Ericboehmia, a new genus segregated from *Ostreichnion* in the *Hysteriaceae*, with the new species *E. saulensis*

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Abstract: A hysteriaceous dothideomycete with conchate ascomata with brittle wall was collected three times in French Guiana on dead corticated twigs of *Caesalpinia pulcherrima*. Examination of morphological characters and phylogenetic analysis of LSU sequences showed its affinities with the genus *Ostreichnion* and particularly with *O. curtisii*, with which it shares an ascospore morphology strongly deviating from that of *O. sassafras*, the type species. Our morphological and molecular results led us to the segregation of the new genus *Ericboehmia* to accommodate those species previously assigned to *Ostreichnion* featuring oblong didymospores. The new species *E. saulensis* is described and illustrated based on these three collections. It is shown to differ from *O. curtisii* by ascospore dimensions and septation and is proposed as type species. In addition, based on our results, six new combinations are proposed to accommodate in *Ericboehmia* some species recently added to *Ostreichnion* or *Hysterium*. A dichotomous key to the species accepted in *Ericboehmia* is proposed.
Keywords: Ascomycota, *Dothideomycetes*, French Guiana, *Hysteriales*, *Mytilinidiaceae*, ribosonomal DNA, taxonomy.

Résumé : une hystériale (*Dothideomycetes*) aux ascomes en forme de bivalve, à paroi fine et fragile, a été récoltée en Guyane française par trois fois sur des brindilles mortes et cortiquées de *Caesalpinia pulcherrima*. Son étude morphologique et l'analyse phylogénétique à partir de séquences LSU ont montré ses affinités avec le genre *Ostreichnion* et en particulier avec *O. curtisii* avec lequel elle partage une morphologie sporale très différente de celle de *O. sassafras*, l'espèce type. Nos résultats morphologiques et moléculaires nous ont conduits à la ségrégation du nouveau genre *Ericboehmia* pour y placer les espèces précédemment attribuées à *Ostreichnion* présentant des didymospores oblongues. L'espèce nouvelle *E. saulensis* est décrite et illustrée à partir de ces trois récoltes. Il est montré qu'elle diffère de *O. curtisii* par les dimensions et la septation des ascospores et elle est proposée comme espèce-type. De plus, nos résultats nous amènent à proposer six nouvelles combinaisons dans *Ericboehmia* pour des espèces récemment ajoutées à *Ostreichnion* et à *Hysterium*. Une clé dichotomique des espèces acceptées dans *Ericboehmia* est proposée.
Mots-clés : ADN ribosomal, Ascomycota, *Dothideomycetes*, Guyane française, *Hysteriales*, *Mytilinidiaceae*, taxinomie.

Introduction

During an inventorial survey of fungi in the vicinity of the village of Saül, set up by the Parc National Amazonien de Guyane in August 2018, a conchate dothideomycetous fungus was collected on dead corticated twigs of *Caesalpinia pulcherrima* (L.) Sw. Given its ascomatal shape, it was first reminiscent of the *Mytilinidiaceae*, a family mostly accommodating genera with conchate ascomata (Barr, 1975; Boehm *et al.*., 2009a), but macro- and microscopic examination strongly suggested affinities with some species placed in *Ostreichnion* Duby (*Hysteriaceae*) as defined by Barr (1975). This genus was thereafter shown to be polyphyletic by Boehm *et al.*. (2009a; 2009b), who likewise underlined that the two species submitted to phylogenetic comparison were different in ascospore morphology. However, by lack of molecular data on the few other species placed in *Ostreichnion*, the status of this genus remained unresolved.

We present here our results based on the morphological and phylogenetic characteristics of these collections and the comparison with ascospore morphology of all known species and LSU sequences of two previously known and two newly added species. As a strong correlation between ascospore morphology and phylogenetic affinities supports the polyphyly of *Ostreichnion* pointed out by Boehm *et al.*. (2009a; 2009b), the segregation of a new genus and the transfer in this genus of several species recently added to *Ostreichnion* and *Hysterium* Pers. appears justified. Therefore, we introduce herein in the *Hysteriaceae* the new genus *Ericboehmia* with *E. saulensis* sp. nov. as type-species and six new combinations in this genus.

