

## 論文の内容の要旨

論文題目 Phylogenetic studies on *Hymenophyllum* subgenus *Mecodium* C. Presl ex Copel. (Hymenophyllaceae) with special focus on the species boundaries of the *Hymenophyllum polyanthos* (Sw.) Sw. complex

(コケシノブ科*Hymenophyllum*属*Mecodium*亜属におけるホソバコケシノブ種複合体の系統・種分化の研究)

名前 TAVARES VASQUES DIEGO

タヴァレス ヴァスケス ジェーゴ

This research targets a subcosmopolitan species of filmy ferns called *Hymenophyllum polyanthos*, which belongs to *Hymenophyllum* subgenus *Mecodium*. The biology and distribution, as well some evidences from previous studies, indicate that the species may correspond to a non-monophyletic lineage. Through collection of several specimens of the subgenus *Mecodium*, this research compares sequences of different markers to distributional and morphological traits, aiming to solve the question regarding the monophyly of the lineage. The study is organized into five chapters, as detailed in the following.

### Chapter 1 – Taxonomical background of *Hymenophyllum* subgenus *Mecodium* C. Presl ex Copel.

This chapter brings a historical summary of the classification system changes within Hymenophyllaceae, with focus on the generic and subgeneric levels. The family Hymenophyllaceae is a subcosmopolitan family of ferns, represented by about 600 species distributed through the tropics. The main characteristic for plants of this family is the extremely thin leaves, composed by 1 to upmost 4 layers of cells, resulting in a filmy appearance. As popularly known, the filmy ferns bear delicate bodies and, for that reason, are mostly restricted to humid and shadowy environments, such as rainy forests. According to recent molecular studies (Ebihara *et al.* 2006), the filmy ferns are divided into 9 genera, being the genus *Hymenophyllum* one of the most representatives of the family, and covering around 250 species. Within *Hymenophyllum*, the subgenus *Mecodium sensu* Ebihara (2006, represented by about 35 species) was the target of several studies regarding its circumscription during the last century (Copeland 1937, 1938, Pichi Sermolli 1977, Morton 1968, Iwatsuki 1984, 1990, Hennequin *et al.* 2003, 2006).

The most recent phylogenetic study on the subgenus *Mecodium* by Hennequin *et al.* (2006) evidenced that it stands as a polyphyletic group, including represents from different subgenera. Additionally, the type species for the subgenus

*Hymenophyllum polyanthos* (Sw.) Sw. did not emerge as a monophyletic clade in Hennequin's (2006) analysis, raising questions about the existence of cryptic species. *Hymenophyllum polyanthos* is distributed Worldwide and lacks traits that can help the identification, such as trichomes, scales or dentations. Although *H. polyanthos* specimens were sampled by Hennequin et al. (2006), the short coverage of the sampling restricts discussions that can lead towards a monophyletic circumscription of the species. To resolve this, a broader sampling of the subgenus is necessary, including both specimens of *H. polyanthos* from several regions of the globe, and also other species included in *Hymenophyllum* subgenus *Mecodium* sensu Ebihara (2006). Like this, both distributional patterns and species limits can be assessed.

Chapter 2 – Molecular plastid markers reveal the actual circumscription of *Hymenophyllum* subgenus *Mecodium* C. Presl ex Copel. and of *Hymenophyllum polyanthos* (Sw.) Sw. (Hymenophyllaceae)

This chapter brings the main discussions of the thesis, corresponding to the polyphyly of *H. polyanthos*. The species is subcosmopolitan, distributed through tropical and subtropical regions of the globe. It is included in subgenus *Mecodium*, which was circumscribed recently by Ebihara et al. (2006) and Hennequin et al. (2006) based on molecular data. At the time of the circumscription, evidences that pointed towards the polyphyly of *H. polyanthos* were also obtained, but further investigations were still necessary to achieve concrete conclusions regarding the species. This chapter focuses on this matter and brings a clarification to the evolutionary history behind the evidences found by Hennequin et al. (2006).

**Sampling** -- Samples included specimens recognized as *H. polyanthos* as well other closely related species of subgenus *Mecodium* sensu Ebihara et al. (2006). 83 samples were

acquired through both collection trips and donations from collaborators. **Markers amplification** -- For the analysis of chloroplast DNA, 6 different markers were used.

