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Analysis of Putative Hybrid between *Anaphalis longifolia* and *A. javanica* (Asteraceae) in Mount Talang, West Sumatra based on Anatomical Characteristics

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Abstract

The putative natural hybrid between *Anaphalis longifolia* and *Anaphalis javanica* from Talang Mountain in West Sumatra has been discovered. The aim of this research is to analyze the anatomical characteristics of the leaf and stem to clarify the status of this putative natural hybrid. The anatomical characteristics of leaves and stem were analyzed by making cross sections and paradermal sections using Johansen's method. The results show seven characteristics of this putative natural hybrid (the stomata density, length, width, and length/width ratio; the number of cortical cell layers, the range of cortex length, and the range of cortex width) that were close to *A. longifolia*, one characteristic that was close to *A. javanica* (the structure of palisade), one characteristic that was intermediate (the structure of spongy layer), and one characteristic that was a combination of both of them (the trichome morphotype). All of the foregoing characteristics can be used to support the identification of a natural hybrid of *A. longifolia* and *A. javanica*.

Abstrak

Analisis Hibrida Dugaan antara *Anaphalis longifolia* dan *A. javanica* (Asteraceae) di Gunung Talang, Sumatera Barat berdasarkan Karakter Anatomi. Beberapa individu yang diduga hibrida alami antara *A. longifolia* dan *A. javanica* dari Gunung Talang di Sumatera Barat telah ditemukan. Tujuan dari penelitian ini adalah untuk menganalisis karakteristik anatomi daun dan batang dalam menjelaskan status dugaan hibrida alami. Karakter anatomi daun dan batang diamati dari sayatan melintang dan paradermal dengan menggunakan metode Johansen. Hasil penelitian menunjukkan tujuh karakteristik dari dugaan hibrida alami (kerapatan stomata, panjang, lebar dan rasio panjang/lebar; jumlah lapisan sel kortikal, kisaran panjang sel korteks dan kisaran lebar sel korteks) dekat dengan *A. longifolia*, satu karakteristik dekat dengan *A. javanica* (susunan palisade), satu karakteristik intermediet (susunan spons) dan satu karakteristik merupakan kombinasi keduanya (jumlah deret sel trikom). Semua karakter tersebut dapat digunakan untuk mendukung identifikasi dugaan hibrida alami dari *A. longifolia* dan *A. javanica*

Keywords: Anaphalis, anatomical characteristics, putative hybrid

Introduction

Anaphalis (known as Edelweis) is a member of the Asteraceae family. In the tropics, *Anaphalis* grows at altitudes of 1,600 to 3,600 meters above sea level in hostile conditions. It can survive in soil with a low nutrient content because *Anaphalis* can be associated with *mycorrhizal* [1]. Mainland Sumatra has only two types of *Anaphalis*: *Anaphalis longifolia* and *Anaphalis javanica* [2]. Taxonomic analysis of *Anaphalis* from Mount Talang in West Sumatra also identified these two types of *Anaphalis* [3]. However, some specimens could not be classified because the morphological structure showed characteristics from both *A. longifolia* and *A. javanica* and appeared to be a putative natural hybrid. Morphological characters of putative natural hybrid showed from both *A. longifolia* and *A. javanica* are distance between nodes and segments, achene width, pattern of leaf growth, leaf margin, trichomes on the underside of the leaves, and color on the tip of the phyllaries. The distance between nodes and segments, achene width, and the pattern of leaf growth are

characteristic of *A. javanica*. However, the leaf margin, the trichomes on the underside of the leaves, and the color on the tip of the phyllaries are more typical of *A. longifolia*.

Morphological structure comparison is often used to find evidence of hybridization [3-9]. Morphologically, hybrids typically display the intermediate of parental characteristics, but it should be noted that a few morphological intermediates may develop through convergent evolution or environmental selection, not through hybridization [6]. However, as environmental factors also influence morphology this is not always a completely accurate method. So, it is very difficult to confirm the hybrids strictly from morphological evidence. Recently, with the use of anatomical methods, a large number of natural hybrids have been confirmed by research. Additional data like anatomical features has been used to establish the status of a putative hybrid in the cases of *Nothofagus obliqua* (Mirb.) Oerst and *N. glauca* (Phil.) Krasser [10]; *Potamogeton crispus* and *P. praelongus* [11]; *Cohniella ascendens* and *C. brachyphylla* [12]. This more accurate dual approach using anatomy established the status of the putative natural hybrid of *Anaphalis* discovered by the authors.