Material and methods

Microscopical observations and measurements were made in water, aqueous chlorazol black and Melzer's reagent were used to investigate ascus and hamathecium morphology. The holotype specimen and paratypes were deposited in LIP herbarium (University of Lille). DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain): Total DNA was extracted from dry specimens blending a portion of them using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65ºC. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers LR0R and LR5 (Vilgalys & Hester, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95 ºC for 5 min, followed by 35 cycles at 94 ºC, 54 ºC and 72 ºC (45, 30 and 45 s respectively) and a final 72 ºC step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected. Analyses were performed online at http://phylogeny.lirmm.fr/phylo_cgi/index.cgi (Dereeper *et al.*., 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (Anisimova & Gascuel, 2006). Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Ericboehmia Gardiennet, Lechat & J. Fourn., *gen. nov.* – MycoBank MB832650

Diagnosis: Morphologically similar to *Ostreichnion* but different in having two-celled ascospores with sometimes secondary, more or less developed distosepta and in being phylogenetically distantly related.

Type species: *Ericboehmia saulensis* Gardiennet, Lechat & J. Fourn.

Etymology: In honour of Dr. Eric Boehm, for his contribution to the knowledge of *Hysteriales* and *Mytilinidiales*.

Ericboehmia saulensis Gardiennet, Lechat & J. Fourn., *sp. nov.* – MycoBank MB832651 – Fig. 2–4

Diagnosis: Differs from *Ericboehmia* species accepted herein in having ascospores 85–113 µm with 6–7 secondary distosepta and occasionally polar appendages.

Holotype: French Guiana, Saül, 3°37'20 N, 53°12'35 W, on dead corticated twigs of *Caesalpinia pulcherrima* (*Caesalpiniaceae*), 22 Aug. 2018, *leg*. A. Gardiennet AG18089 (LIP 0001658); GenBank LSU sequence MN338581.

Etymology: the epithet *saulensis* refers to Saül, the locality where this fungus was collected.

Ascomata superficial, scattered, erumpent through the bark, conchate, laterally flattened with a convex upper ridge, narrowing at base, black, matt to slightly shiny, 400–650 µm long, 270–360 µm wide, 300–360 µm high (n=20), smooth to very slightly striate along the length, with a longitudinal slit on upper ridge. **Peridium** carbonaceous, brittle, 30–70 µm thick, laterally up to 90 µm thick, narrowing upwards, composed of small sclerenchymatous cells. **Periphyses** numerous, less than 1 µm wide. **Hamathecium** trabeculate, consisting of numerous pseudoparaphyses at least as long as the asci, sometimes ramified and twisted, 0.8–1.6 µm wide, embed-

