Reaffirming the phyllocladoid affinities of *Huncocladus laubenfelsii* (Podocarpaceae) from the early Eocene of Patagonia – a comment on Dörken et al. (2021)

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Running title: On the affinities of *Huncocladus*

Abstract

We discuss a recent assessment by Dörken et al. (2021) regarding the affinities of the Eocene fossil species *Huncocladus laubenfelsii* from Laguna del Hunco (Patagonia, Argentina). We originally (Andruchow-Colombo et al., 2019) assigned this species to the conifer family Podocarpaceae as the first certain South American macrofossil record of the phyllocladoid lineage (*Huncocladus* + *Phyllocladus*), based on a combination of numerous macro- and micromorphological vegetative characters. However, Dörken et al. (2021) rejected the podocarpaceous affinity of *H. laubenfelsii* and considered it to be more closely related to the cycad genera *Bowenia* or *Eobowenia*. Their assessment was based almost entirely on two cuticular characters, with only superficial consideration of the abundant additional evidence available that included several diagnostic macromorphological features. We review the two characters mentioned by these authors and other features and find that their suggestion is strongly contradicted by the available evidence, maintaining our original assignment. Critical characters include presence/absence of a midvein, secondary venation pattern, arrangement and general morphology of the photosynthetic structures, and morphology and disposition of epidermal cells.

In a recent publication in this journal, Dörken, Hill, Jordan & Parsons (2021) described the photosynthetic-structure morphology of juvenile and adult plants of *Phyllocladus aspleniifolius* (Labill.) Hook. f. and adult plants of *P. trichomanoides* D. Don. Dörken et al. interpreted these data and provided a valuable hypothesis of phylloclade evolution in the Podocarpaceae. Towards the end of their discussion, Dörken et al. briefly reviewed the fossil record of *Phyllocladus* Rich. ex Mirb. and morphologically associated genera, and they provided lists of “accepted” and “invalid” fossil taxa related to the phyllocladoid lineage (Dörken et al., 2021, tables 1, 2), neither of which was discussed in depth. Among the so-called “invalid” records, Dörken et al. included the recently described fossil species *Huncocladus laubenfelsii* A.Andruchow-Colombo, Wilf, I. Escapa (2019) from the early Eocene of Laguna del Hunco (Patagonia, Argentina). *Huncocladus laubenfelsii* is significant as the only phyllocladoid macrofossil known from South America, other than wood records of possible phyllocladoid affinity that include occurrences at Laguna del Hunco (e.g., Pujana et al., 2020). As a side note, the fossil record of the phyllocladoids is also represented in Argentina by palynological remains included in *Microalatidites*, an organ genus associated with the genus *Phyllocladus* (Heredia et al., 2012; Macphail & Cantrill, 2006). This combination of macro- and microfossil remains constitute rather strong evidence of the presence of this lineage in the fossil record of southern South America.

Dörken et al. argued that *H. laubenfelsii* was probably not related to the extant Podocarpaceae genus *Phyllocladus*, as we had proposed, but to the cycad genus *Bowenia* Hook. ex Hook. f. or its extinct relative *Eobowenia* M.Coiro et C.Pott (2017). Even though the lone specimen of *Huncocladus* A.Andruchow-Colombo, Wilf, I. Escapa is a well-preserved branch bearing several attached phylloclades and shows a large number of morphological characters, this conclusion was based almost entirely on two micromorphological characters: (1) the morphology of alleged trichome bases and (2) the "general arrangement" of epidermal cells.

Here, we argue in defense of the podocarpaceous and phyllocladoid affinities of *Huncocladus* by first discussing the two features just listed. We also suggest that the second character actually shows a closer relationship with *Phyllocladus* than with *Bowenia* and *Eobowenia* species. We then refer to the much larger number of macromorphological characters already included in the original publication of *H. laubenfelsii* that Dörken et al. overlooked, all of which support podocarp affinity and clearly contradict their asserted affinity to cycads, to which *Huncocladus* bears no resemblance whatsoever.

