



**New molecular and morphological evidences favor a combination of *Blechnum bakeri* C.Ch. in *Cranfillia* Gasper & V.A.O.Dittrich (Blechnaceae, Polypodiopsida), thus extending the distribution of *Cranfillia* to Madagascar and East Africa**

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*Here we remember and record our gratitude for the life of Jose María Gabriel y Galán, botany mentor and promoter of research, who left us in the process of this work. He will never stop accompanying us every step of the way as we try to advance our various projects.*

Rubén Vázquez & Sonia Molino

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## ABSTRACT

In the fern family Blechnaceae, *Cranfillia* Gasper & V.A.O.Dittrich and *Austroblechnum* Gasper & V.A.O.Dittrich are two genera recently described from the splitting of *Blechnum* L. The assignation of species to *Cranfillia* or *Austroblechnum* could be difficult due to their morphological similarities. Focusing on *Austroblechnum bakeri* (C.Chr.) Gasper & V.A.O.Dittrich, and based on new molecular and morphological evidence, we show that *Austroblechnum bakeri* actually belongs to the genus *Cranfillia* as *Cranfillia bakeri* (C.Chr.) Vázquez Ferreira & S. Molino, comb. nov. This new combination extends the distribution of the genus *Cranfillia* to Madagascar and East Africa. Additionally, we propose that partially adnate basal pairs of pinnae and deflexed first pair of pinnae could represent new diagnostic characters to distinguish *Cranfillia* from *Austroblechnum*. Finally, we provide a taxonomic treatment of *Cranfillia bakeri* with all recognized synonyms, and conduct detailed lectotypification for *Blechnum bakeri* C.Chr.

## KEY WORDS

Pteridophytes,  
ferns,  
*Cranfillia*,  
*Austroblechnum*,  
Africa,  
Madagascar,  
spores,  
new combination,  
lectotypification.

## RÉSUMÉ

*De nouvelles données moléculaires et morphologiques soutiennent une combinaison de Blechnum bakeri C.Chr. dans Cranfillia Gasper & V.A.O.Dittrich, élargissant ainsi la répartition de Cranfillia à Madagascar et à l'Afrique de l'Est.*

Au sein de la famille de fougères Blechnaceae, *Cranfillia* Gasper & V.A.O.Dittrich et *Austroblechnum* Gasper & V.A.O.Dittrich sont deux genres récemment décrits à partir du fractionnement du genre *Blechnum* L. L'attribution des espèces au genre *Cranfillia* ou *Austroblechnum* peut être rendue difficile en raison de leurs similarités morphologiques. En nous focalisant sur *Austroblechnum bakeri* (C.Chr.) Gasper & V.A.O.Dittrich, et en se basant sur de nouvelles données moléculaires et morphologiques, nous montrons que *Austroblechnum bakeri* appartient en fait au genre *Cranfillia* en tant que *Cranfillia bakeri* (C.Chr.) Vázquez Ferreira & S. Molino, comb. nov. Cette nouvelle combinaison élargit la répartition du genre *Cranfillia* à Madagascar et à l'Afrique de l'Est. De plus, nous proposons que les paires de pennes basales partiellement adnées ainsi que les premières paires de pennes réfléchies pourraient représenter de nouveaux caractères diagnostiques discriminant *Cranfillia* de *Austroblechnum*. Enfin, nous apportons un traitement taxonomique de *Cranfillia bakeri* incluant tous les synonymes reconnus, et effectuons une lectotypification détaillée pour *Blechnum bakeri* C.Chr.

## MOTS CLÉS

Pteridophytes,  
fougères,  
*Cranfillia*,  
*Austroblechnum*,  
Afrique,  
Madagascar,  
spores,  
combinaison nouvelle,  
lectotypification.

## INTRODUCTION

Blechnaceae is a leptosporangiate fern family (eupolypods II, PPGI 2016) with a subcosmopolitan distribution, comprising 25 genera and about 250 species based on recent treatments and analyses (Gasper *et al.* 2016, Molino *et al.* 2019). *Cranfillia* Gasper & V.A.O.Dittrich occurs in the Neotropics and from Malesia to the Pacific area and was recently described from the splitting of the large genus *Blechnum* L., including 12 species (Gasper *et al.* 2016). New combinations and a recent review of the *Cranfillia* *vulcanica* (Blume) Gasper & V.A.O.Dittrich complex increased the species number to 21 (Dittrich *et al.* 2017; Chambers & Wilson, 2019; Lange & Parris, 2019). According to the last molecular phylogenetic studies (Perrie *et al.* 2014; Gasper *et al.* 2017; Bauret 2017), *Cranfillia* is a monophyletic genus, sister group to *Austroblechnum* Gasper & V.A.O.Dittrich and *Blechnum* s.s.

Diagnostic characters proposed for *Cranfillia* include: terrestrial habit, suberect or erect rhizomes, oblong entire and

concolorous scales which vary from brown to black, rachises and costae pilose, with uniseriate, septate, multicellular, black or hyaline trichomes, and dimorphic fronds (Gasper *et al.* 2016). Since many of these characters overlap those proposed for *Austroblechnum* (while *Blechnum* s.s. species are easily distinguished by their monomorphic fronds), the genus *Cranfillia* is morpho-anatomically poorly circumscribed. Moreover, species suspected to be closely related due to shared morpho-anatomical similarities are phylogenetically placed in both *Cranfillia* and *Austroblechnum*, like *Cranfillia fluvialis* (R.Br.) Gasper & V.A.O.Dittrich and *Austroblechnum membranaceum* (Colenso ex Hook.) Gasper & V.A.O.Dittrich (Chambers & Farrar 2001). One species has also been recombined from *Austroblechnum* to *Cranfillia* since the classification of Gasper *et al.* (2016; *Cranfillia nukuhivensis* (E.D.Br.) de Lange & Parris; Lange & Parris 2019).

