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## Cultivation experiments with *Aneura pinguis*. A contribution to the separation of *Aneura maxima* and *A. pinguis*

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**Summary:** The habitat as well as the unicellular thallus margins of *Aneura maxima* lead to the assumption that this species could be a hygromorphosis of *A. pinguis*. Therefore specimens of *A. pinguis* were cultivated in water. Even after four months the multicellular thallus margins were retained, which proved to be a stable character to separate *A. pinguis* from *A. maxima*. Therefore plants even with a small unistratose border, which are attributed to aquatic forms of *A. pinguis*, may belong to *A. maxima*.

It is supposed that *A. maxima* was overlooked in Europe but regarded as aquatic forms of *A. pinguis* named as *fo. rivularis*. Such plants show unicellular thallus margins and are regarded as hygrophytic form of *A. maxima*. The presence viz. absence of an unicellular thallus margin seems to be a sufficient character to separate both species.

The discussion about the width of the unistratose thallus margin is useless because the type of *A. maxima* has only 2-4 rows of unicellular cells and not 6-12 or more as attributed by European hepaticologists. This raises doubts whether the European material is identical with *A. maxima* rather with *A. pellioides* from Japan.

*Aneura maxima* is a species which was described from Java and recognized in North America (Schuster 1992), reported only twenty years ago from Europe in the Belgian Ardennes (Andriessen et al. 1992) and recorded from numerous other countries. This species has probably ever existed in Europe but was overlooked as indicated by literature citations in the past, in which it was described as *fo. rivularis* or *fo. fluitans*.

The circumscription of this species as well as the segregation from *A. pinguis* is subject of discussion, which raises the question whether *A. maxima* is a modification of *A. pinguis* in wet habitats. A major character of *A. maxima* is the presence of unicellular thallus wings (which lack in plants of *A. pinguis* growing on soil or rotten wood). This character could be a result of hygromorphism in wet growth conditions and the variation of this character an expression of different moisture.

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How can be decided whether *A. maxima* is a modification or not? There are principally different ways.

1. The observation of mixed tufts. If two different phenotypes grow aside in the same habitat, they are different genotypes, since they live under the same ecological conditions. The value of this simple method is largely forgotten or ignored. Pictures of different phenotypes of *Hypnum cupressiforme* growing in mixed tufts revealed that these are genotypes which gain taxonomic recognition (Frahm 2009).

This method cannot be applied to the problem of *Aneura maxima* and *A. pinguis*, because they exist under different ecological conditions.

2. Molecular analysis principally allows a separation of genotypes, however in the case of *A. pinguis*, the morphological uniform species *Aneura pinguis* consists of various different hidden segregates that this method cannot be applied. According to Martin Nebel and Dietmar Quandt (pers. comm.), *A. maxima* is not distinguishable in molecular analyses.

3. Cultivation experiments allow to test whether characters remain stable under different ecological conditions. Especially cultures under hyperhygric conditions can easily be performed, in which plants are kept in closed plastic boxes. If a character stays, it is genetically fixed and not a modification induced by the extreme situation.

Especially the fact that *A. maxima* cannot be distinguished in molecular analyses raises doubts whether this is a good species or merely a modification of *A. pinguis*. Therefore cultivation experiments were performed to test this hypothesis (case 1), completed by observations in the field (case 2).

#### CASE 1

##### **Methods**

To test whether *Aneura maxima* is a modification of wet habitats, plants of *A. pinguis* were kept in hydroculture to see whether they can be performed to *A. maxima*. Two samples of *A. pinguis* were collected by Michael Siemsen and Thomas Homm during a fieldtrip of the Mecklenburger Moosfloristen on April, 2012 on the island of Zingst at the coast of the Baltic Sea in the state Mecklenburg-Vorpommern, East Germany. The plants were roughly cleaned from soil and transferred into mineral water in open freezer-plastic-boxes and kept at a N-exposed window at room temperature (about 20°). Evaporated water was replaced by sparkling mineral water, of which the CO<sub>2</sub> had a positive impact on the growth of the plants. The changes of the plants were recorded in monthly intervals.

