



On the presence of *Asplenium ×alternifolium* nsubsp. *heufleri* (Aspleniaceae) in Belgium

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Illustrations: T. Gyselinck [Fig. 2 adapted from Pangua *et al.* (1989)].

ABSTRACT. – In 2010, a specimen of *A. ×alternifolium* nsubsp. *heufleri* was found in Carrière de la Falize (Aywaille). This taxon is a hybrid of *A. trichomanes* subsp. *quadrivalens* (Maidenhair Spleenwort) and *A. septentrionale* subsp. *septentrionale* (Northern Spleenwort). Here, we describe the phylogeny, morphology and ecology of this hybrid and its parents. Despite the absence of this taxon in the Standard Flora of Belgium, it is probably not new to the flora of Belgium.

RÉSUMÉ. – Sur la présence d'*Asplenium ×alternifolium* nsubsp. *heufleri* (Aspleniaceae) en Belgique. En 2010, un spécimen d'*A. ×alternifolium* nsubsp. *heufleri* a été trouvé dans la Carrière de la Falize (Aywaille). Ce taxon est un hybride entre *A. trichomanes* subsp. *quadrivalens* (Fausse capillaire) et *A. septentrionale* subsp. *septentrionale* (Doradille du Nord). Nous décrivons ici la phylogénie, la morphologie et l'écologie de cet hybride et de ses parents. Malgré l'absence de ce taxon dans la *Nouvelle Flore de la Belgique*, il ne s'agit probablement pas d'une première trouvaille sur le territoire belge.

SAMENVATTING. – Over het voorkomen van *Asplenium ×alternifolium* nsubsp. *heufleri* (Aspleniaceae) in België. In 2010 werd een exemplaar van *A. ×alternifolium* nsubsp. *heufleri* gevonden in de Carrière de la Falize (Aywaille). Dit taxon ontstaat na hybridisatie van *A. trichomanes* subsp. *quadrivalens* (Steenbreekvaren) met *A. septentrionale* subsp. *septentrionale* (Noordse streepvaren). Hier beschrijven we de fylogenie, morfologie, ecologie en verspreiding van zowel deze hybride als haar oudersoorten. Ondanks het ontbreken van dit taxon in de Standaardflora van België, is het waarschijnlijk niet nieuw voor de flora van België.

Introduction: discovery of the plant

In 2010, a group of biology students visited the Carrière de la Falize ($50^{\circ} 28' 43''$ N, $5^{\circ} 41' 07''$ E), a nature reserve situated next to the river Amblève near the village of Aywaille (S. Jacobs pers. comm., 2010). This former quarry is part of a larger area called Heid des Gattes, which is renowned for its geology with silicate rocks alternating with limestone synclines (Mottequin & Marion 2012). This exceptional combination of both acid and alkaline soils allowed the development of a unique vegetation (Lejeune 1811, Darimont 1945, Saintenoy-Simon & Duvigneaud 1996).

One well-represented plant family in Heid des Gattes is the spleenworts (Aspleniaceae). Here, a rare combination of both basophilous species, such as *Asplenium ceterach*

L., *A. scolopendrium* L. and *A. ruta-muraria* L., and typical acidophiles, such as *A. septentrionale* (L.) Hoffmann and *A. adiantum-nigrum* L., can be observed. In addition, high densities of plants belonging to the *A. trichomanes* L. complex can be found.

Futhermore, the students noticed a fern displaying intermediate characteristics between *A. septentrionale* and *A. trichomanes*. After consulting *Flora van België*... (Lambinon *et al.* 1998), they identified it as the rare *A. ×alternifolium* Wulfen nsubsp. *alternifolium*. Photographs taken during a later visit, in March 2011, were sent to Prof. Em. Dr. R. Viane (Research Group Pteridology, Ghent University). He revealed that the finding most likely belonged to a much more rare taxon, *A. ×alternifolium* nsubsp. *heufleri* (Reichardt) Aizpuru, Catalan & Sal-



Figure 1. *Asplenium ×alternifolium nsubsp. heufleri* from the Carrière de la Falize (Aywaille, Liège). W. Tavernier identified the specimens based on these pictures.

vo, which is missing from the latest versions of both the Dutch and French editions of the standard Belgian Flora (Lambinon *et al.* 1998, Lambinon & Verloove 2012; Viane pers. comm. 2011). This same comment was given independently by the third author, after investigating more recent pictures taken in November 2017 (Fig. 1). In this article, we describe the phylogeny and morphology of *A. ×alternifolium* nsubsp. *alternifolium* and nsubsp. *heufleri*, as well as their ecology and distribution. Next, we discuss whether *A. ×alternifolium* nsubsp. *heufleri* should be considered as new for the flora of Belgium.