*Blechnum bakeri* C.Chr. is a species occurring in Madagascar and East Africa, terrestrial or epilithic, in evergreen forests. The lectotype (first step) was designated by Schelpe

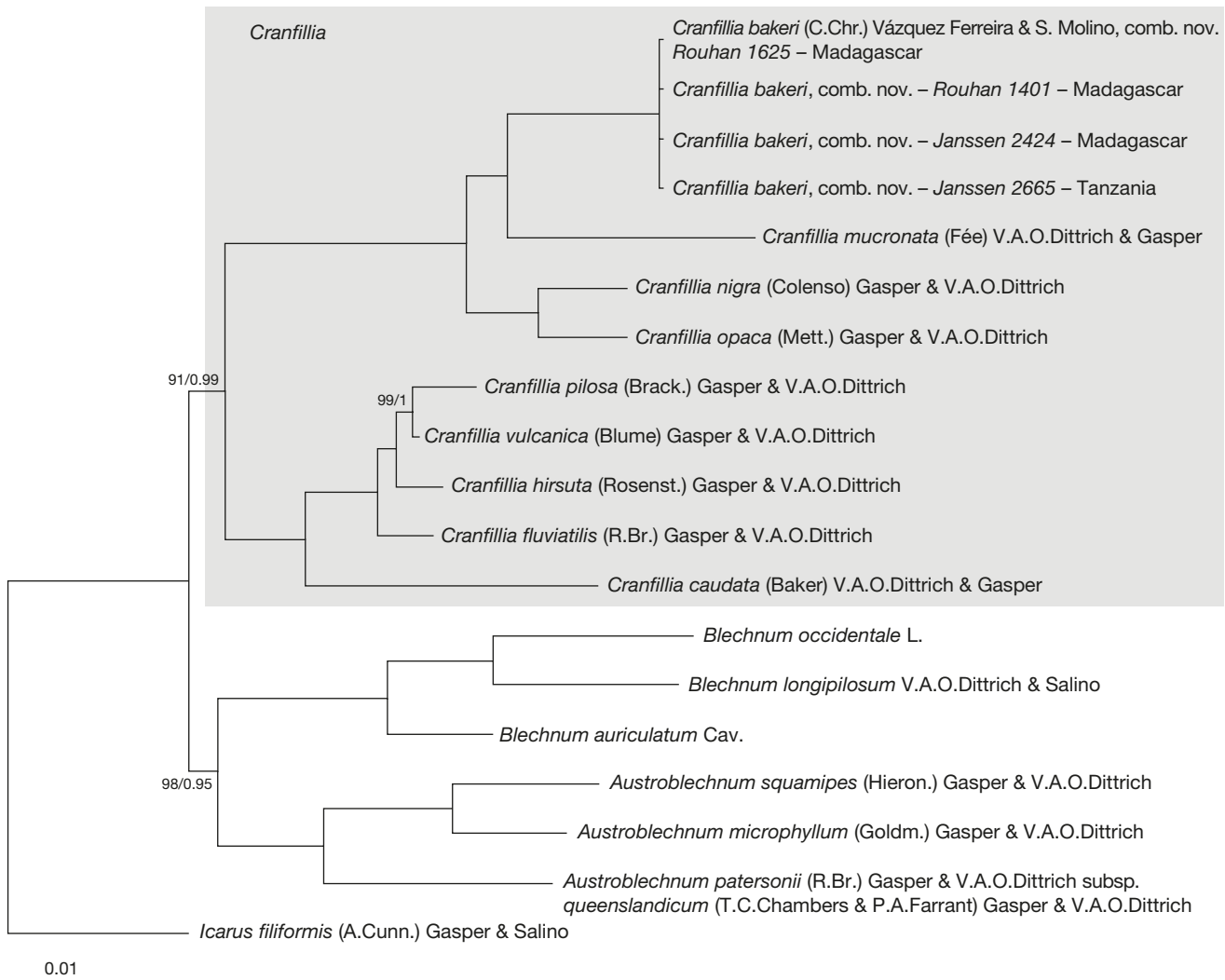


FIG. 1. — Majority rule consensus phylogenetic tree for the genus *Cranfillia* estimated by Bayesian inference on the combined plastid DNA dataset (*rbcl*, *rps4*, *rps4-trnS*, *trnL/trnL-trnF*), with support values from the Maximum likelihood method and Bayesian inference. Unless mentioned next to the nodes, support values are bootstraps (BS) = 100 and posterior probabilities (PP) = 1. Scale bar is for branch lengths of the phylogram (substitutions/site).

(1967) as two herbarium sheets, with mention that each sheet included a mixture of fronds of this species, *Blechnum ivohibense* C.Chr. and *Blechnum microbasis* (Baker) C.Chr. The only difference separating *B. ivohibense* from *B. bakeri* was that *B. ivohibense* was glabrous. Rakotondrainibe *et al.* (2013) later considered *B. ivohibense* as synonym of *B. bakeri* based on the variability of the hair density, with numerous intermediate forms from densely hairy to glabrous, and the overlapping distribution of the species that they observed. *Blechnum bakeri* was then combined into *Austroblechnum* by Gasper *et al.* (2016).

However, preliminary data from morphological observations (R. Vázquez, S. Molino) and molecular data (Bauret 2017) led us to consider *B. bakeri* as rather belonging to *Cranfillia*. Here, we present the molecular and morphological evidence supporting the phylogenetic placement of *Blechnum bakeri* in the genus *Cranfillia*, followed by its taxonomic treatment including the resulting new combination.

## MATERIAL AND METHODS

### TAXONOMIC SAMPLING, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

We included eight species of *Cranfillia*, with one specimen per species. *Blechnum bakeri*, hypothesized as being part of *Cranfillia*, was represented by four specimens from Madagascar and Tanzania (Appendix 1) in order to also assess the monophyly of this species. Seven species, representing the closest genera to *Cranfillia*, were included as outgroups: three species of *Blechnum s.s.*, three of *Austroblechnum*, and the monospecific genus *Icarus* Gasper & Salino. Sequences were obtained from previous studies (Shepherd *et al.* 2007; Perrie *et al.* 2014; Gasper *et al.* 2017), except those of *Blechnum bakeri* which were newly generated for the present study. We found unnecessary here to present the results in a broader phylogenetic context, as it was already done with the same samples in Bauret (2017).

