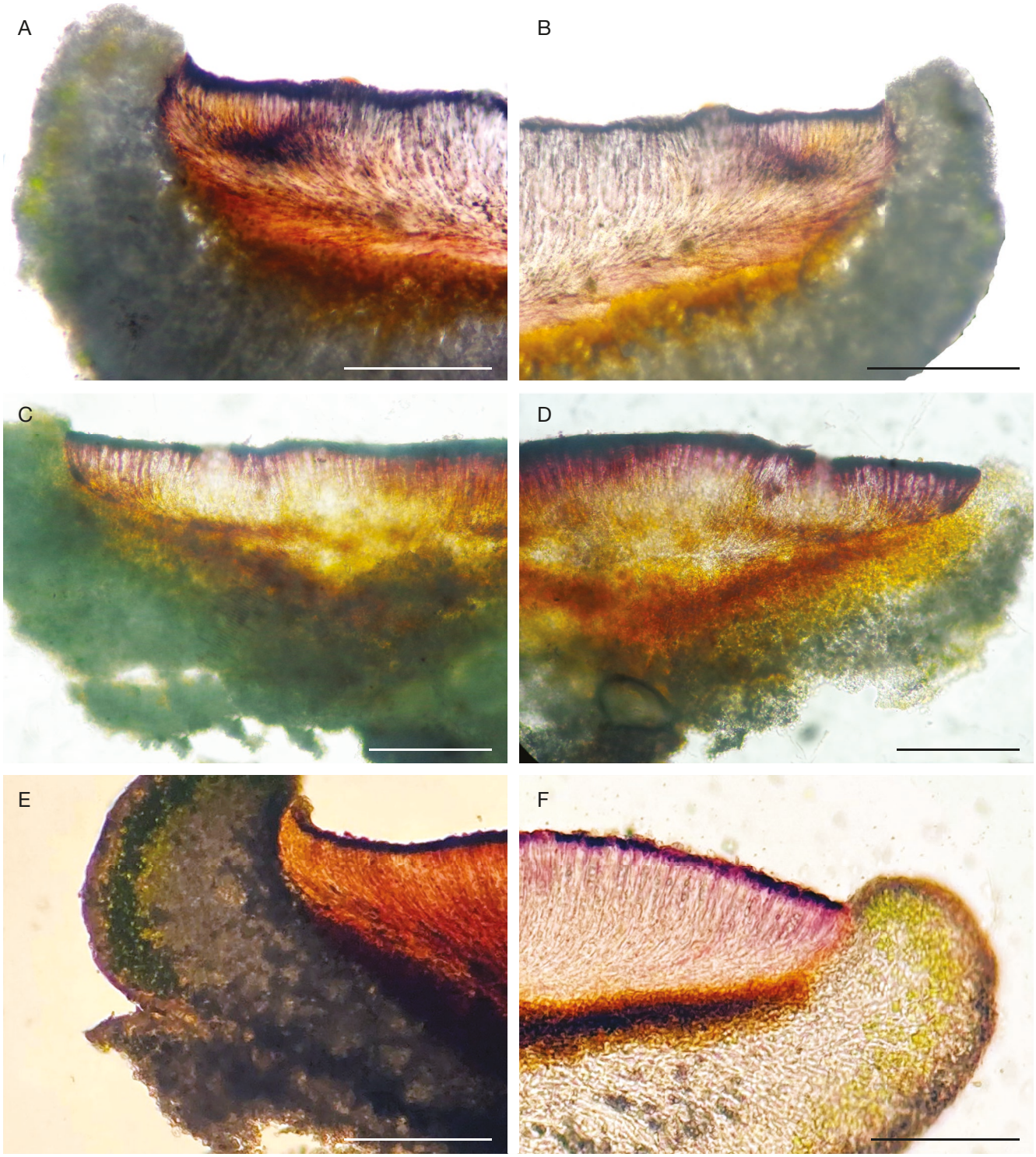


FIG. 4. — Section ascomata: **A**, **B**, *Tephromela obesimarginata* L.A.Santos, Aptroot & M.Cáceres, sp. nov. (holotype), before and after KOH treatment; **C**, **D**, *Tephromela vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov. (holotype), before and after KOH treatment; **E**, **F**, *Tephromela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

with similar colorations as the hymenium. Asci slightly clavate, 65-55 µm tall, 8-spored. Ascospores simple, hyaline, ellipsoid, 9-13 × 5-6 µm, wall 1-2 µm 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.