Taxon description and ecology

The genus *Asplenium* L. (Aspleniaceae) is known for its high number of polyploid taxa (Schneider *et al.* 2017). This explains the high amount of hybrids within this taxon (Meyer 1960, Reichstein 1982, Sleep 1983). One of the most intensively studied *Asplenium* hybrids is *A. ×alternifolium*, which results from a hybridisation between *A. septentrionale* and *A. trichomanes* (Ceutrot 1926, Reichstein 1982, Barrett *et al.* 1998, Stöhr 2010).

Asplenium septentrionale is a rhizomatous herbaceous species with simple to sparsely forked wintergreen leaves (Lawalrée 1950, Lambinon *et al.* 1998, Stace 2010; see Fig. 2b). They dichotomously divide up to 3 times into linear to narrow elliptic segments. The petiole is green and 2-3 times the length of the lamina. Fronds are 5-15 cm in size. *Asplenium septentrionale* occurs in Europe, North America and Asia (Rünk *et al.* 2014, GBIF 2017). In Europe it has a widespread distribution with the highest densities in the west (Tutin *et al.* 1993). Within Belgium, the species is rare and restricted to the valleys of the river Meuse and its tributaries (Delvosalle 2010, Lambinon & Verloove 2012), except for one locality on the walls of an

old abbey near Bornem, Antwerp (De Kesel 1991, Van Landuyt *et al.* 2006).

Two cytotypes of *A. septentrionale* have been described: a tetraploid *A. septentrionale* subsp. *septentrionale* ($2n = 144$) and a diploid *A. septentrionale* subsp. *caucasicum* Fraser-Jenkins & Lovis ($2n = 72$) (Fraser-Jenkins & Lovis 1980). The former has a widespread distribution, including Belgium, while the latter is currently only known from southwestern Asia (Viane & Reichstein 2003). Morphologically, there is almost no difference between them (Viane & Reichstein 2003). Both are restricted to acidic soils like rock surfaces and old walls, always with full sunlight exposure (Benlioğlu *et al.* 1998, Rünk *et al.* 2014). Only the tetraploid form of *A. septentrionale* is known to hybridize with *A. trichomanes* (Barrett *et al.* 1998).

Asplenium trichomanes is a rhizomatous herbaceous species with 1-pinnate linear wintergreen leaves (Lawalrée 1950, Lambinon *et al.* 1998, Stace 2010; see Fig. 2a and c). The rachis is dark brown to black and the petiole is about $\frac{1}{4}$ th as long as the lamina. Fronds generally reach up to a length of 4-20(-45) cm. The species has a worldwide distribution and is found growing on rock surfaces and old walls (Lovis 1958). *Asplenium trichomanes* occurs in nearly all temperate regions and at high altitudes in the tropics. It is found throughout Europe (Tutin *et al.* 1993). In Belgium, it is common, especially in the Meuse, Ardenne and Lorraine flora districts. It is uncommon in the Brabant district, while it is rare in other parts of the country (Delvosalle 2010, Lambinon & Verloove 2012).

Several subspecific cytotypes of *A. trichomanes* have been described, of which six are known in Europe and three in Belgium (Leurquin 2007, Christenhusz & von Raab-Straube 2013). They mainly diverge in their eco-