We used the Qiagen DNeasy Plant Mini Kit (Valencia, CA, USA) for DNA extraction from silica-dried leaf mate-

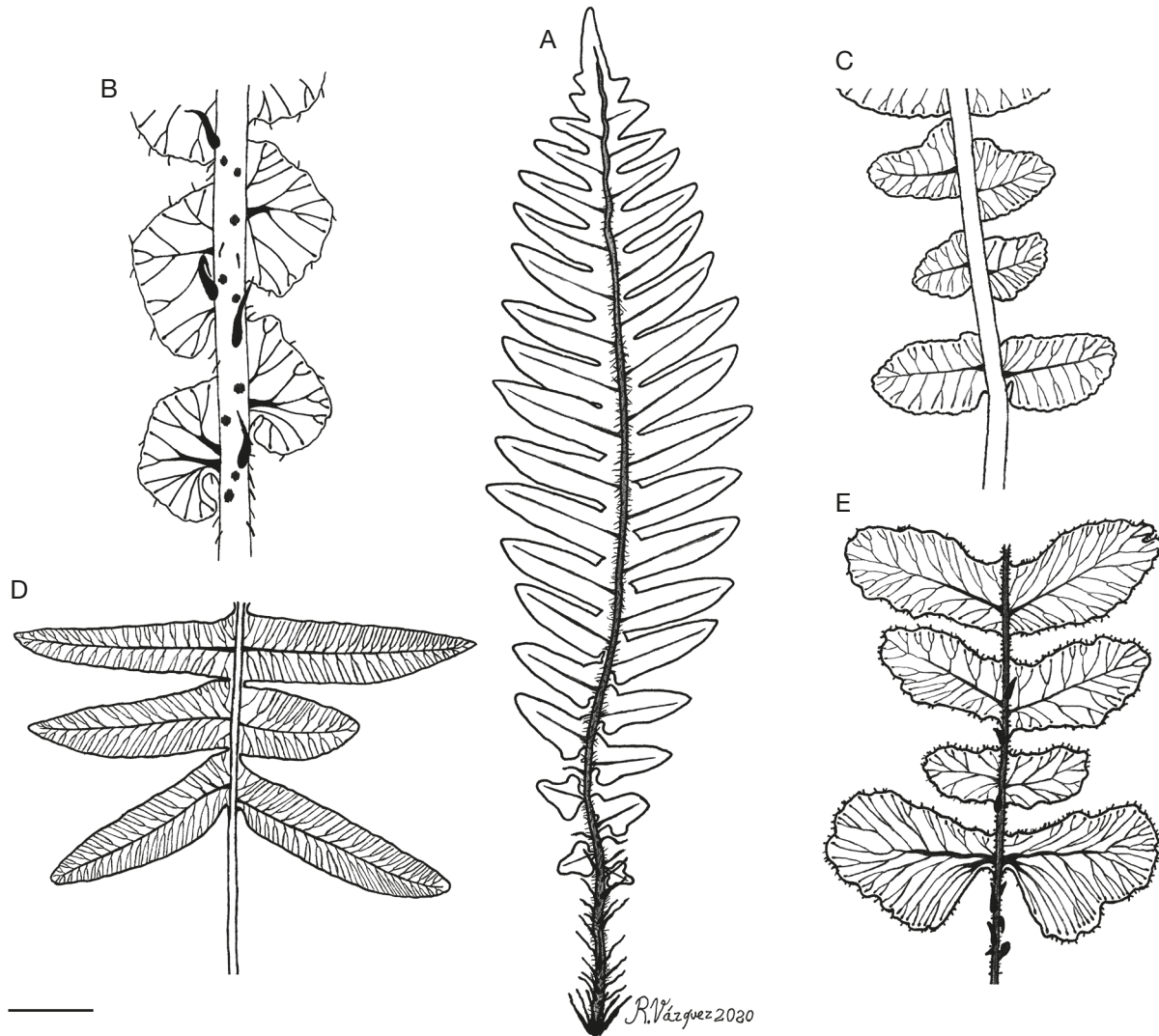


FIG. 2. — Morphology of sterile pinnae in *Cranfillia* species: **A**, Sterile frond of *C. fullagari* (K001092750); **B-E**, Basal pinnae morphology; **B**, *C. opaca* (US1431859); **C**, *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. (P00483198); **D**, *C. mucronata* (P01389538); **E**, *C. nigra* (K001092713). Scale bar: A, 28 mm; B, 6 mm; C, 10 mm; D, 17 mm; E, 7 mm.

rial. Four plastid DNA regions were amplified by PCR and sequenced: two genes, *rbcL* and *rps4*, and two non-coding regions, the intergenic spacer *rps4-trnS* and the *trnL* intron plus *trnL-trnF* intergenic spacer (*trnL-trnL-trnF*). All PCRs were carried out in a 25 µL volume containing 1x PCR buffer, 2.5 mM MgCl<sub>2</sub>, 250 µM of each dNTP, 1M betaine, 0.4 µM of each primer, 0.75 U Taq polymerase (Taq CORE kit; MP Biomedicals, Illkirch, France), and 1 µL of template, non-diluted genomic DNA.

For *rbcL* and *trnL-trnL-trnF*, primers and thermal conditions were the same as in Bauret *et al.* (2017). For *rps4* and *rps4-trnS*, that were amplified and sequenced together, we used the primers *rps4.5'* 5'-ATG TCS CGT TAY CGA GGA CCT-3' (Small *et al.* 2005) and *trnS-r* 5'-TAC CGA GGG TTC GAA TC-3' (Smith & Cranfill 2002) and the following thermal cycling conditions: 5 min 94°C / 5 × (30 s 94°C / 30 s 65-55°C / 1 min 30 s 72°C) / 35 × (30 s 94°C / 30 s 55°C / 1 min 30 s 72°C) / 10 min 72°C. For the first five

cycles, 65-55°C stands for a temperature that was decreased by 2.5° at each cycle. We checked the PCR products on a 1% agarose gel and they were sequenced in both directions by Eurofins (Evry, France), using the amplification primers and additional internal primers for *rbcL*. Sequencer 4.9 (Gene Codes Corporation, Ann Arbor, Michigan, USA) was used to edit and assemble the DNA strands. Genbank accession numbers of the newly obtained consensus sequences are available in Appendix 1.