##### **Results**

The specimens of *A. pinguis* were comparably small when collected, only 5-8 mm long and thick and fleshy (figs. 1-4). Perhaps they had just regenerated after the winter. Within 4 months, they developed new narrow shoots about 1 cm long under water (fig. 5-6). The newly developed thallus was considerably thinner, however, it retained its multicellular margin (fig. 8) and did not develop hyaline unicellular margins as typical for *A. maxima*.

#### CASE 2

In some cases, the nature is the experimentator. For more than twenty years I am observing a population of "*Aneura pinguis* f. *rivularis*" growing under water in a mill pond in the Vosges

Mountains, France. The thalli are very narrow, up to 5 mm wide, but to 10 cm long and more und irregularly branched (fig. 9) These plants are growing permanently in hydroculture and have changed their appearance accordingly. The thalli are sometimes slightly sinuose and have an unicellular margin up to 8 cells wide. The margin is chlorophyllose and only sometimes hyaline. The width of the thalli may be a result of nutrient poor water (similar as the linear thalli of var. *angustior* growing on peat or dune sand). Grown on wet sand, this forms retains the unistratose border in the innovations and does not develop multistratose borders like in *A. pinguis*.

## Discussion

At first, *Aneura maxima* was supposed to be introduced by North American troupes during World War II (Andriessen et al. 1995). However, records of this species from many parts of Europe during the next years raises the question whether *Aneura maxima* was in fact native in Europe but overlooked or not recognized. The latter hypothesis is supported by the indication of *Aneura pinguis* fo. *rivularis* by Koppe (1935), who wrote: "Different [from *A. pinguis* s.str.] is fo. *rivularis* with large, up to 10 cm long thalli, which are strongly sinuose at margins. Perhaps they correspond to fo. *fluitans*, which Beckhaus mentioned in 1856. I observed it in cool mountainous streams" (translated from German). Müller (1954) listed no infraspecific taxa but mentioned that the species is extremely variable and that the extreme modifications can hardly be taken for members of the same species.

The separation of *A. maxima* from *A. pinguis* differs from author to author. Schuster (1992) characterizes *A. maxima* by the presence of unistratose thallus wings and *A. pinguis* by a thallus "pluristratose to the edge (occasionally with an unistratose margin one cell wide)", and furthermore the number of oil bodies (8-15 in *A. pinguis*, 30-50 in *A. maxima*). These characters are quite clear cut. Paton (1999) relates the differences with the statement "that several British gatherings of robust aquatic thalli indicate that they represent an extreme form of *A. pinguis* and are not referable to *A. maxima*". She reports forms of *A. pinguis* with unistratose wings of 1-6(10) cells, but why do such forms not belong to *A. maxima*? It is hardly understandable how authors can decide how many rows of hyaline cells has *A. maxima* and how many *A. pinguis* grown in a swamp, that specimens with less than 6 rows are *A. pinguis* and more than 6 rows are *A. maxima*. Schuster (1992) states that Schiffner in his original description of *A. maxima* indicates an unistratose border of only 2-4 cells, which is in strong contrast to Paton (1999). Damsholt (2002) again separates *A. pinguis* from *A. maxima* by unistratose thallus wings 4-5 cells wide or 6-12 cells wide, although he illustrates a transverse section of *A. pinguis* without unistratose border.

The present cultivation experiment shows clearly that aquatic forms of *A. pinguis* do not develop unicellular thallus wings with the consequence that plants with 6 or less unistratose rows cannot be attributed to *A. aquatic* forms of *A. pinguis* but must be referred to *A. maxima*.