Materials and Methods

The investigation was conducted from February to September 2014 and took place in the Laboratory of Plant Structure and Development, Department of Biology, Andalas University, Padang. Samples of fresh leaves and stems from *A. longifolia*, *A. javanica*, and the putative hybrid plants were collected from Talang Mountain. A total of nine (9) individuals were collected: three (3) individuals were *A. longifolia*, three (3) individuals were *A. javanica*, and three (3) individuals were hybrids. Leaf materials and stems were prepared from mature plants.

Fresh paradermal leaf sections were used for analysis. Transverse sections of leaves and stems were prepared using Johansen's method. These specimens were fixed for 24 hours in FAA solvent (glacial acetic acid: formalin: alcohol 96%: aquadest = 5:10:50:35) and then dehydrated with Johansen solvent [13]. The specimens were then infiltrated with a soft paraffin solution and solid paraffin. The preparation was then cut with a rotary microtome into 12 µm sections and stained with Safranin-Fastgreen.

The transverse sections were used to study the type and position of stomata, the type of trichomes, the morphotype of the trichomes cells, the palisade structure and position, and the spongy layer structure of the leaves were studied using the transverse sections. Paradermal sections furnished information on the length of stomata, its width, density, and the width/length ratio. Subsequently, the transverse stem sections were used to determine the number of cortical cell layers and the range of values for length and width of the cortical cells.

Results and Discussion

Of the fourteen anatomical characteristics measured, ten characteristics of the putative hybrid plants differed from *A. longifolia* and *A. javanica*. These include both quantitative and qualitative characteristics as shown in Table 1.

Seven of the quantitative anatomical characteristics found in the putative hybrid plants can be classified as characteristic of *A. longifolia*. From three qualitative anatomy characteristics that were found, one was close to *A. javanica*; one was intermediate between that of *A. longifolia* and *A. javanica,* and the other was a combination of both putative parent plants.

The anatomical characteristics of the hybrid plants that were close to *A. longifolia* included the stomata density, its length, width, ratio, the number of cortical cell layers, the range of cortex length and width (Table 1, Figure 1 and Figure 2). One anatomical characteristic of the putative hybrid plants close to *A. javanica* was the palisade structure (Table 1). Like *A. javanica*, the structure was less dense and neat. This differs from the palisade structure of *A. longifolia* which is tight, cohesive, and neat; there are no spaces between the cells within the palisade layer (Figure 3).

The intermediate anatomical characteristic that was found in the hybrid plants was the spongy structure (Table 1). The spongy structure in the hybrid plants showed only narrow spaces between the cells. *A. longifolia* has a tighter, more cohesive, and neater spongy structure with no spaces between the cells while *A. javanica* has much larger spaces between the cells within the spongy layer (Figure 3) than the putative hybrid.

The morphotype characteristics of the trichomes in the putative hybrid plant appear to be a combination of both *A. longifolia* and *A. javanica*. *A. longifolia* has multicellular-unicellular-*capitate* glandular trichomes, while *A. javanica* has a unicellular-*capitate* glandular pattern (Table 1 and Figure 4). The putative hybrid exhibits both of these morphologies.

Previous studies of naturally-occurring hybrids have investigated the relationship between the anatomical characteristics of putative hybrids and the putative parent species. Putative *Nothofagus leoni* hybrids have a tendency to show anatomical similarities with one putative parent and characteristics that were of an intermediate character between the two putative parent plants. The researchers found that five out of ten characteristics of the putative hybrid were close to *N. glauca*, three characteristics resembled *N. obliqua*, while the other

two were identified as intermediate characteristics [10]. A more recent study by the researchers also found that five out of nine anatomical characteristics of the putative hybrid of *Cohniella* were close to *C. ascendens;* while one of the characteristics was close to *C. brachyphylla* [12].