**Acknowledgements**
The authors thank Conselho Nacional de Pesquisa e Desenvolvimento (CNPQ) for financially supporting this work with a PhD's scholarship to LAS, a research grant to MESC (307569/2019-5), financial support for the field trips (process: 437097/2018-8), and a collaborative project with RL (PVE 314570/2014-4). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 with a Visiting Professor grant to AA. The curator of TUR is thanked

## 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 α-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 α-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; epiphymenium bluish; hymenium hyaline or mottled bluish and brownish ..... 6

6. Subhymenium reddish brown; epiphymenium and hymenium mottled bluish and brownish .....
..... *T. carassensis* (Vain.) Aptroot & Lücking, comb. nov.
— Subhymenium dark orangish brown; epiphymenium 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 × 6-8 µm; medulla UV+ bluish-green (α-collatolic acid) .....
..... *T. buelliana* (Müll. Arg.) Kalb
— Ascospores 10-11 × 6-7 µm; medulla UV- (colensoic acid) .....
..... *T. colensoica* Rambold & Knoph

9. Ascospores 7-10 × 3.5-4.5 µm; medulla UV+ white (loxodellonic and glomelliferic acids) .....
..... *T. matogrossensis* Kalb & Elix
— Ascospores 10-12 × 8-9 µm; 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 × 5-7.5 µm ..... 16

12. Medulla UV- (no substances in medulla); ascospores long ellipsoid, 10-12 × 3-4 µm .....
..... *T. americana* (Fée) Kalb
— Medulla UV+ greenish-white or UV+ bluish-green; ascospores ellipsoid, at least 5 µm wide ..... 13

13. Apothecial margin UV+ yellow (lichexanthone), medulla UV+ bluish green (α-collatolic acid); fatty acids absend; ascospores 14-16 × 5-6.5 µm ..... *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 × 5-6 µm; in mangroves ..... *T. rhizophorae* Kalb
— Ascospores 10-17 × 5-9 µm; in inland ecosystems ..... 15

15. Fatty acids present (TLC); ascospores 10-13 × 5-8 µm .....
..... *T. vinacea* L.A.Santos, Aptroot, Lücking & M.Cáceres, sp. nov.
— Fatty acids absent (TLC); ascospores 12-17 × 6-9 µm ..... *T. alectoronica* Kalb s.lat.

16. On Vellozia stems; ascospores 9-12 × 5-7 µm ..... *T. velloziae* Kalb
— On tree bark; ascospores 14-16 × 6.5-7.5 µm .....
..... *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.

for sending specimens of Vainio types on loan. We are thankful to the Laboratory of Mycorrhiza (UFPE), for helping with the logistics during the sampling expeditions, and to the laboratories of Genetics and Molecular Biotechnology (GMBIO) at Federal University of Sergipe (UFS) and Natural Products at Federal University of Pernambuco (UFPE) for yielding the structure and equipment to carry out DNA and secondary metabolite analysis, respectively. Thanks also to the Administration of Santuário do Caraça RPPN for the collecting permits and to the employees for helping during the field work. The reviewers are also acknowledged for their valuable contributions.

## REFERENCES

BINHUM. 2021.— Biodiversitätsnetzwerk des Humboldt-Rings. Available at https://www.binhum.net/ (accessed 13 April 2021).

CESTARO L., TØNSBERG T. & MUGGIA L. 2016. — Phylogenetic data and chemical traits characterize a new species in the lichen genus *Tephromela*. *Herzogia* 29 (2): 383-402. https://doi.org/10.13158/heia.29.2.2016.383

CHOISY M. 1929. — Genres nouveaux pour la lichénologie dans le groupe des Lécanoracées. *Bulletin de la Société botanique de France* 76 (3): 521-527. https://doi.org/10.1080/00378941.1929.10837179

ELIX J. A. 2012. — Four new species and a new record of *Tephromela* (lichenized Ascomycota) from Australia. *Australasian Lichenology* 71: 3-11.

ELIX J. A. 2013. — Further new species and new records of *Tephromela* (lichenized Ascomycota) from Australia. *Australasian Lichenology* 71: 20-31.