**Sequences alignment and phylogenetic analysis** -- Acquired forward and reverse sequences were assembled, aligned and compared under a Bayesian inference analysis and a Maximum Likelihood analysis. As outgroup lineages, sequences of *Hymenophyllum* species belonging to subgenera other than *Mecodium* were used.

The results indicate that the species within the subgenus have diversified initially into 2 big lineages: one lineage composed of plants from Malesia, Pacific and South America; and another composed of plants from Africa, Asia, Malesia and the Neotropics (Fig. 1). This distributional pattern was suggested before by Hennequin

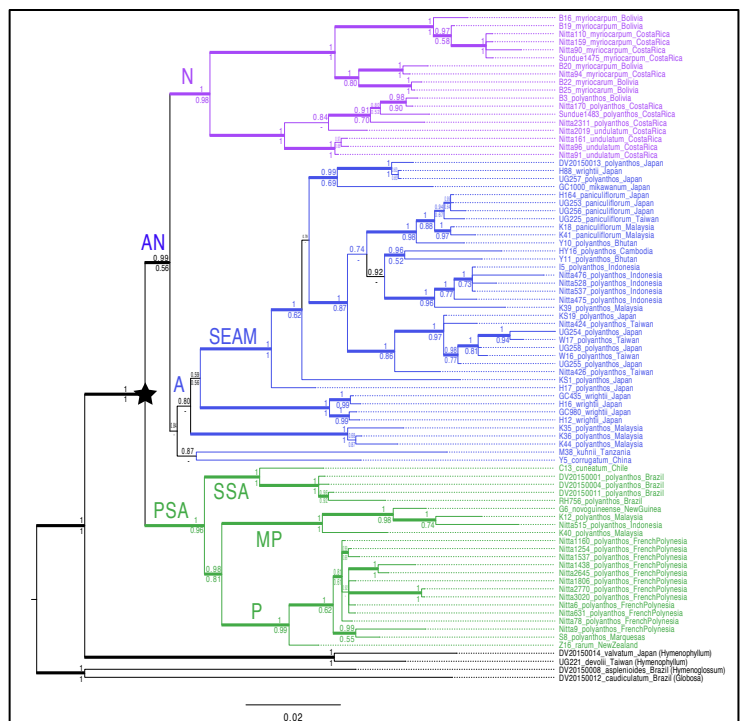


Fig. 1: Phylogenetic tree for *Hymenophyllum* subgenus *Mecodium* C. Presl ex Copel. based on plastid markers.

*et al.* (2006), but with few pieces of evidence at the time. Furthermore, the phylogenetic analysis based on plastidial markers indicates that, as expected, *Hymenophyllum polyanthos sensu lato* does not correspond to a monophyletic lineage. The subcosmopolitan species emerged interspersed to other represents of subgenus *Mecodium*, thus being described as a “polyphyletic species”.

### Chapter 3 – Further insights on the circumscription of *Hymenophyllum polyanthos* (Sw.) Sw. complex (Hymenophyllaceae) based on the nuclear *LEAFY* marker

This chapter investigates the possible reasons for the polyphyletic condition of *H. polyanthos*. In order to address the occurrence of gene introgressions within the complex, the nuclear marker *LEAFY* is applied on a phylogenetic analysis, and the result is compared to the trees obtained in chapter 2. From this comparison, possible explanations for the diversity within *Hymenophyllum* subgenus *Mecodium* and the *H. polyanthos* complex are explored.

**Sampling** -- In total, 31 different samples were cloned and at least 10 different clone colonies were sequenced for each sample. Ultimately, 66 different sequences were obtained. **Markers amplification** -- For the nuclear DNA, a region of ca. 550bp for the *LEAFY* gene was chosen for amplification. Direct sequencing of the nuclear region was not possible, and cloning experiments were performed in order to isolate each copy of the marker. **Sequences alignment and phylogenetic analysis** – Similarly to the plastid markers analysis, acquired forward and reverse sequences were assembled, aligned and compared under a Bayesian inference analysis and a Maximum Likelihood analysis. As outgroup lineages, sequences of *Hymenophyllum caudiculatum* were used.

With a few exceptions, the results of chapter 3 suggest that gene introgression events are not the most likely reason to explain the patterns obtained in chapter 2, indicating that *H. polyanthos*' polyphyletic state may have been a result of poorly distinguishable traits between global specimens, what caused the inclusion of different lineages under the same name. Further research is still necessary and additional evidence from other nuclear markers, or of different regions of the *LEAFY* marker may confirm this hypothesis in the future.

