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# Desiccation tolerance of Hymenophyllacea filmy ferns is mediated by constitutive and non-inducible cellular mechanisms

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ABSTRACT

The Hymenophyllaceae is a primitive family within the Filicopsidae. One of the most exceptional features of this family of ferns is the presence of fronds with one or just a few cell layers (hence their name of filmy ferns), and the absence of stomata. *Hymenophyllum caudiculatum* and *Hymenophyllum dentatum* are able to lose more than 82% of their fully hydrated water content, to remain dry for extended periods of time (days or weeks), and to survive and remain viable following rehydration. The aim of this work was to understand whether the adaptive strategy of the Hymenophyllaceae for desiccation tolerance is constitutive or inducible. A proteomic approach was adopted in combination with physiological parameters to assess whether there were changes in the protein content during dehydration and following rehydration. Detached fronds were used to monitor the rates of photosynthesis in desiccation experiments, sugar accumulation, and high-resolution 2-DE to analyze proteome variation during a desiccation–rehydration cycle. The analyzed proteome exhibited little variation (3–4%) between hydrated and desiccated states, while variation was greater between the desiccated and rehydrated states (8.7–10%). Eighty-two discrete proteins were analyzed by MS/MS, and 65 were identified. About 21% of the analyzed proteins (17) were mixtures of two or more different polypeptides. Of the identified proteins, more than a half (33 spots, 55%) had functions related to energy-photosynthesis. The second largest category with known function (five spots, 8%) was related to cell rescue, defense, and virulence. More than one in every four proteins analyzed belonged to a group of hypothetical proteins (18 spots, 28%). The results suggest that the Hymenophyllaceae represent an example of a change in adaptive strategy from a typical vascular to the poikilohydric homoiochlorophyllous adaptation, which they share with the bryophytes that grow in profusion in the same habitats. The speed at which desiccation takes place therefore precludes the induction of protective systems, suggesting a constitutive mechanism of cellular protection.



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## 1. Introduction

The Hymenophyllaceae is one of the most primitive families belonging to the class Filicopsida, Pteridophyta [1,2]. The central ecological niche of filmy ferns is a shady humid forest, at moderate altitudes in the tropics (1,500 to 300 m) or at comparable climatic conditions in lower altitudes in temperate hyperoceanic regions, such as Atlantic western Europe, southern Chile, south-east Australia, and New Zealand [3]. Filmy ferns are very common epiphytes in the temperate rain forest of southern Chile, and the most diverse genus is *Hymenophyllum* with 19 species [4]. One of the most remarkable characteristics of this fern group is that the frond, apart from the vascular tissue, is made of a single cell layer (hence the name filmy ferns). For this reason, they do not have a need for stomata [5]. Filmy ferns are poikilohydric organisms. They have a rudimentary ability to control water loss, and depend on high environmental humidity to be well hydrated [6]. There are some species of this filmy fern group that are able to tolerate considerable desiccation, and some of them even exhibit the ability to resurrect (the ability to lose more than 95% content of water, remain in this anhydrobiotic state for a period of time and survive after the rehydration). Very recently, Saldaña et al. [7], using whole plants reported a desiccation recovery after 60 minutes for *Hymenophyllum caudiculatum* and *Hymenophyllum dentatum*.

Desiccation tolerance is most frequently observed in mature seeds or pollen of higher plants, but very rarely observed in vegetative tissues [8]. Desiccation-tolerant plants fall into two main categories: fully desiccation-tolerant plants that can withstand the total loss of free protoplasmic water at any rate, and modified desiccation-tolerant plants that can only survive such a stress if water loss is slow. Plant complexity appears to influence which category a plant belongs to. All fully desiccation-tolerant plants studied to date are of the less complex groups of plants; algae, bryophytes, or lichens [9]. Modified desiccation-tolerant plants tend to be more complex (fern, fern allies, and angiosperms). Proteome differential expression [10] and sugar accumulation are well-known inducible desiccation tolerance mechanisms in vascularized desiccation-tolerant plants [11,12]. Using ecophysiological data, the Hymenophyllaceae have been recently described as a rare example of an evolutionary shift of adaptive strategy from that typical for vascular plants to that of poikilohydry typical for bryophytes [3]. It is hypothesized that in poikilohydrous filmy ferns where desiccation can occur very rapidly, desiccation tolerance is largely associated with constitutive mechanisms of cellular protection. Therefore, during a desiccation–rehydration cycle, it is hypothesized that the proteome and soluble sugars content should remain largely unchanged, with no significant variations.

The aim of the present study is to analyze the proteomic and physiological status of two filmy ferns, *H. caudiculatum* and *H. dentatum*, comparing hydrated, desiccated and rehydrated states, in order to understand the type of adaptive strategy the Hymenophyllaceae possesses with regard to desiccation tolerance.

## 2. Material and methods

### 2.1. Tissue sampling and desiccation treatments

*H. caudiculatum* (Mart.) var. *productum* (K. Presl.) and *H. dentatum* (Cav.) plants were collected from second-growth forest stands in the southern temperate rainforest in the Cordillera de Quillaipe, Katalapi Park. This site of collection has been extensively characterized previously [7]. A nursery area with automatic watering and shade cloth was previously established in order to maintain epiphytic filmy ferns taken from the field. Fronds detached from the fully hydrated plants were dehydrated at room temperature, at 70–80% relative humidity and moderate illumination for 24 h. For rehydration, the 24-h-dried fronds were placed in Petri dishes with distilled water for 24 h (Fig. 1).

**Fig. 1.** (Color online). Desiccation and rehydration of detached fronds of *Hymenophyllum caudiculatum* (left panel) and *Hymenophyllum dentatum* (right panel). Fronds detached from fully hydrated plants were dried under 70–80% relative humidity and moderate illumination for 24 h (RWC 18%) and subsequently rehydrated for 24 h in Petri dishes with distilled water (RWC 82%).