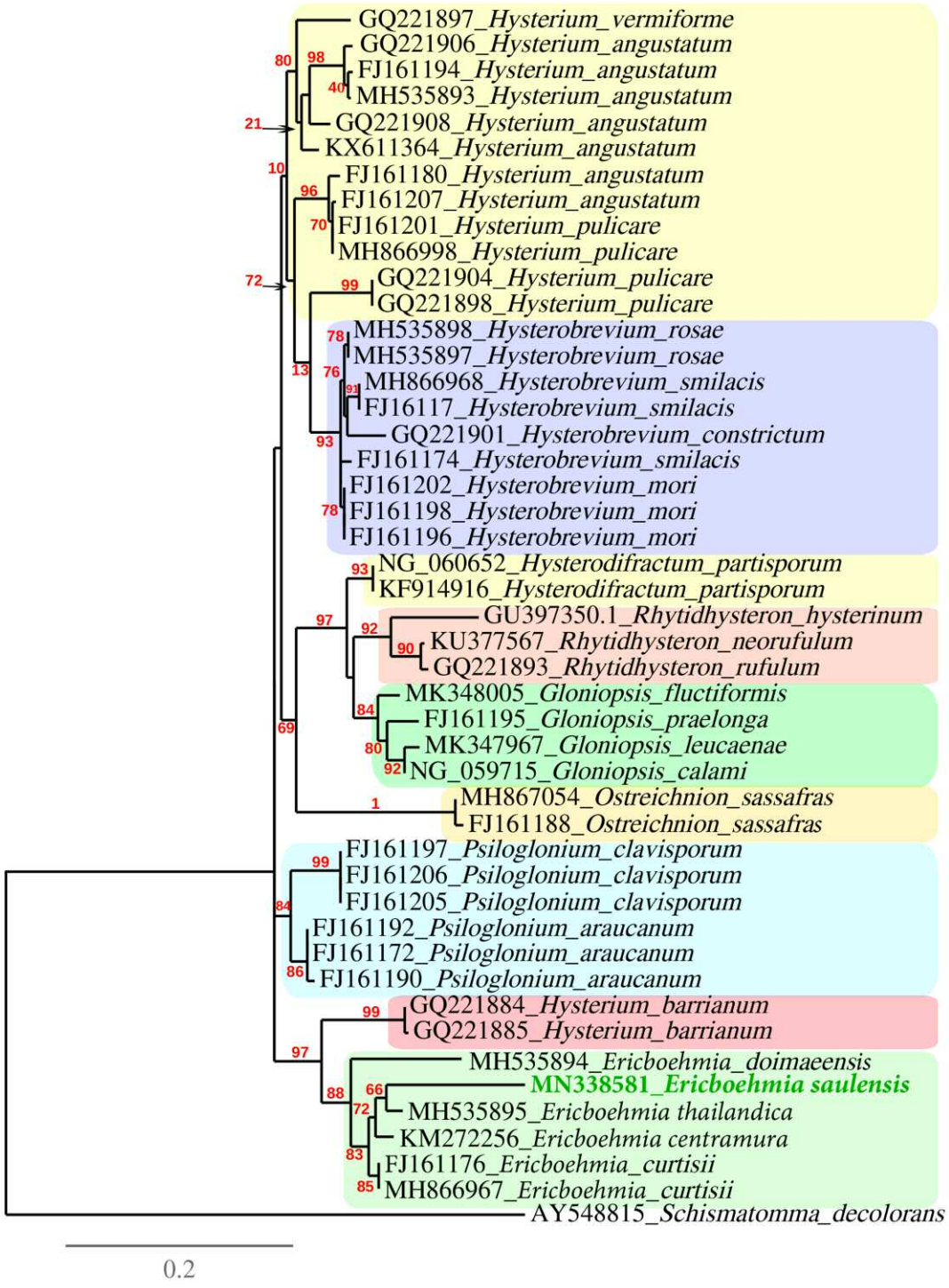

Fig. 1 – Maximum likelihood phylogeny (-lnL =3468.67621) of *Ericboehmia saulensis* within *Hysteriaceae*, inferred by PhyML 3.0, model HKY85 from a 955 bp matrix of LSU sequences, rooted with *Schismatomma decolorans*.

ded in a gel matrix. **Asci** bitunicate, oblong to clavate, shortly stipitate, with an ocular chamber, 225–305 × 38–58 µm, containing eight biseriate to multiseriate ascospores. **Ascospores** 85–113 × 17–23 µm (n=50), l/w = 4.7–5.7, hyaline, then slightly pale brown to light yellow, slightly fusiform when young, becoming oblong, smooth-walled, first 1-septate, strongly constricted at this primary median to submedian septum, with 6–7 secondary distosepta, rarely with a thin longitudinal pseudoseptum, occasionally showing polar appendages at maturity; when observed in India ink, ascospores may show mucilaginous remnants just after being released from the ascus but lack a well-defined sheath.

Asexual morph: unknown.

Fig. 2 – a-e: *Ericboehmia saulensis* (Holotype AG18089). a-e ascomata on natural substrate. Scale bars: a-b = 1 mm; c-e = 500 µm.