## PHYLOGENETIC ANALYSES

We aligned the sequences with Muscle 3.8.425 (Edgar 2004), checked and revised them manually when necessary. Analyses were conducted independently for each DNA region, to detect conflicts between the topologies, and the four alignments were then concatenated using Sequence Matrix 1.7.8



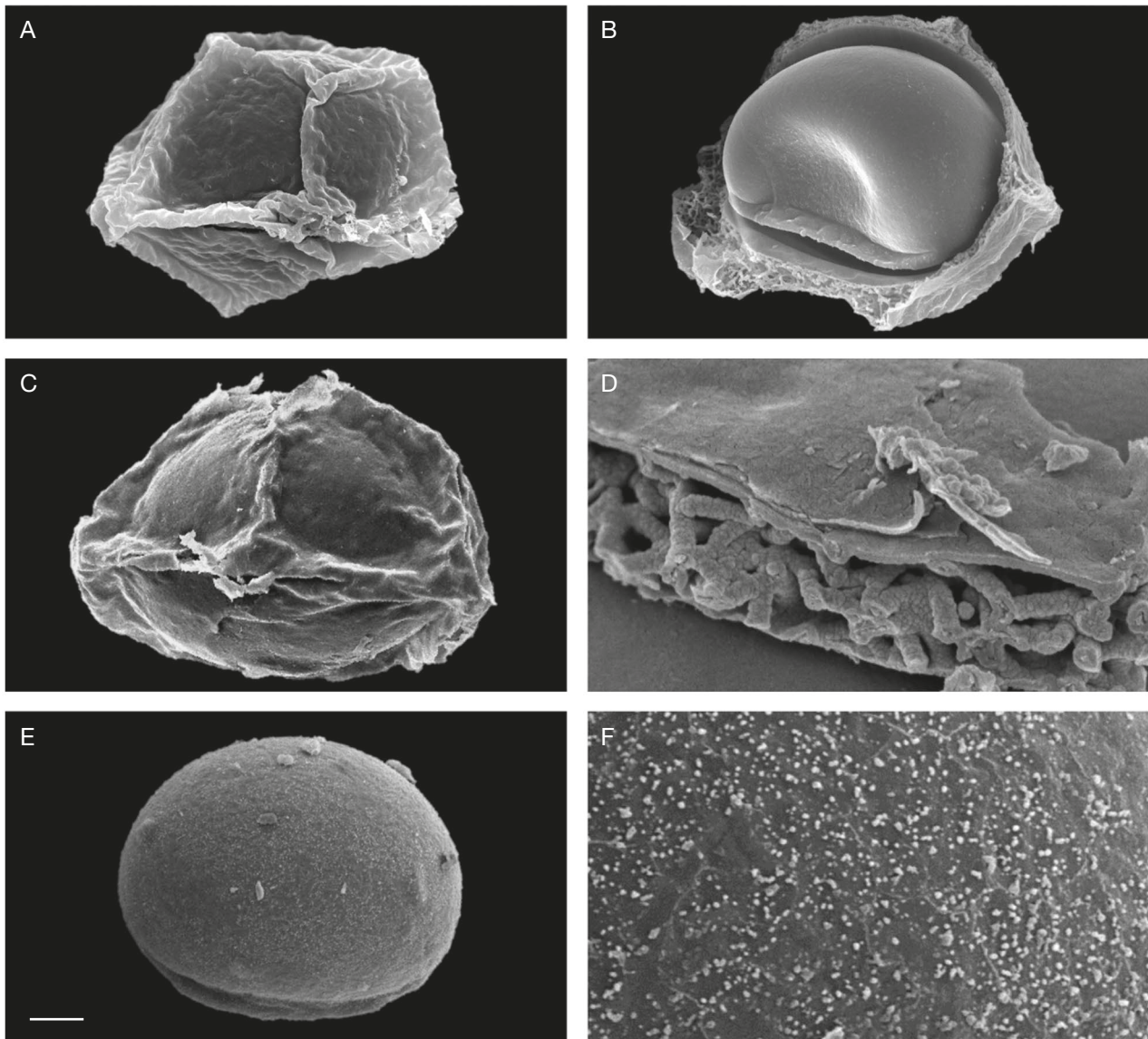


FIG. 3. — Spores of *Cranfillia* and *Austroblechnum* as observed with SEM: **A, B, C.** *bakeri* Vázquez Ferreira & S.Molino, comb. nov. (MA389177); **C, D.** *C. mucronata* (MA655870 & UC1615719, respectively); **E, F.** *A. lherminieri* (Bory ex Kunze) Gasper & V.A.O.Dittrich (BA57587). Thick and straight muri are visible in *Cranfillia*, as well as the spongy-trabecular middle layer of the perine (**B** and **D**). Smooth and micro-granulated perine ornamentation is observed in *A. lherminieri*. Scale bar: A, 17  $\mu$ m; B, 9  $\mu$ m; C, 7  $\mu$ m; D, 1  $\mu$ m; E, 8  $\mu$ m; F, 2  $\mu$ m.

(Vaidya *et al.* 2011). In this final dataset, we defined each region as an independent partition and gaps were treated as missing data.

Bayesian inference (BI) and Maximum likelihood (ML) approaches were used to infer the phylogenetic relationships, and conducted on CIPRES science gateway (Miller *et al.* 2010). We performed the BI using a Metropolis-coupled Markov chain Monte Carlo (MCMC) method implemented in MrBayes 3.2.6 (Ronquist *et al.* 2012). For each region, we identified the best model of nucleotide substitution by using a reversible jump MCMC (rjMCMC; Huelsenbeck *et al.* 2004). We allowed the rjMCMC to move across models with +I+ $\Gamma$ . The analysis was conducted with two independent runs of one million generations each, with four chains (one cold and three incrementally heated at a temperature

of 0.1) sampled every 100 generations, resulting in 10 000 sampled trees. The convergence of the two runs to the same stationary distribution was checked and the burn-in length was determined with Tracer 1.6.0 (Rambaut 2014). We discarded the first 2500 (25%) trees as burn-in and the 7500 remaining trees were pooled in a 50% majority-rule consensus in MrBayes.