Table 1 shows that the quantitative anatomical characteristics of the putative hybrid of *A. longifolia* and *A. javanica* resembles that of one of the putative parent species. The qualitative anatomical characteristics of the putative hybrid were intermediate between both putative

Table 1. Anatomical Characteristics of a Putative Natural Hybrid of *A. longifolia* and *A. javanica*

Characteristics	*A. longifolia*	Putative Hybrid	*A. javanica*
1. Leaves			
Stomata			
Stomata density (N/mm^2)	67 ± 6.11	65 ± 8.08	34 ± 2
Length (µm)	86.28 ± 4.86	87.55 ± 4.00	115.18 ± 5.14
Width (µm)	75.65 ± 5.95	76.92 ± 5.14	87.55 ± 4.00
Ratio (µm)	1.14 ± 0.82	1.14 ± 0.78	1.32 ± 1.28
Trichomes			
Trichome morphotype	Multicellular-unicellular-capitate glandular	Unicellular-capitate glandular Multicellular-unicellular-capitate glandular	Unicellular-capitate glandular
Mesophyll			
Palisade structure	Tight, cohesive, and neat. No space between cells	Less dense and neat	Less dense and neat
Spongy structure	Strict, more cohesive, and more tidy spongy structure with no spaces between the cells	Small spaces between cells	Large spaces between cells
2. Stem			
Number of cortical cell layers	5–8	4–8	8–9
The range of values for cortex length (µm)	34–170	42.5–170	25.5–110.5
The range of values for cortex width (µm)	25.5–102	25.5– 10.5	25.5–85

Note: Observations of stomata were conducted with three replicates for each individual.

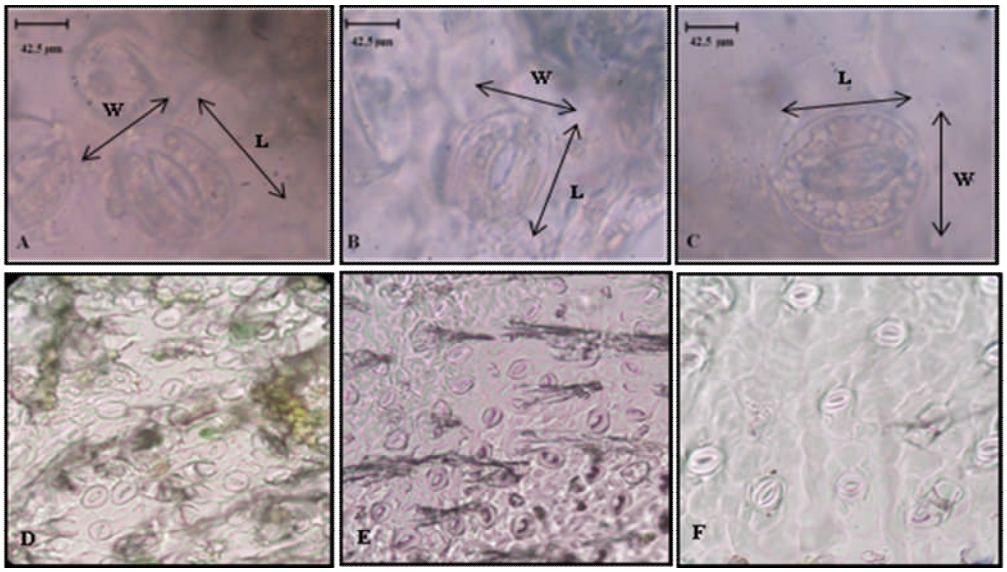

Figure 1. Paradermal Leaf Section of *Anaphalis* (A). Length and Width of Stomata *A. longifolia* (86.28 ± 4.86 µm and 75.65 ± 5.95 µm); (B). Length and Width of Stomata Putative Hybrid (87.55 ± 4.00 µm and 76.92 ± 5.14 µm); (C). Length and Width of Stomata *A. javanica* (115.18 ± 5.14 µm and 87.55 ± 4.00 µm); (D). Stomatal Density of *A. longifolia* (67 ± 6.11 N/mm^2); Stomatal Density of Putative Hybrid (65 ± 8.08 N/mm^2); (F). Stomatal Density of *A. javanica* (34 ± 2 N/mm^2)