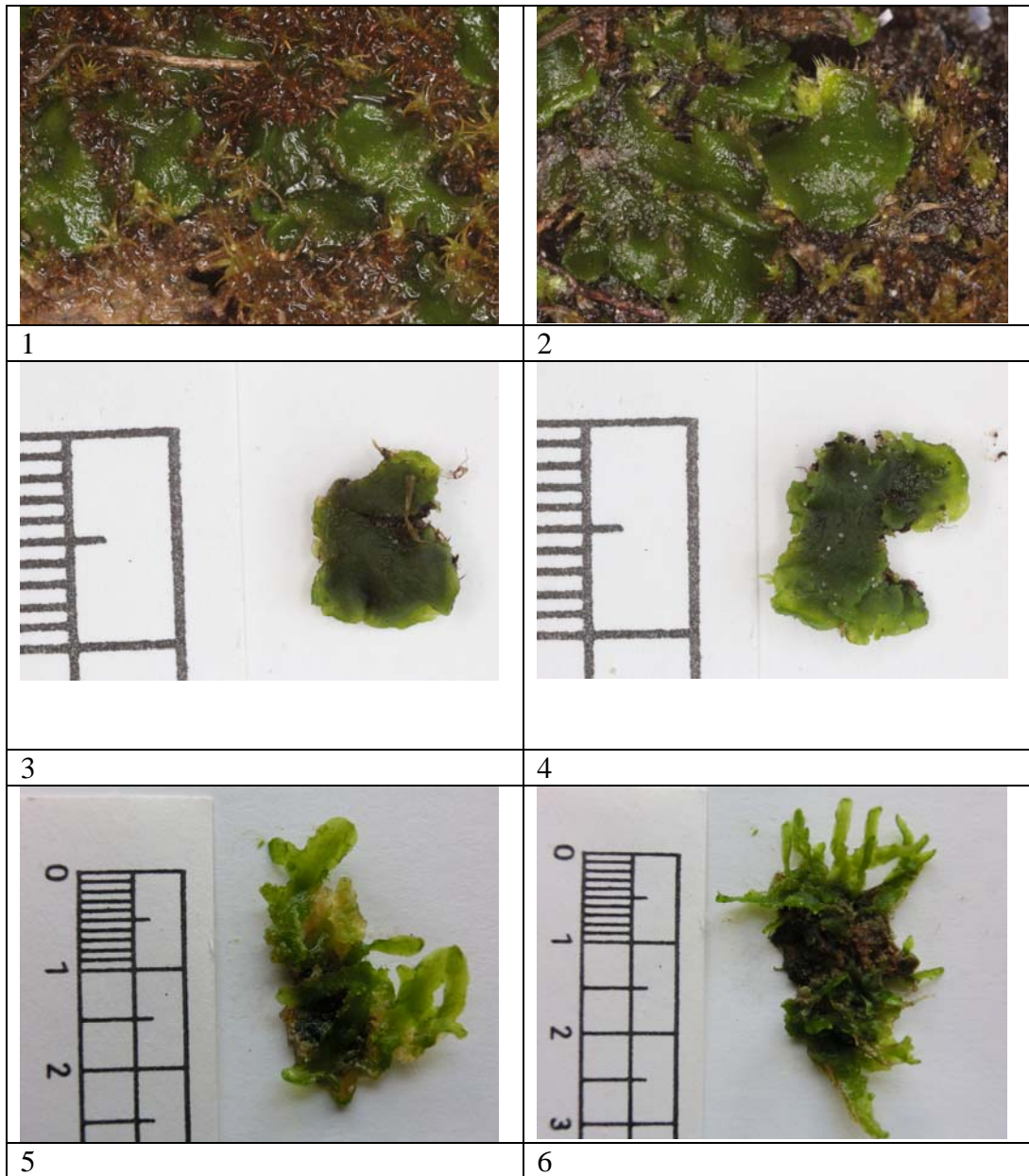
The number of oil bodies seems to vary widely (cf. Frahm 2011) and is not practical since only fresh material can be identified with the help of this character.

The application of the epithet *maxima* to the European specimens is still doubtful because Schuster (1992) has no doubt that the North American plants are identical with *A. pellioides* from Japan but has "considerable reservations" that *A. pellioides* is synonymous with *A. maxima*.

## Literature

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Figs. 1-4: Material of *Aneura pinguis* at the begin of the experiment and after four months (5-6)