The ML analysis was performed using RAxML-HPC2 8.2.6 (Stamatakis 2014), using the same four partitions by DNA regions as in the BI analysis, and with the GTRGAMMA+I model of nucleotide substitution. We performed 1000 rapid bootstrap (BS) replicates and searched for the best-scoring ML tree.

The BI 50% majority-rule consensus tree and ML best tree were visualized in FigTree 1.4.2 (Rambaut 2014).



TABLE 1. — Sequence summary statistics for the four plastid DNA regions and combined dataset.

| DNA region     | Number of sequences | Aligned length (in base pairs) | Percentage of variable characters | Percentage of informative characters |
|----------------|---------------------|--------------------------------|-----------------------------------|--------------------------------------|
| rbcl           | 19                  | 1252                           | 12.9                              | 7.3                                  |
| rps4           | 19                  | 570                            | 14.4                              | 8.4                                  |
| rps4-trnS      | 19                  | 438                            | 30.8                              | 17.8                                 |
| trnL+trnL-trnF | 19                  | 916                            | 28.6                              | 14.7                                 |
| Combined       | 19                  | 3176                           | 20.2                              | 11.1                                 |

TABLE 2. — Morphological and anatomical characters in *Cranfillia bakeri* Vázquez Ferreira & S.Molino, comb. nov., compared to close species.

| Characters                 | <i>Cranfillia bakeri</i> (C.Chr.) Vázquez Ferreira & S.Molino, comb. nov.                                               | <i>Cranfillia mucronata</i> (Fée) V.A.O.Dittrich & Gasper                                                      | <i>Cranfillia nigra</i> (Colenso) Gasper & V.A.O.Dittrich                 | <i>Cranfillia opaca</i> (Mett.) Gasper & V.A.O.Dittrich                        |
|----------------------------|-------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Rhizome scales             | Linear-lanceolate, entire, atropurpureous scales                                                                        | Identical to <i>C. bakeri</i> , comb. nov.                                                                     | Ovate-lanceolate, entire, light brown scales                              | Identical to <i>C. bakeri</i> , comb. nov.                                     |
| First basal pair of pinnae | Ovate pinnae with both acroscopic and basiscopic faces not completely adnate in all their width (sometimes almost free) | Linear-lanceolate pinnae with its basiscopic half from almost free to almost adnate; pinnae sometimes deflexed | Obcordate pinnae with both acroscopic and basiscopic faces almost free    | Vestigial semi-circular pinnae with its basiscopic half partially free         |
| Lamina margin              | Finely denticulate                                                                                                      | Identical to <i>C. bakeri</i> , comb. nov.                                                                     | Identical to <i>C. bakeri</i> , comb. nov.                                | Identical to <i>C. bakeri</i> , comb. nov.                                     |
| Trichomes                  | Short (0.4-1.0 mm), tuberculate uniseriate with 4-6 cells, and brown; some specimens glabrous                           | Almost glabrous, some individuals with sparse brown hairs (pers. obs.)                                         | Very short (0.3-0.5 mm), tuberculate uniseriate with 2-4 cells, and brown | Short (0.4-1.1 mm), tuberculate uniseriate with 3-8 cells, very atropurpureous |
| Perine ornamentation       | Thick straight muri which delimit areoles; trabecular middle layer observed                                             | Thick straight muri which delimit areoles; trabecular middle layer observed                                    | Slim and more or less curly muri which delimit areoles                    | Thick straight muri which delimit areoles                                      |

## MORPHOLOGICAL STUDY

Morpho-anatomical characters were observed with a Leica stereomicroscope (Stereozoom S9i model), and photographed and measured with the software Leica Application Suite version 4.12.0. Scales and trichomes were sampled from herbarium specimens, and rehydrated with a drop of water on a microscope slide for better observations. Micro-anatomical characters were observed with a Nikon microscope (Eclipse Ci-L model), and photographed and measured with the software NIS-Elements F version 4.00.00.

While most of the trichome cells were easily observed and counted, a treatment with diluted bleach was required for the dark opaque trichomes of *C. opaca*. To facilitate observation of cells at lamina margin, epidermal tissue was sampled by hand under the stereomicroscope. Then, extracted epidermal tissues were bleached, washed with pure water, and stained with toluidine blue for observation under microscope.

Spores for scanning electron microscope (SEM) were transferred with dissecting needles from herbarium specimens to metal stubs with double-sided tape, coated with gold/palladium under vacuum in a sputter-coater, and photographed with a Jeol/EO JSM 6360 (15 kV) SEM (Jeol Ltd, Madrid, Spain).

The list of the studied material is available in Appendix 2.

## RESULTS AND DISCUSSION

### MOLECULAR PHYLOGENY

We obtained a final dataset including 19 specimens and 3176 characters (see summary statistics in Table 1). Similar topologies were inferred by BI and ML analyses (Fig. 1), supporting the monophyly of *Cranfillia* (BS=91; PP=0.99). *Blechnum* and *Austroblechnum* were sister groups (BS=98; PP=0.95) and together formed the sister group to *Cranfillia* (BS=100; PP=1), confirming the previously recovered relationships (Perrie *et al.* 2014, Bauret 2017, Gasper *et al.* 2017). All four *Blechnum bakeri* specimens from Madagascar and East Africa were found in the same clade (BS=100; PP=1), and nested within *Cranfillia* as sister group to *Cranfillia mucronata* (Fée) V.A.O.Dittrich & Gasper (BS=100; PP=1). Based on these phylogenetic results, *Blechnum bakeri* should therefore be combined in *Cranfillia*.

### MORPHOLOGY AND ANATOMY

*Cranfillia bakeri* Vázquez Ferreira & S.Molino, comb. nov. and its sister lineage *C. mucronata* occur in distinct geographic areas: *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. occurs in Madagascar and East Africa (Rakotondrainibe *et al.*

IDENTIFICATION KEY TO THE SPECIES CLOSELY RELATED TO *CRANFILLIA BAKERI* (C.CHR.) VÁZQUEZ FERREIRA & S.MOLINO, COMB. NOV.