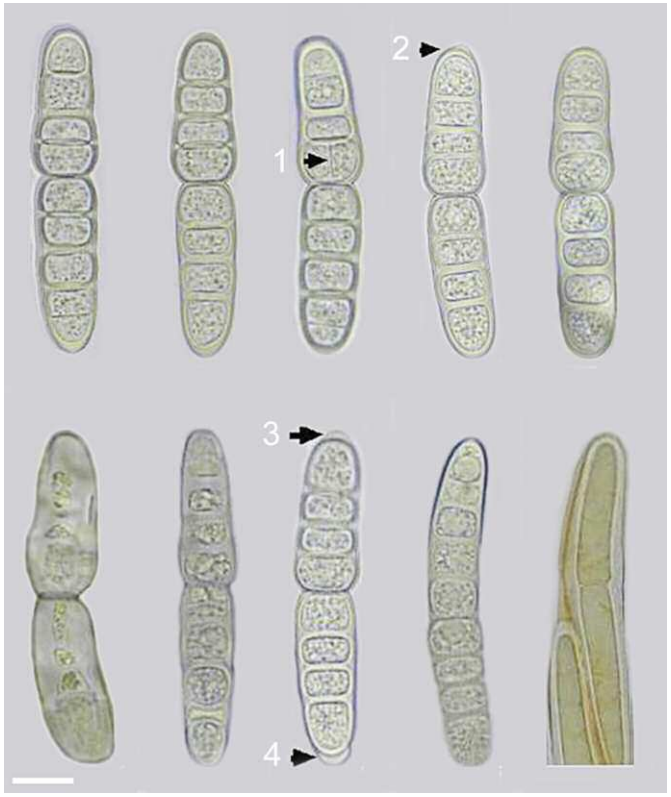

Fig. 3 – *Ericboehmia saulensis* (Holotype AG18089): ascospores all observed in water except the last one in Melzer's reagent. Arrows showing longitudinal septum (1) and appendages (2,3,4). Scale bar: 20 µm.

Discussion

To understand our concept of the genus *Ericboehmia*, it is necessary to review the history of *Ostreichnion*, which was well documented by Boehm *et al.*. (2009a; 2009b). At that time the three species placed in *Ostreichnion*, namely *O. sassafras* (Schwein.) M.E. Barr (the type-species), *O. curtisii* (Duby) M.E. Barr and *O. nova-caesariense* (Ellis) M.E. Barr were supposed to belong to *Mytilinidiaceae* because of their conchate ascomata with brittle wall. The multigene phylogeny of *Hysteriales* carried out by Boehm *et al.*. (2009a; 2009b) unexpectedly showed that *Ostreichion* was a member of *Hysteriaceae*, which was strongly counter-intuitive because of the deviating ascomatal morphology. Moreover, *O. curtisii*, characterized by 1-septate ascospores, was only distantly related to *O. sassafras*, a species with dictyoseptate ascospores, suggesting to these authors the conclusion that *Ostreichnion* was perhaps "artificial".

Several additions of new species with conchate ascomata fitting *Ostreichnion* and resembling *O. curtisii* in ascospore morphology recently occurred, broadening insight into this genus. Three of them were described from Thailand and placed in *Hysterium* based on phylogenetic grounds, namely *H. centramurum* Senan. (Tipbromma *et al.*., 2017), *H. doimaeensis* Jayasiri & K.D. Hyde and *H. thailandica* Jayasiri & K.D. Hyde (Jayasiri *et al.*., 2018). Based on strong phylogenetic affinities of these three species with *O. curtisii*, Jayasiri *et al.*. (2018) re-instated *O. curtisii* in *Hysterium* and proposed a key to these four supposed *Hysterium* species. In both phylograms published by Boehm *et al.*. (2009a; 2009b), *Ostreichnion* is in a sister clade to *Hysterium* but distant from *H. angustatum* Pers., the type species. This is even more obvious in our LSU-based phylogram (Fig. 1) where *O. sassafras* appears on an isolated branch basal to the clade representing the *Hysteriaceae* and the new species from Thailand clustering in our *Ericboehmia* clade along with *O. curtisii* and clearly distant from the *Hysterium* clade. The misplacement of the three species from Thailand in *Hysterium* by Tipbromma *et al.*. (2017) and Jayasiri *et al.*. (2018) is confirmed by morphological characters distinguishing both genera: hysterothecia with thick pseudoparenchymatous wall and hamathecium of cellular pseudoparaphyses for *Hysterium*, vs. conchate ascomata with brittle sclerenchymatous wall and trabeculate hamathecium for *Ericboehmia* and *Ostreichnion*. This is why we propose to exclude these species from *Hysterium* and to accommodate them in *Ericboehmia*. Ascospore septation in *E. centramura* is difficult to evaluate because of discrepancies between the description and the illustrations in the protologue (Tipbromma *et al.*., 2017). The cytoplasm was reported as "subdivided in numerous compartments in Melzer's and cotton blue, muriform when mature" while images show some rather narrow transverse well-defined septa, and do not show a muriform pattern of septation.