Trichomes and stomatal apparatuses. Dörken et al. interpreted as trichomes the structures that we described as stomatal apparatuses (Andruchow-Colombo et al., 2019 fig. 6 G-I). However, those structures clearly and repeatedly show the subsidiary cells and, in some cases, also guard cells, which are all features of stomata and not trichomes. Moreover, in our original publication, trichome bases were found, although scarce and poorly preserved (Andruchow-Colombo et al., 2019, fig. 6 J), and they markedly differ in morphology from the stomatal apparatuses on the same specimen. Because of the poor preservation of these structures there is not much additional information that can be extracted from them. Nonetheless, the disposition of the cells around the trichome bases of *Huncocladus* strongly contrasts with those observed in species of *Bowenia* (Hill et al., 2019), being markedly chaotic in *Huncocladus* (Andruchow-Colombo et al., 2019 fig. 6 G-I), whereas in *Bowenia* the epidermal cells surrounding the trichome show slight to no deviations from their arrangement in rows (see Hill et al., 2019 fig. 3). The chaotic configuration of the cells surrounding the *Huncocladus* trichomes is also completely different from the well-organized stomatal complexes.

Epidermal cell morphology and disposition. Dörken et al. stated that *Huncocladus laubenfelsii* resembled *Bowenia* and *Eobowenia* in the “general arrangement” of epidermal cells. However, epidermal cells in those two cycadalean genera are mostly spindle-shaped and elongated (Greguss, 1968; Pant & Nautiyal, 1981; Coiro & Pott, 2017; Hill et al., 2019), whereas epidermal cells in *H. laubenfelsii* are mostly rectangular and elongated. Moreover, epidermal cells in *Bowenia eocenica* R.S. Hill, the Australian fossil species that Dörken et al. compared with *H. laubenfelsii*, show a V-shaped pattern of arrangement in the stomatal zones (Hill et al., 2019, fig. 3) that strongly contrasts with the organization in rows shown by *Huncocladus* (Andruchow-Colombo et al., 2019, fig. 6). Furthermore, darker epidermal cells (due to the thicker cuticle), which typically occur together with ordinary epidermal cells in *Bowenia* and *Eobowenia* (Pant & Nautiyal, 1981; Coiro & Pott, 2017; Hill et al., 2019), were not detected in the *Huncocladus* cuticle. As we originally stated, the shape and arrangement of epidermal cells in *Huncocladus laubenfelsii* are consistent with extant *Phyllocladus* species and do not resemble cycads (see images of epidermal morphology of *P. aspleniifolius*, courtesy of Dörken et al. coauthor G. Jordan, that we included as supplementary information S1 of Andruchow-Colombo et al., 2019).

Arrangement of photosynthetic structures. Dörken et al. interpreted the single specimen of *H. laubenfelsii* as a frond similar to that of the Early Cretaceous *Eobowenia*. However, *Eobowenia* shows subopposite leaflets (Archangelsy, 1966; Artabe & Stevenson, 1999; Coiro & Pott, 2017), whereas *H. laubenfelsii* has its photosynthetic structures (interpreted as pinnae by Dörken et al. and as phylloclades by us) spirally arranged over the branch (interpreted as the rachis of the frond by Dörken et al.). The spiral arrangement of the photosynthetic units (Andruchow-Colombo et al., 2019 fig. 3, 4) strongly contradicts the hypothesis of the whole structure being a frond rather than a branch bearing phylloclades. No cycad known, living or fossil, has its leaflets spirally arranged on a rachis (Greguss, 1968).

Presence of a prominent midvein. *Huncocladus laubenfelsii* phylloclades — like those of *Phyllocladus* — show a prominent midvein, from which the secondary venation emerges (Andruchow-Colombo et al., 2019 figs. 3-5). In contrast, *Eobowenia* only has a delicate midrib, and *Bowenia* frond segments either lack a midvein or show a rudimentary one that is only present at the base of the pinnule (Greguss, 1968; Stevenson et al., 1996; Coiro & Pott, 2017; Hill et al., 2019).

Secondary venation pattern of the photosynthetic units. *Huncocladus laubenfelsii* shows the characteristic secondary venation pattern of *Phyllocladus*, precisely as schematized by Dörken et al. (compare figure 5A-C in Andruchow-Colombo et al., 2019 and figure 3D, F in Dörken et al., 2021). Both those genera have a basal unbranched vein (marked with white arrowheads in figure 5B, C of Andruchow-Colombo et al., 2019 and in light blue in figure 3F of Dörken et al., 2021) and a distal, pinnately-branched vein (marked with black arrowheads in figure 5B, C Andruchow-Colombo et al., 2019 and in red in figure 3F of Dörken et al., 2021) entering each phylloclade lobe.

The venation pattern shown by *Phyllocladus* and *Huncocladus* strongly contrasts with that of *Bowenia* (see Plate XCV fig. 1 in Greguss, 1968 and fig. 2 in Hill et al., 2018) and *Eobowenia*

(Archangelsky, 1966; Coiro & Pott, 2017), or any cycad. In those two cycadalean genera, multiple veins arise from the pinnule base, dichotomize and become mostly parallel towards the mid-portion of the leaflet (Greguss, 1968; Coiro & Pott, 2017; Hill et al., 2018).