1. Sterile laminae with fully adnate pinnae in distal half, and with partially free pinnae in proximal half (sometimes only in 1/3 proximal of its length) [Lord Howe Island] ..... *C. fullagari* (F. Muell.) Gasper & V.A.O.Dittrich
- Sterile laminae with fully adnate pinnae all along the length, except for the first 1-2 (-3) most basal pinnae pairs, which are partially free at least basiscopically ..... 2
2. Scales from nigrescent to light brown; perine ornamentation with curly thin muri, which do not give the spore a polyhedral shape [N. Zealand] ..... *C. nigra* (Colenso) Gasper & V.A.O.Dittrich
- Scales notably atropurpureous; perine ornamentation with straight thick muri which give the spore a polyhedral shape ..... 3
3. Trichomes remarkably opaque and atropurpureous, from dense to scattered but always present at least on the abaxial surfaces [New Caledonia, Fiji, Vanuatu] ..... *C. opaca* (Mett.) Gasper & V.A.O.Dittrich
- Trichomes when present, light brown (never atropurpureous); often completely glabrous individuals ..... 4
4. Sterile blade triangular-ovate; basal segments lanceolate to linear-elliptic; apical segments obtuse, sometimes slightly acute [Brazil] ..... *C. mucronata* (Fée) V.A.O.Dittrich & Gasper
- Sterile blade lanceolate-ovate; basal segments semicircular, sometimes ovate; apical segments acute to acuminate [East Africa, Madagascar] ..... *C. bakeri* (C.Ch.) Vázquez Ferreira & S.Molino, comb. nov.

2013), while *C. mucronata* is endemic to the Southern Brazil (Dittrich *et al.* 2017). Despite their non-overlapping distribution areas, we found morpho-anatomical resemblances (Table 2) between them, and with two other closely related *Cranfillia* species: *Cranfillia nigra* (Colenso) Gasper & V.A.O.Dittrich and *Cranfillia opaca* (Mett.) Gasper & V.A.O.Dittrich. *Cranfillia fullagari* (F.Muell.) Gasper & V.A.O.Dittrich should also be considered as closely related, but we could not conduct a proper anatomical work on this species due to its scarcity in herbaria. Nonetheless, we considered its macro-morphology based on virtual herbarium observations and illustrations in a local flora (Flora of Australia, 1994).

### Pinnae

All studied species had at least their first basal pair of pinnae partially adnate to the rachis, and sometimes deflexed (Fig. 2). This was especially true for *C. fullagari* and *C. nigra* (Fig. 2A, E), in which the basal pair of pinnae was morphologically very different from the medial and apical pairs that were completely adnate. *Cranfillia opaca* and *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. have their basal pairs almost adnate, the free width being sometimes hard to observe but at least visible basiscopically (Fig. 2C, D). In *C. mucronata*, the basal pinnae are highly variable, from half-free to almost adnate, but they are always deflexed compared to the other pinnae (Table 2). As this was also observed in other *Cranfillia* species but not in *Austroblechnum*, we suggest that partially adnate basal pairs of pinnae and deflexed first pair of pinnae could be diagnostic characters to distinguish *Cranfillia* from *Austroblechnum*.

### Hairs

In *Cranfillia*, hairs usually grow abaxially along the rachises, costae, veins and over the laminar tissue, and plants often have densely pilose margins. In *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. and its three close relatives, trichomes

were observed as relatively short (compared to other species of the genus, such as *C. vulcanica*) and brown (similar to those of *C. mucronata* and *C. nigra*, Table 2) whereas *C. opaca* has opaque atropurpureous hairs (Table 2). Among *Cranfillia* species, completely glabrous specimens were only reported in *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. and *C. mucronata*, but although *C. mucronata* is often glabrous, some specimens showed varying minute hairs (Dittrich *et al.* 2017; Gasper, pers. obs.). The absence of hairs for some specimens of *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. notably led Christensen to describe *Blechnum ivohibense* as a new species (1928), followed by Schelpe (1967), although it is now recognized as a synonym of *B. bakeri* (Rakotondrainibe *et al.* 2013).

### Spore ornamentation

Another taxonomically significant character was spore ornamentation (Fig. 3), with a similar perine ornamentation observed in *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. and other *Cranfillia* species, whereas *Austroblechnum* species had a different ornamentation. Precisely, spores of *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. (Fig. 3A, B) and *C. mucronata* (Fig. 3C, D) are characterized by a perine with more or less thick and straight muri, which delimit areoles, giving the spores a polyhedral shape. This was also observed in *C. opaca* and *C. nigra*, *C. nigra* being the most different one with more irregular, slim and more or less curly muri that did not give a polyhedral shape to the spores (Passarelli *et al.* 2010; Moran *et al.* 2018, Silva *et al.* 2019). Also, we observed a perine middle layer with a trabecular structure in *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. and *C. mucronata*. Spores of *Austroblechnum* also revealed that *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov. differs from other species of this genus: perine is broadly folded in *C. bakeri* Vázquez Ferreira & S.Molino, comb. nov.

(Fig. 3A, B) and its closest relatives, whereas those from *Austroblechnum* are unfolded (Fig. 3E, F), as previously reported by Moran *et al.* (2018).

Therefore, *C. bakeri* Vázquez Ferreira & S. Molino, comb. nov. appears as morpho-anatomically close to *C. mucronata*, *C. nigra* and *C. opaca*, and it clearly differs from *Austroblechnum* spp. based on several spores and pinnae characters.

## SYSTEMATICS

Our results on molecular phylogeny and morpho-anatomy led us to combine the species name *Blechnum bakeri* into *Cranfillia*, thus extending the distribution of this latter genus to Madagascar and East Africa. The following list of synonyms was modified from Rakotondrainibe *et al.* (2013), but typifications are here completed for some names.

### *Cranfillia bakeri* (C. Chr.)

Vázquez Ferreira & S. Molino, comb. nov.

*Blechnum bakeri* C. Chr., *Index Filicum*: 151 (1905), *nom. nov.* for *Lomaria pubescens* Baker; *Journal of the Linnean Society, Botany* 15: 415 (1876), *non* Kunze (1843); *nec* *Blechnum pubescens* Desv. (1827), *nec* Hooker (1837). — *Austroblechnum bakeri* (C. Chr.) Gasper & V.A.O. Dittrich, *Phytotaxa* 275 (3): 191–227 (2016). — Type: Madagascar, Antananarivo, *W. Pool s.n.* p.p. (lecto-, K[K000424012]!), first-step designated by Schelpe [1967: 348], second-step designated here; isolecto-, K[K000424011]! excl. specim.: fronds annotated as G and H).