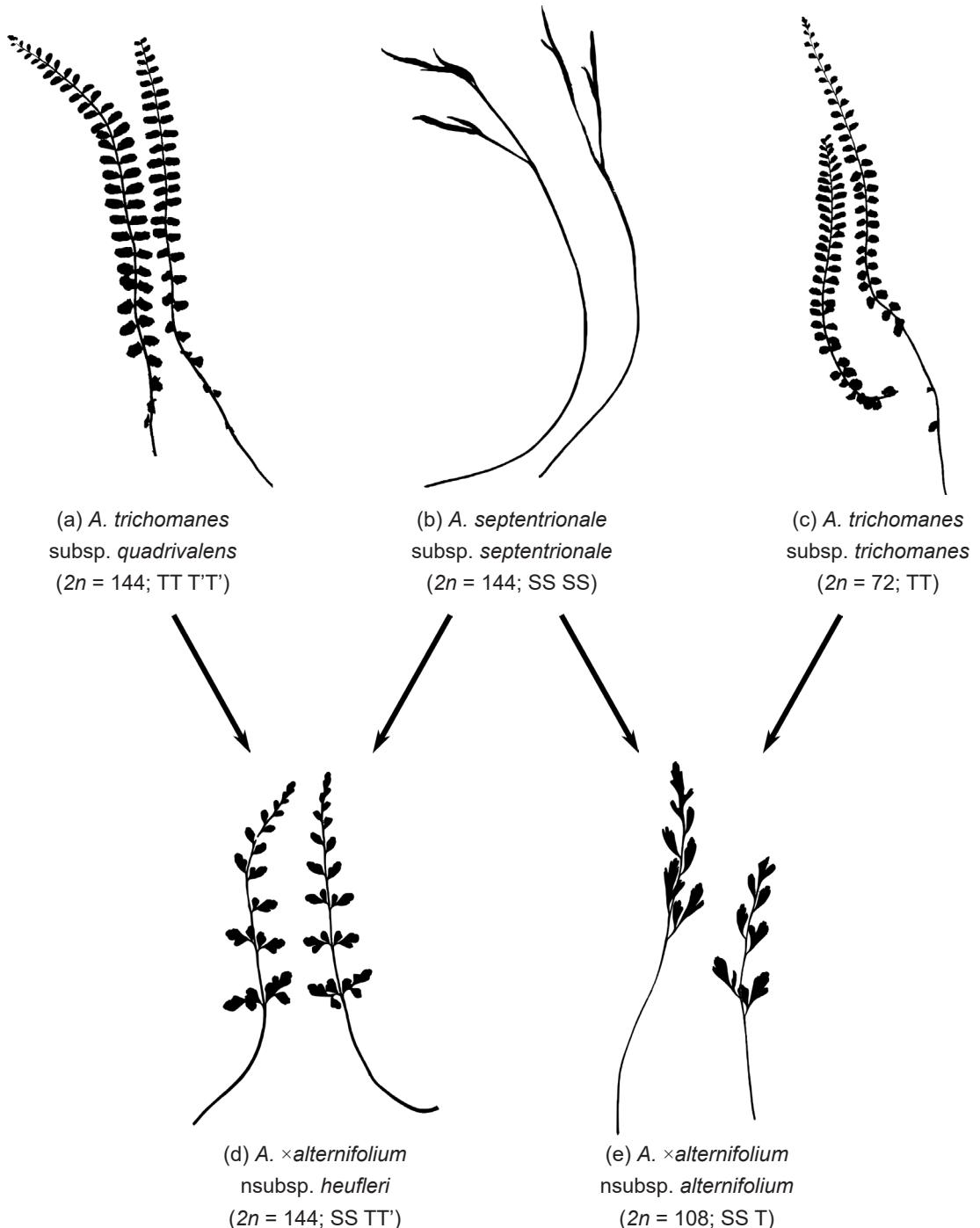


Figure 2. Hybridization scheme of *Asplenium × alternifolium* adapted from Barrett et al. (1998), with images (a) and (c) adapted from Pangua et al. (1989), and (b), (d) and (e) collected by the first author in Thayatal National Park, Austria. Note that the images are not scaled and that they show fertile fronds.

logical preferences and show low levels of morphological variation (Ekrt & Štech 2008). Only two of them are currently known to hybridize with *A. septentrionale*: a diploid *A. trichomanes* subsp. *trichomanes* ($2n = 72$; Fig. 2c) and a tetraploid *A. trichomanes* subsp. *quadrivalens* D.E. Mey. ($2n = 144$; Fig. 2a) (Barrett et al. 1998). Both cytotypes occur throughout the species' range (Tutin et al. 1993). The diploid cytotype is bound to open and sunny non-calcareous rocky habitats (Tutin et al. 1993, Leurquin

2007) similarly to *A. septentrionale* (Barrett et al. 1998). In Belgium, it is restricted to the Ardenne flora district (Lambinon & Verloove 2012). The tetraploid cytotype *A. trichomanes* subsp. *quadrivalens* has a wider ecological amplitude, varying from siliceous to calcareous habitats (Tutin et al. 1993, Leurquin 2007), but prefers, in contrast to *A. septentrionale*, more shaded locations (Barrett et al. 1998). *Asplenium trichomanes* subsp. *quadrivalens* is the most common subspecies of *A. trichomanes* and occurs

Table 1. Morphological characteristics of *A. ×alternifolium* nsubsp. *heufleri* and *A. ×alternifolium* nsubsp. *alternifolium*, adapted from Bouharmont (1966) and Krause (1996)