Although apparently informative for phylogenetic investigations, possibly paralog sequences were also acquired in the analysis, requiring further investigations to fully address this marker. The development of specific primers for the acquired sequences may solve the problem with the emergence of paralog sequences and bring, at last, conclusive results to the polyphyly of *H. polyanthos*. Nevertheless, this is the first time *LEAFY* marker sequences are acquired for Hymenophyllaceae, what brings new possibilities for phylogenetic studies with the family.

### Chapter 4 – Morphological and distributional patterns within the *Hymenophyllum polyanthos* (Sw.) Sw. complex

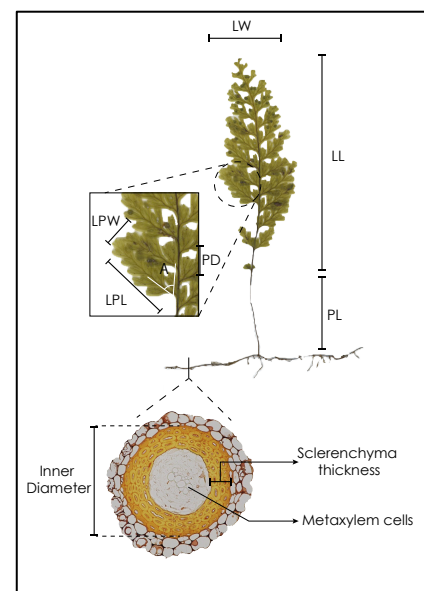


Fig. 2: Morphological and anatomical parameters used in the analysis.

Based on the previous chapters discussions, chapter 4 brings morphological and anatomical comparisons between the samples analyzed, taking as a premise the phylogenetic groups obtained in those chapters. Seven morphological parameters from the leaves (petiole length, lamina length, lamina width, lateral pinnae length, lateral pinnae width, pinnae distance and lateral pinnae insertion angle) and three anatomical parameters from the rhizomes (inner diameter, sclerenchyma thickness and metaxylem cells number) are analytically compared to the phylogenetic groups in this chapter (Fig. 2).

Although the simple body of filmy ferns imposes limits to the description of taxonomically informative traits, the principal component analysis applied here brings insights on possible diagnostic traits for the obtained lineages. The most correlate parameter here was the insertion angle of pinnae on the leaf, showing that plants from PSA clade have more closely appressed pinnae than those from AN clade. The assays on the anatomy of the rhizome of these plants, however, showed no potential correlation of parameters with the acquired groups.

#### Chapter 5 – Taxonomical treatment for the subgenus *Mecodium* including the re-validation of *H. sturmi* Bosch. in Brazil

All of the results of the previous chapters are merged in chapter five into a novel taxonomical treatment for *Hymenophyllum* subgenus *Mecodium*. As a result, traits of the leaf of the plants support the suggestion of the creation of two new sections within the subgenus *Mecodium*: section *Cuneatae*, represented by plants from Malesia, Australia, Pacific islands and South America; and section *Mecodium*, represented by plants from Africa, Asia and Neotropics and including the type clade for *H. polyanthos*.

In this study, the combination of molecular, morphological and distributional data analysis with the revision of the taxonomical transformations within the subgenus *Mecodium* have brought a new level of detail to the comprehension of the diversity within the group. Basing the discussion on the results of the phylogenetic analyses applied here, it is evident that *H. polyanthos s.l.* corresponds to a non-monophyletic grouping and that further treatment of the subgenus *Mecodium* can bring a better solution to the taxonomy of the group. In the near future, the comparison of additional nuclear sequences to this analysis may bring to a conclusion the phylogenetic condition of *H. polyanthos s.l.*, opening ways to novel works towards a taxonomical revision of the complex.

#### General conclusions

- Based on molecular evidence obtained with this study, *Hymenophyllum* subgenus *Mecodium* phylogeny is made clear, suggesting the diversification of 2 main lineages within the subgenus;
- Additionally, *Hymenophyllum polyanthos s.l.* emerges as a polyphyletic species with support of both plastid and nuclear markers data;
- Comparison of morphological and anatomical features with the obtained phylogenetic groups bring insights on putative diagnostic traits within the subgenus;
- A taxonomic treatment for the subgenus, including the re-validation of *H. sturmi* Bosch, is suggested.