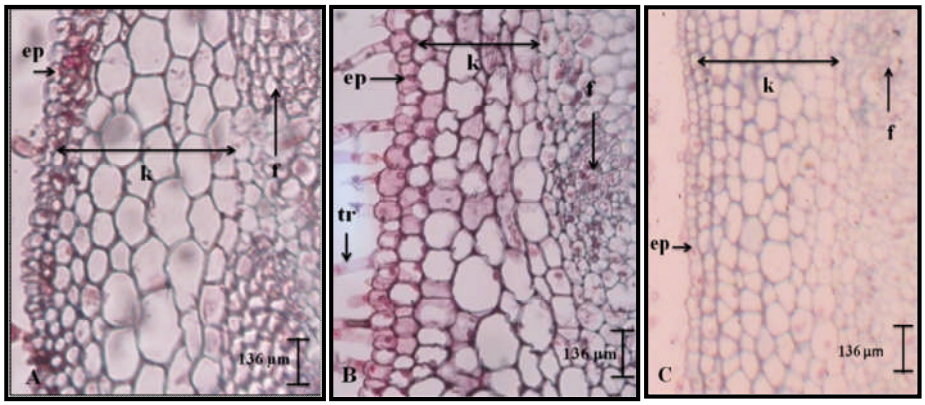

Figure 2. Transverse Section Stem of *Anaphalis*: *A. longifolia* (A), Putative Hybrid (B), *A. javanica* (C). Shown are Epidermis (ep), Cortical Layer (k), Phloem (f), and Trichomes (tr). The Number of Cortical Cell Layers of *A. longifolia* and Putative Hybrid Inclined Slightly, so that the Size of Cortical Cell Layers looked Larger, while the Number of Cortical Cell Layers of *A. javanica* Tend to be Numerous so that the Size of Cortical Cell Layers Seemed Small

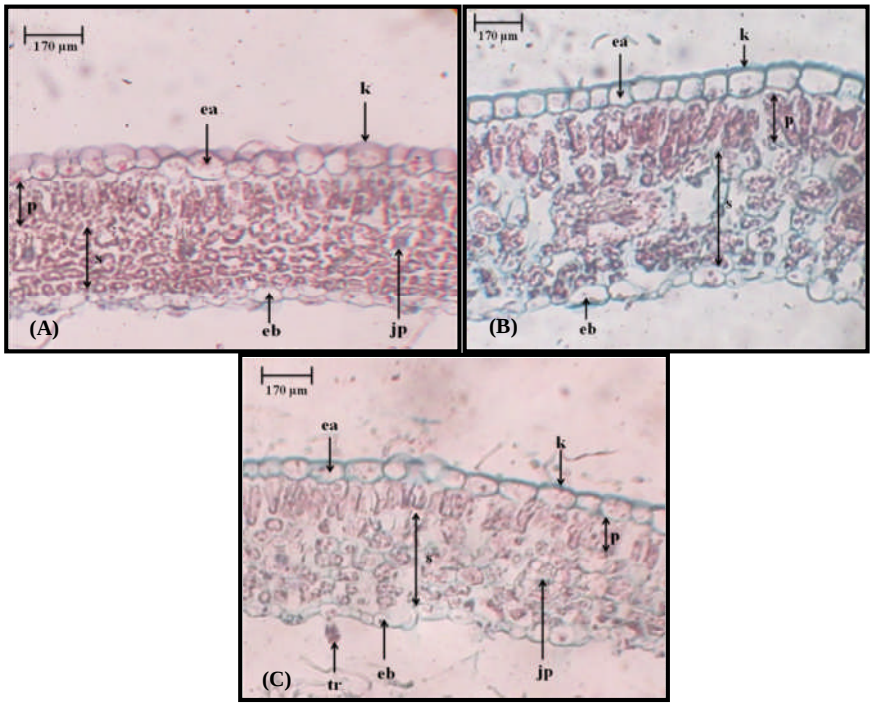

Figure 3. Transverse Section Leaf of *Anaphalis*: *A. longifolia* (A), Putative Hybrid (B), and *A. javanica* (C). Shown are cuticles (k), Upper Epidermis (ea), Palisade Layer (p), Spongy Layer (s), Vascular Layer (jp), Lower Epidermis (eb), and Trichomes (tr). The Spongy Structure in the Hybrid Plants showed only Narrow Spaces between the Cells. *A. longifolia* has a Strict, more Cohesive, and Tidier Spongy Structure with no Spaces between the Cells while *A. javanica*, has much Larger Spaces between the Cells within the Spongy Layer than the Putative Hybrid. The Palisade Structure in the Hybrid, like *A. javanica*, was less Dense and Neat. This Differs from the Palisade Structure of *A. longifolia* which is Tight, Cohesive, and Neat; there are no Spaces between the Cells within the Palisade Layer

parent species. Studies investigating a putative hybrid cross between *Cucurbita ficifolia* x *C. maxima* reported that a quantitative morphological characteristic of that putative hybrid cross resembled *C. maxima*, while the qualitative morphological characteristics such as leaf margin, shape, and the color of the fruit were intermediate characteristics between *C. ficifolia* and *C. maxima* [14].