Two further additions were made based on morphological similarities with *Ostreichnion*, respectively *O. appendiculatum* R.M. Sánchez & Bianchin. from Argentina (Sánchez *et al.*., 2018) and *O. beejakoshae* M. Niranjan & V.V. Sarma from India (Niranjan & Sarma, 2018).

Sánchez *et al.*. (2018) introduced *O. appendiculatum*, justifying its placement in *Ostreichnion* and pointing out a greater similarity with *O. curtisii* as to ascospore morphology than with *O. sassafras* and *O. nova-caesariense*. We therefore interpret *O. appendiculatum* as a species of *Ericboehmia*, its appendaged ascospores being noteworthy; ascospores of *E. saulensis* may occasionally feature polar appendages that, in contrast, are not consistently conspicuous.

Niranjan & Sarma (2018) introduced *O. beejakoshae* from Indian Andaman islands, which appears to fit well our concept of *Ericboehmia* because of its conchate ascomata, hamathecium composed of thin pseudoparaphyses and long cylindrical light brown to grey ascospores with a strongly constricted median septum. Its medium-sized ascospores with strongly thickened apical wall and lacking secondary septa or cytoplasmic subdivisions set it apart from other known species. Morphological similarities of these two species with our concept of *Ericboehmia* strongly suggest that they can be accepted in the new genus, pending molecular support in future.

The taxonomic status of *H. barrianum* E. Boehm, A.N. Mill., Mugambi, Huhndorf & C.L. Schoch compared to that of *Ericboehmia* must be discussed because it appears close to *O. curtisii* in the phylogram published by Boehm *et al.*. (2009b) and close to *Ericboehmia* in our phylogram (Fig. 1). This North American species differs from a typical *Hysterium* by unusually high and laterally compressed hysterothecia and differs from *Ericboehmia* by cellular pseudoparaphyses and narrowly fusiform, curved, thin-walled phragmospores. We therefore do not include it in *Ericboehmia* and we suggest it might deserve to be accommodated in a different genus.

As a result, only two species with dictyoseptate ascospores are retained herein in *Ostreichnion*: *O. sassafras* and *O. nova-caesariense*. Finally, the new genus *Ericboehmia* currently includes seven species and is probably widespread.

In contrast with *Ostreichnion s. str.* having dictyoseptate ascospores, we have defined the genus *Ericboehmia* by its species consistently having phragmospores. All species of *Ericboehmia* feature first a primary septum and it should be noted that the spores are conspicuously constricted at this level. In all species, with the possible exceptions of *E. beejakoshae* and *E. appendiculata*, secondary septa then develop upon maturation; they remain rudimentary or few in most species but when fully developed, as in *E. saulensis*, they have the characteristics of distosepta. We consider that the occasional presence of a thin, hyaline longitudinal additional septum in some ascospores of *E. curtisii* and *E. saulensis* does not justify to term them "dictyoseptate" or "muriform", in comparison with those typically encountered in *Ostreichnion*.