*Blechnum polypodioides* (Sw.) Kuhn var. *holstii* Hieron., *Pflanzenwelt Ost-Afrikas und der Nachbargebiete* 1, C: 81 (1895). — Type: “usb., Mbaramu”, *C. Holst* 2479 (lecto-, B200031479!, designated by Parris (2006: 4); isolecto-, P[P00113566]!).

*Blechnum bakeri* C. Chr. var. *glabrum* Bonap., *Notes ptéridologiques* 7: 210 (1918) (as “glabra”). — Type: Madagascar. Mandraka, *C. d’Alleizette* 82 in Herb. Bonaparte (holo-, P[P00483198]!).

*Blechnum ivohibense* C. Chr., *Archives de Botanique* (Caen), *Bulletin mensuel* 2: 211–212 (1928). — Type: Madagascar. Pic d’Ivohibe, Bara, *H. Humbert* 3300 p.p. in Herb. C. Christensen (holo-, BM[BM001066246 photo]!; iso-, B[B200031506 photo]! p.p., excl. specim.: two small individuals on the right and left; excl. specim. P01625371!). See Rakotondrainibe *et al.* (2013) for details.

*Blechnum umbrosum* Peter, *Repertorium specierum novarum regni vegetabilis*, Beiheft 40 (1), *Lieferung* 1: 82; *Descriptiones*: 9, t. 3: 5–8 (1929). — Type: Tanzania. West Usambara, Kisimba above Mazumbai, IV.1916, *A. Peter* 16489 (lecto-, B[B200034334 photo]!, designated by Parris (2006: 4); isolecto-, BM[BM000585222 photo]!, BR[BR0000008054487 photo]!, GOET007151 photo!, K[K001092775 photo]!, K[K001092776 photo]!, P[P00483201]!, US00135471 photo!).

*Blechnum ivohibense* C. Chr. var. *hirsutum* C. Chr., *Dansk Botanisk Arkiv* 7: 106 (1932). — Type: Madagascar. Pic d’Ivohibe, *H. Humbert* 3299 (lecto-, BM[BM001066247 photo]!; designated by Rakotondrainibe *et al.* (2013: 151–193); isolecto-, P[P01625808]!); remaining syntypes: Analamazaotra, *H. Perrier de la Bâthie* 7550 (syn-, P[P00835613]!, P01625804!); *H. Perrier de la Bâthie* 7625 (syn-, P[P01625802]!, P01625803!)).

## NOMENCLATURE COMMENTS

Schelpe (1967) cited two sheets at K as the lectotype of *Blechnum bakeri*, and only conducted the valid first-step lectotypification of the name (Art. 9.17 of ICN; Turland *et al.* 2018). Since those two sheets at K are not clearly labeled as being part of a single specimen (Art. 8.3 of ICN; Turland *et al.* 2018), we here conducted the second-step lectotypification, selecting the sheet with two isolated sterile fronds (annotated E, F). From the same sheet K000424012, the two fertile fronds (annotated G, H) are excluded from the lectotype, as they must be following the first-step lectotypification (Schelpe 1967); we however ascribed the fertile frond H to *Cranfillia bakeri* Vázquez Ferreira & S. Molino, comb. nov., while frond G was ascribed to *Lomariidium bifforme* (Baker) Gasper & V.A.O. Dittrich.

In the protologue of *Blechnum bakeri* var. *glabrum*, Bonaparte (1918) cited *C. d’Alleizette* 82 (in the Bonaparte’s Herbarium) as the type. This gathering is represented by a single sheet at P, which is the holotype; Rakotondrainibe *et al.* (2013) erroneously cited P00483199 as an isotype, as this latter sheet in fact belongs to the distinct gathering *C. d’Alleizette* 82m (i.e. including “m” as a suffix).

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## APPENDICES

APPENDIX 1. — List of specimens used for the phylogenetic analysis, with Genbank accessions numbers for *rbcL*, *rps4/rps4-trnS*, *trnL/trnL-trnF* sequences. Sequences newly obtained for this study are highlighted by \*.

- Cranfillia bakeri* (C.Chr.) Vázquez Ferreira & S. Molino, Rouhan *et al.* 1625 (P02435151), Madagascar, MT521069\*, MT521073\*, MT521077\*; Rouhan *et al.* 1401 (P02433013), Madagascar, MT521070\*, MT521074\*, MT521078\*; Janssen *et al.* 2424 (P00590664, P00590665), Madagascar, MT521071\*, MT521075\*, MT521079\*; Janssen *et al.* 2665 (P00590785, P00590786), Tanzania, MT521072\*, MT521076\*, MT521080\*. — *Cranfillia fluviatilis* (R.Br.) Gasper & V.A.O.Dittrich, Wilson 64 (MELU), Australia, KJ170791, KJ170818, KJ170845. — *Cranfillia hirsuta* (Rosenst.) Gasper & V.A.O.Dittrich, Perrie NC 2012 196 (WELT), New Caledonia, KF975718, KF975748, KF975792. — *Cranfillia nigra* (Colenso) Gasper & V.A.O.Dittrich, Perrie 3454 (WELT), New Zealand, DQ683399, KF975756, KF975800. — *Cranfillia opaca* (Mett.) Gasper & V.A.O.Dittrich, Perrie NC 2012 167 (WELT), New Caledonia, KF975725, KF975761, KF975805. — *Cranfillia pilosa* (Brack.) Gasper & V.A.O.Dittrich, Perrie & Brownsey FJ2011181 (WELT), Fiji, KF975728, KF975765, KF975809. — *Cranfillia mucronata* (Fée) V. A. O.Dittrich & Gasper, Salino 13818 (BHCB), Brazil, KU898572, KU898685, KU898629. — *Cranfillia caudata* (Baker) V. A. O.Dittrich & Gasper, Jiménez 1743 (LPB), Bolivia, KU898573, KU898630, KU898686. — *Blechnum occidentale* L., Funez 548 (FURB), Brazil, KU898568, KU898623, KU898681. — *Blechnum longipilosum* V.A.O.Dittrich & Salino, Salino 15570 (BHCB), Brazil, KU898570, KU898626, KU898683. — *Blechnum auriculatum* Cav., Funez 2162 (FURB), Brazil, KU898561, KU898616, KU898675. — *Austroblechnum squamipes* (Hieron.) Gasper & V.A.O.Dittrich, Salino 14737 (BHCB), Brazil, KU898555, KU898610, KU898669. — *Austroblechnum microphyllum* (Goldm.) Gasper & V.A.O.Dittrich, Biganzoli 2141 (SI), Argentina, KU898552, KU898607, KU898666. — *Austroblechnum patersonii* (R.Br.) Gasper & V.A.O.Dittrich subsp. *queenslandicum* (T.C.Chambers & P.A.Farrant) Gasper & V.A.O.Dittrich, Ohlsen BB144 (BRI), Australia, KJ170796, KJ170823, KJ170850. — *Icarus filiformis* (A.Cunn.) Gasper & Salino, Perrie 3459 (WELT), New Zealand, DQ683385, KF975745, KF975789.