Most anatomical characteristics from the putative hybrid of *A. longifolia* and *A. javanica* resembled that of *A. longifolia*, including the stomata density, its length, width, ratio, the number of cortical cell layers, the range of cortex length and width. This corresponds to the wider distribution of *A. longifolia* compared to *A. javanica*. *A. longifolia* is found on Mount Talang between 1,185–2,501

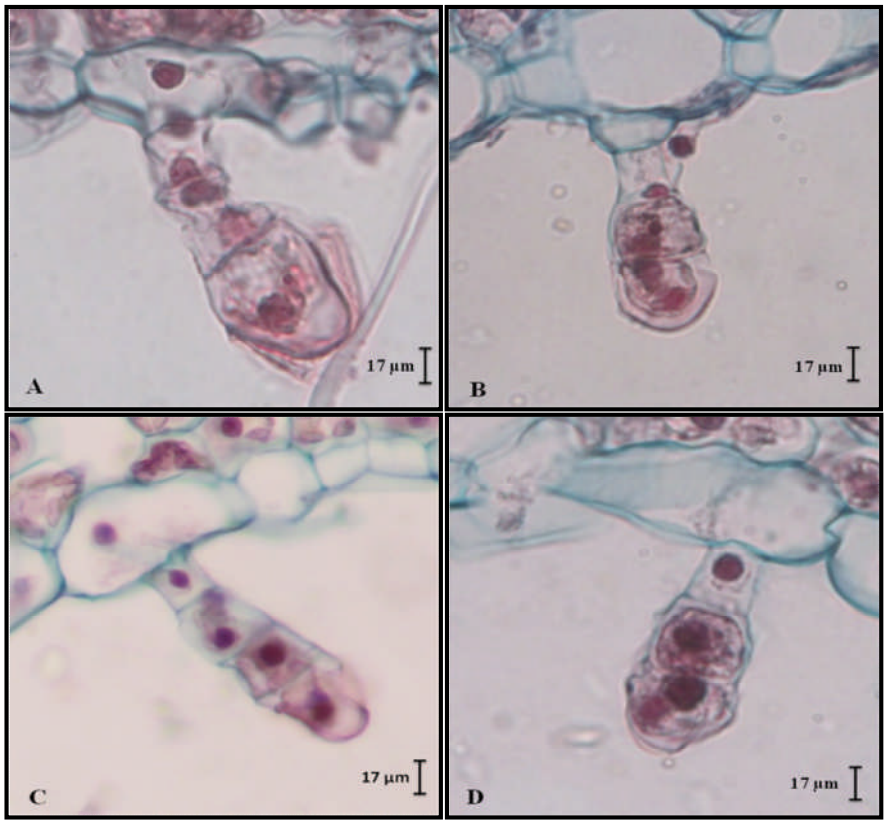

Figure 4. ***Anaphalis*** **Leaf Trichomes: *A. longifolia* (A), *A. javanica* (B), and Putative Hybrid (C and D). *A. longifolia* has Multicellular-unicellular *capitates* Glandular Trichomes, while *A. javanica* has a unicellular-*capitate* Glandular Pattern. The Putative Hybrid Shows both of these Morphologies**

meters above sea level, while *A. javanica* grows between 2,330–2,797 meters above sea level. The wider distribution of *A. longifolia* suggests this species has more dominant features that enable the plant to survive natural selection [15]. This reflects the observation that *N. glauca* also dominated its habitat having a wider distribution than *N. oblique* hence the anatomical characteristics present in its putative hybrid reflected this with a closer resemblance to *N. glauca* [10].

Conclusions

The characteristics of stomatal density, length, width, length/width ratio; trichome morphotype; palisade structure; spongy structure; the number of cortical cell layers; and the range of values for cortex length and width can be used to support the identification of this plant as a natural hybrid between *A. longifolia* and *A. javanica.*

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