The pattern of septation and the type of secondary septa thus appear as the key features separating *Ericboehmia* from *Ostreichnion* but they may vary during maturation and their interpretation remains in some cases arguable. Ascospores of species formerly

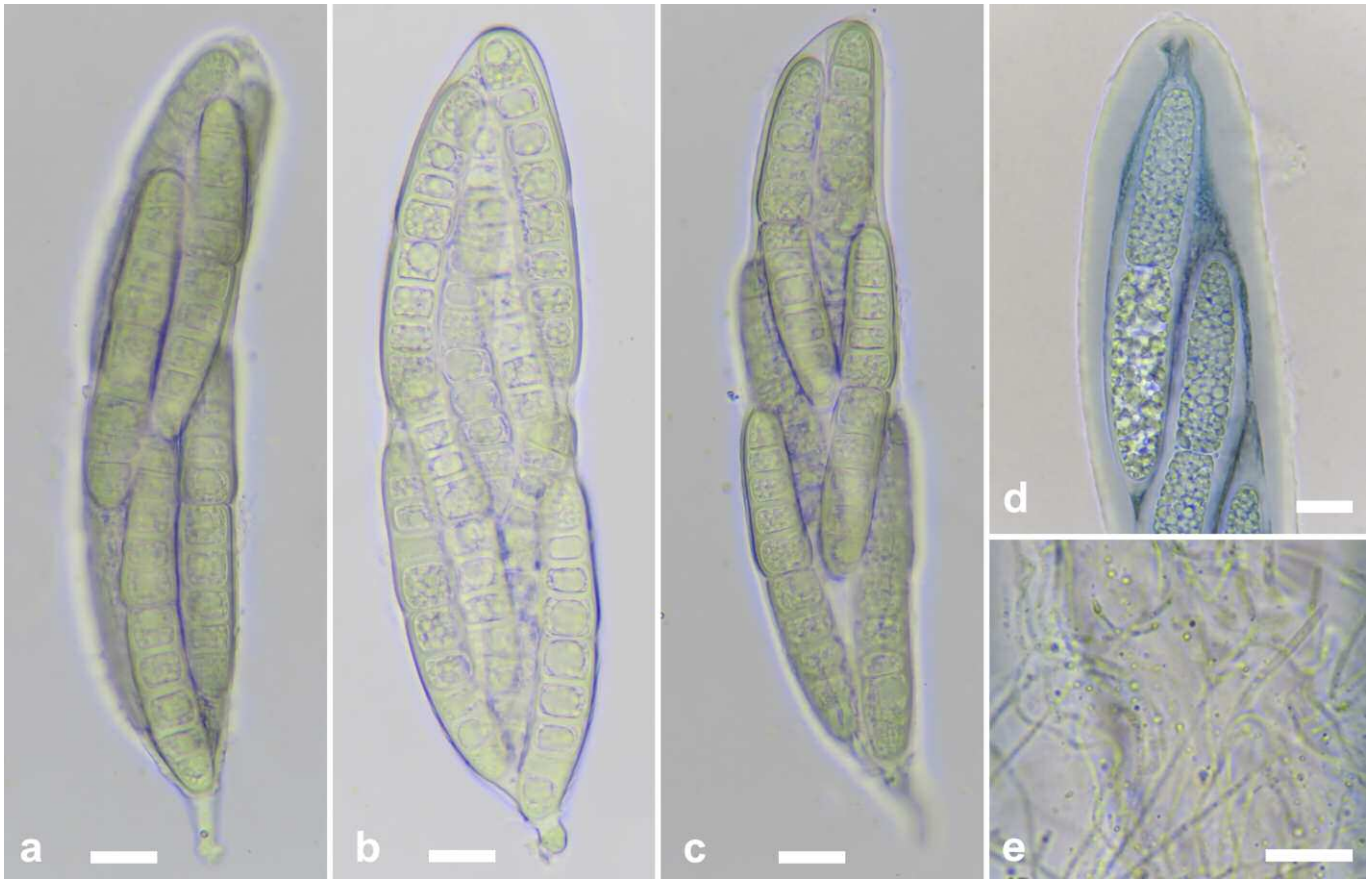

Fig. 4 – a-e: *Ericboehmia saulensis* (Holotype AG18089). a-b: asci in water; c: ascus in chlorazol black after 1 min; d: immature ascus in chlorazol black after 10 min; e: pseudoparaphyses in chlorazol black after 5 min. Scale bars: a-d = 20 µm; e = 10 µm.

placed in *Ostreichnion* and their septation have been well illustrated by Sánchez *et al.*. (2018).