FRYDAY A. M. 2011. — New species and combinations in *Calvitimela* and *Tephromela* from the southern subpolar region. *Lichenologist* 43 (3): 225-239. https://doi.org/10.1017/S0024282911000065

FRYDAY A. M. 2019. — Eleven new species of crustose lichenized fungi from the Falkland Islands (Islas Malvinas). *Lichenologist* 51 (3): 235-267. https://doi.org/10.1017/S0024282919000185

GARDES M. & BRUNS T. D. 1993. — ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2 (2): 113-118. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x

HAFELLNER J. 1984. — Studien in Richtung einer natürlicheren Gliederung der Sammelfamilien Lecanoraceae und Lecideaceae. *Beiheft zur Nova Hedwigia* 79: 241-371.

HALL T. A. 1999. — BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

HUNECK S. & YOSHIMURA I. 1996. — *Identification of Lichen Substances*. Springer, Berlin, Heidelberg, 493 p. https://doi.org/10.1007/978-3-642-85243-5_2

KALB K. 1983. — *Lichenes Neotropici ausgegeben von Klaus Kalb*. Fasc. VII (nos 251-300). Published by the author, Neumarkt/Opf.

KALB K. 1984. — *Lichenes Neotropici ausgegeben von Klaus Kalb*. Fasc. VIII (nos 301-350). Published by the author, Neumarkt/Opf.

KALB K. 2008. — New or otherwise interesting Lichens. IV. *Sauteria* 15: 239-248.

KANTVILAS G. 2015. — Observations on the genus *Tephromela* (lichenised Ascomycetes) in Tasmania, with the description of a new species. *Herzogia* 28: 430-444. https://doi.org/10.13158/heia.28.2.2015.430

KANTVILAS G. & ELIX J. A. 2017. — *Tephromela baudiniana* sp. nov. (lichenised Ascomycetes) from Kangaroo Island. *Swainsona* 31: 27-30.

KATOH K., ASIMENOS G. & TOH H. 2009. — Multiple alignment of DNA sequences with MAFFT. *Methods in Molecular Biology* 537: 39-64. https://doi.org/10.1007/978-1-59745-251-9_3

KATOH S. 2013. — MAFFT: Multiple sequence alignment software version 7: improvements in performance and usability (outlines version 7). *Molecular Biology and Evolution* 30 (4): 772-780. https://doi.org/10.1093/molbev/mst010

LARENA I., SALAZAR O., GONZÁLEZ V., JULIÁN M. C. & RUBIO V. 1999. — Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. *Journal of Biotechnology* 75 (2-3): 187-194. https://doi.org/10.1016/s0168-1656(99)00154-6

LÜCKING R., HODKINSON B. P. & LEAVITT S. D. 2017. — The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist* 119 (4): 361-416. https://doi.org/10.1639/0007-2745-119.4.361

MUGGIA L., PEREZ-ORTEGA S., FRYDAY A., SPRIBILLE T. & GRUBE M. 2014. — Global assessment of genetic variation and phenotypic plasticity in the lichen-forming species *Tephromela atra*. *Fungal Diversity* 64: 233-251. https://doi.org/10.1007/s13225-013-0271-4

ORANGE A., JAMES P. W. & WHITE F. J. 2001. — *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London, 101 p.

SPRIBILLE T., GOFFINET B., KLUG B., MUGGIA L., OBERMAYER W. & MAYRHOFER H. 2011. — Molecular support for the recognition of the *Mycoblastus fucatus* group as the new genus *Violella* (Tephromelataceae, Lecanorales). *The Lichenologist* 43 (5): 445-466. https://doi.org/10.1017/S0024282911000478

STAMATAKIS A. 2006. — RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22 (21): 2688-2690. https://doi.org/10.1093/bioinformatics/btl446

VAINIO E. A. 1890. — Étude sur la classification et la morphologie des lichens du Brésil. I. *Acta Societatis pro Fauna et Flora Fennica* 7 (1): 1-247.

WHITE F. J. & JAMES P. W. 1985. — *A New Guide to Microchemical Techniques for the Identification of Lichen Substances*. British Lichen Society, London, 41 p.

ZHAO X., LEAVITT S. D., ZHAO Z. T., ZHANG L. L., ARUP U., GRUBE M., PÉREZ-ORTEGA S., PRINTZEN C., ŚLIWA L., KRAICHAK E., DIVAKAR P. K., CRESPO A. & LUMBSCH H. T. 2016. — Towards a revised generic classification of lecanoroid lichens (Lecanoraceae, Ascomycota) based on molecular, morphological and chemical evidence. *Fungal Diversity* 78: 293-304. https://doi.org/10.1007/s13225-015-0354-5

*Submitted on 21 February 2022;*
*accepted on 29 November 2022;*
*published on 22 February 2023.*

## 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