Photosynthetic unit morphology. The general morphology of *Huncocladus* phylloclades includes a symmetrical, lanceolate general outline, delicately lobed near the apex, the lobes becoming more incised towards the middle portion and pinnatifid towards the base (Andruchow-Colombo et al., 2019 figs. 3, 4). Both the general outline and the lobing pattern closely resemble those of *Phyllocladus*, as we described extensively (see Andruchow-Colombo et al., 2019 figs. 1, 2; also Dörken et al. fig. 4A). These features strongly contrast with the asymmetrical, oblong to rhomboidal outline with dentate or serrate margins of *Bowenia* and *Eobowenia* frond segments (Greguss, 1968; Coiro & Pott, 2017; Hill et al., 2019).

There is an additional factor to consider in Dorken et al.'s comparison of the morphology of photosynthetic structures in *Huncocladus* and *Eobowenia*. This is an apparent misinterpretation of the holotype material of *Eobowenia incrassata* (S.Archang.) M.Coiro et C.Pott made by Hill et al. (2019, see their fig. 5A) and consequently, possibly, by Dörken et al. Next to the holotype frond portion of *E. incrassata*, and aligned with it but at a different microstratigraphic level, are the remains of another, unidentified plant that shows a general outline similar to that of *Huncocladus* phylloclades and is markedly different from *Eobowenia* (M. Coiro pers. comm.). Hill et al. (2019), apparently, considered the separate, unidentified plant as part of *E. incrassata* because they referred to it as a "terminal pinna" of the *Eobowenia* specimen. This idea could have contributed to Dorken et al.'s interpretation of *Eobowenia* as somehow related to *Huncocladus laubenfelsii*. However, as stated in the preceding paragraph, the macromorphology of *Huncocladus* strongly differs from that of *Bowenia* and *Eobowenia*. The interpretation outlined here for the holotype of *Eobowenia incrassatta* (or *Almargemia incrassata* prior to the taxonomic treatment by Coiro & Pott, 2017) is supported by several independent interpretations of the material made by different authors in the past, none of whom included the purported "terminal pinna" in descriptions or interpretations of the cycad specimen (see diagnosis and description of the material in Archangelsky, 1966; fig. 2C in Artabe & Stevenson, 1999; diagnosis and description in Coiro & Pott, 2017; Coiro pers. comm.).

Concluding remarks.

One of the advantages of having macrofossil and cuticle remains connected is that it generally provides a means of testing the consistency of hypotheses of affinity. If the general morphology of *Huncocladus laubenfelsii* is considered alone, the *Bowenia* or *Eobowenia* affinities proposed by Dörken et al. cannot be supported due to a long list of phyllocladoid characters and a total absence of cycad characters, as detailed above. These include venation pattern (presence/absence of a midvein, and secondary venation pattern) and the arrangement and general morphology of the photosynthetic structures, all of which support phyllocladoid affinity. Likewise, the cuticle characters in the same specimen of *Huncocladus* also contradict the Dörken et al. hypothesis and support the original phyllocladoid affinity, including the morphology and disposition of epidermal cells. Furthermore, as shown here, their idea that the stomata of *Huncocladus* are actually trichome bases is not supportable.

In sum, the cycadalean affinities proposed for *Huncocladus* by Dörken et al. (2021) do not make sense in light of all the information available for this significant Patagonian species. Their critique of the genus is based on superficial and selective use of the evidence, and we find it incorrect. The phyllocladoid affinities of *Huncocladus* remain supported by the scientific evidence from numerous macro- and micro-morphological characters, as originally detailed, and from the resulting phylogenetic position as published (Andruchow-Colombo et al. 2019). Thus we maintain the taxonomic and phylogenetic position of *Huncocladus laubenfelsii* as originally described.

Fossils species have a central role in macroevolutionary and biogeographical studies (e.g., Leslie et al., 2012, 2018; Klaus & Matzke, 2019). It is then highly important to provide an alpha taxonomy supported in the largest possible number of characters, because the affinity is the primary hypothesis on which lie calibrations, and therefore all additional inferences. This is why we took seriously to discuss in depth the characters for and against each considered affinity. This been said, we continue collecting at the Laguna del Hunco locality, from which the Museo Paleontológico Egidio Feruglio has more than 8000 specimens to date, only one of which is *Huncocladus*, and we hope we will be able to find in the future more specimens and organs that help us further investigate all the hypotheses about this fossil plant.

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