Taxonomic novelties

Ericboehmia saulensis Gardiennet, Lechat & J. Fourn., *sp. nov.* – MycoBank MB832651

Ericboehmia appendiculata (R.M. Sánchez & Bianchin.) Gardiennet, Lechat & J. Fourn., *comb. nov.* – MycoBank MB832652

Basionym: *Ostreichnion appendiculatum* R.M. Sánchez & Bianchin., *Darwiniana, n.s.*, 6 (1): 49 (2018) – MycoBank MB822921.

Ericboehmia beejakoshae (M. Niranjan and V.V. Sarma) Gardiennet, Lechat & J. Fourn., *comb. nov*. – MycoBank MB832653

Basionym: *Ostreichnion beejakoshae* M. Niranjan & V.V. Sarma, *Kavaka*, 50: 91 (2018) – MycoBank MB822688

Ericboehmia centramura (Senan.) Gardiennet, Lechat & J. Fourn., *comb. nov*. – MycoBank MB832654

Basionym: *Hysterium centramurum* Senan., *Fungal Div*., 83: 22 (2017) – MycoBank MB552708.

Ericboehmia curtisii (Duby) Gardiennet, Lechat & J. Fourn., *comb. nov.* – MycoBank MB832655

Basionym: *Hysterium curtisii* Duby, *Mém. Soc. Physique Hist. nat. Genève*, 16 (1): 42 (1862) – MycoBank MB319045.

= *Gloniella curtisii* (Duby) Sacc., *Syll. Fung*., 2: 766 (1883); *Glonium curtisii* (Duby) M.L. Lohman, *Bull. Torrey Bot. Club*, 64: 66 (1937); *Hysteroglonium curtisii* (Duby) Earle, *Bull. Alabama Agri. Exp. Station*, 64: 163 (1937) ; *Ostreichnion curtisii* (Duby) M.E. Barr, *Mycotaxon*, 3 (1): 86 (1975).

Ericboehmia doimaeensis (Jayasiri & K.D. Hyde) Gardiennet, Lechat & J. Fourn., *comb. nov.* – MycoBank MB832656

Basionym: *Hysterium doimaeensis* Jayasiri & K.D. Hyde, *Mycosphere*, 9 (4): 815 (2018) – MycoBank MB554456.

Ericboehmia thailandica (Jayasiri & K.D. Hyde) Gardiennet, Lechat & J. Fourn., *comb. nov*. – MycoBank MB832657

Basionym: *Hysterium thailandica* Jayasiri & K.D. Hyde, *Mycosphere*, 9 (4): 817 (2018) – MycoBank MB554455.

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Dichotomous key to species of *Ericboehmia*

1. Ascospores 85–130 µm long **2**
1. Ascospores shorter, less than 80 µm long **3**
2. Ascomata 0.4–0.5 mm long; ascospores 85–113 × 17–23 µm, occasionally appendaged, lacking a hyaline sheath ..... ***E. saulensis***
2. Ascomata 0.9–1.8 mm long; ascospores 110–130 × 20–25 µm lacking appendages but with a hyaline sheath ..... ***E. centramura***
3. Ascospore length less than 60 µm **4**
3. Ascospore length more than 60 µm **5**
4. Ascospores with secondary septa at maturity; on average less than 11 µm wide ..... ***E. thailandica***
4. Ascospores without secondary septa at maturity; on average more than 11 µm wide ..... ***E. beejakoshae***
5. Ascospores with appendages and more than 15 µm wide ..... ***E. appendiculata***
5. Ascospore without appendages and less than 15 µm wide **6**
6. Ascospores with obscure, up to 7 secondary septa and occasionally with a thin colourless longitudinal septum in one cell ..... ***E. curtisii***
6. Ascospores with up to 2 secondary septa and lacking longitudinal septa ..... ***E. doimaeensis***

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