## APPENDIX 2. — List of specimens studied for morphology and anatomy.

- C. bakeri*: East Africa, Kenya, Meru, Marimba, Ithangune forest, road along the base of volcanic cone Kirue, 10 miles from Nkubu, 2335 m, 22.VI.1969, *R.B. Faden et al.* 69/773 (US2614126); Tanzania, Tanga, Morogoro, Uluguru – Gebirge, Nordwestseite, Lupanga-Berg, Nebelwald, 2100 m, 10.XI.1932, *H.J. Schlieben* 2959 (MA389177); West Usambara, IV.1916, *G.A. Petter* 16489 (US1755107); Madagascar, Ambatofitorahana, à 35 km au sud d'Ambositra, 1700 m, 21.I.1970, *Frère M. Onraedt* 70M77 (US3005446); Antananarivo, Forest remnants 5 km E of Anjozorobe, 1250 m, 06.XI.1992, *H. van der Werff et al.* 12849 (UC1604664); Mandraka, 15.VIII.1906, *C. Alleizette* 82 (P00483198 [photo]). — *C. fullagari*: Lord Howe Island, Mount Gower, no date, *C. Moore* 15 (K001092750); *ibid.*, *J.P. Fullagar* 15 (MEL54009); *ibid.*, *J.P. Fullagar & Lind* 26 (MEL54005, holotype); *ibid.*, *J.P. Fullagar & Lind s.n.* (MEL54006, isotype); *ibid.* (MEL51551, isotype); *ibid.*, 2650 m, 27.VIII.1969, *J. Game* 69/234 or 235 (UC1952108 [photo]). — *C. mucronata*: Brazil, Paraná, Serra Piramirim, Estrada Itupava (Mun. Piraquara), 09.IV.1996, *J.M. Silva et al.* 1651 (MA655870); Rio de Janeiro, *P. Rosenstock* 20812 (UC441995); Serra do Leão (Mun. Morretes), 1000 m, 10.VI.1969, *G. Hatschbach* 2161 (UC1365989); Serra de Araraquara (Mun. Guaratuba), 100 m, 04.I.1968, *G. Hatschbach* 18243 (UC1330736); Serra Piramirim, Estrada Itupava (Mun. Piraquara), 09.IV. 1996, *J.M. Silva et al.* 1651 (UC1615719); Trilha para a Torre da Prata (Mun. Paranaguá), 01.VII.2003, *J.M. Silva et al.* 3767 (UC1783508); 1872, *M. Glaziov s.n.* (P01389538 [photo]). — *C. nigra*: New Zealand, Auckland, Mt. Wainui, west coast near Wellington, 1700ft, 15.IX.1962, *W. Burke* 39 (UC1216922); Coromandel, no date, *T. Kirk* 603 (US816226); Farnsammlung, 1870, *F. Helms s.n.* (US2416348); Nelson, Westhaven inlet, 28.XII.1958, *D.M. Calder s.n.* (US2360742); North Island, Le Aroha Mountain, 1884, *T.F. Cheeseman s.n.* (US no voucher, barcode: 01528011); *ibid.*, I.1884, *T.F. Cheeseman s.n.* (US816227); *ibid.*, Orongorongo Valley, Water Reserve, Rumutaha Range, east of Wellington, 14.II.1949, *F.R. Fosberg* 30481 (US2584908A); Stewart Island: Fern Gully near Halfmoon Bay. Second growth *Dacrydium-Weinmannia* forest, 15.III.1949, *E.H. Walker* 4887 (US2015875); *ibid.*, Oban. In fern gully 1.5 miles northwest of Oban. Growing in wet soil in deep shade of the fern gully, 20.II.1935, *T.T. Earle et al.* 336 (UC1368910); Wai Pounamu, Nelson, 1886, *E. Rosenstock* 38 (UC398232); Waitakeri, VI.1911, collector unknown *s.n.* (US652881); 1847, *J.W. Colenso* 331 (K001092713 [photo]). — *C. opaca*: Vanuatu, Aneityum Island, Anelgauhat Bay, 2000ft, 05.III.1929, *S.F. Kajewski* 879 (US1550771); New Caledonia, donated by *M. J.A.I. Pancher*, 1870, unknown collector (US483253); Dogny, La Foa, *I. Franc* 45 (US1096842); Mont Mou, XI.1908, *G.H. Bonati* 2049 (US2427225); *ibid.*, *I. Franc* 2049 (US1205019); Mt. Panié, dense very wet forest, 500 m, 15.XII.1995, *D.R. Hodel et al.* 1436 (UC1606655); Province du Sud, Mt. Dzumac. Forest patches in scrub, 900 m, 14.XI.1999, *H. van der Werff & G. McPherson* 16107 (UC1750393); Vanuatu, New Hebrides-Aneiteum, II.1859, 47 (US1431859); *ibid.*, mountain woods, II.1859, *M. Gillivray* 47 (US1431860); *ibid.*, II.1859, *M. Gillivray* 47a (US2987104); 01.XI.1908, *I. Franc* 2049 (UC394309); 1907, 2049 (UC393022). — *Austroblechnum lherminieri*: Guadeloupe, no date, *F. L'Herminier s.n.* (BA57587).