	<i>A. ×a. nsubsp. heufleri</i>	<i>A. ×a. nsubsp. alternifolium</i>
Tuft	thin, with only a few fronds	dense, with numerous fronds
Nr. of leaf segments at each frond side	3 – 9 (– 12)	2 – 5
Leaf segment shape	broad, round to rhomboid	elongated; lower leaves with 2 – 3 lobes
Positioning of leave segments	(nearly) opposite	alternate
Petiole colour	dark brown	dull brown
Ventral side rachis	shiny brown over more than half the length	dull brown at the base

throughout the Belgian distribution range of the species (Lambinon & Verloove 2012). One morphological difference between both subspecies is the petiole colour, which is reddish-brown in *A. trichomanes* subsp. *trichomanes* and brown-black in *A. trichomanes* subsp. *quadrivalens* (Leurquin 2007), a difference which can be found in their respective hybrid offspring *A. ×alternifolium* (Table 1; Bouharmont 1966).

A. ×alternifolium (*A. ×a.*) occurs as two nothosubspecies, depending on which subspecies of *A. trichomanes* crosses with *A. septentrionale* (Fig. 2d, e; Table 1; Barrett *et al.* 1998). In most cases, a triploid *A. ×a. nsubsp. alternifolium* ($2n = 108$; Fig. 2e) is obtained, which arises when *A. septentrionale* hybridizes with *A. trichomanes* subsp. *trichomanes*. In very rare occasions, however, a tetraploid *A. ×a. nsubsp. heufleri* ($2n = 144$; Fig. 2d) is formed when *A. septentrionale* hybridises with *A. trichomanes* subsp. *quadrivalens*. Although both *A. trichomanes* subsp. *trichomanes* and *A. trichomanes* subsp. *quadrivalens* grow together with *A. septentrionale* in many localities throughout Europe, it seems that nearly all individuals of *A. ×a.* are triploid (Bouharmont 1966, Barrett *et al.* 1998). The rarity of the tetraploid *A. ×a. nsubsp. heufleri* is most likely explained by a dissimilarity in micro-niches (shaded vs. open) between both parent taxa, which can in turn affect phenology (Barrett *et al.* 1998). Morphologically, the differences between the two *A. ×a.* nothosubspecies are more pronounced than between *A. trichomanes* subsp. *trichomanes* and *A. trichomanes* subsp. *quadrivalens* (Fig. 2, Table 1). This is a result of the differing ploidy levels between the nothosubspecies, as the tetraploid *A. ×a.* has an extra set of *A. trichomanes* chromosomes and thus resembles it more closely (Bouharmont 1966).

Herbarium: Aywwaille, carrière du Goiveux, 08.2018, Thomas Gyselinck s.n. (BR0000025616644V; available on line at: <https://www.botanicalcollections.be/specimen/BR0000025616644V>). [The herbarium label was established by the fourth author, based on data from Waarnemingen.be. The latter erroneously situates the *Asplenium* in the nearby Carrière du Goiveux.]

New for the Belgian flora?

Lawalrée (1950) reported the presence of two growth forms of *Asplenium ×breynei* Koch, the former name of *A. ×a.* (Reichstein 1982), in Belgium. He indicated eight

localities where *A. ×a. nsubsp. heufleri* was found. More recently, cytological research on two neighbouring individuals from Les Rochers du Collyre at Ciergnon (Vallée de la Lesse, Belgium), revealed one triploid and one tetraploid individual (Bouharmont 1966). Subsequently, all 128 herbarium specimens in Meise Botanic Garden, including the eight specimens reported by Lawalrée (1950), were checked using morphological characteristics derived from these two specimens (Table 1). However, only two individuals of *A. ×a. nsubsp. heufleri* could be identified, none of which mentioned by Lawalrée (1950). One tetraploid individual was also found on Les Rochers du Collyre and one in the Grand Duchy of Luxembourg (location not further specified). Thus, this taxon is not new for the Belgian flora.

Interestingly, however, *A. ×a. nsubsp. heufleri* is only mentioned in the 4th edition of the *Nouvelle Flore de la Belgique...* (Lambinon *et al.* 1992), while all other editions, including the most recent ones, have missed this taxon (De Langhe *et al.* 1967, 1973, 1983, 1988; Lambinon *et al.* 1998, 2004; Lambinon & Verloove 2012). Although the authors of the various editions of these floras have motivated each adjustment, nothing can be found regarding the removal of this taxon from the 4th French edition (Lambinon *et al.* 1994a, 1994b). We therefore recommend the inclusion of *A. ×a. nsubsp. heufleri* in future editions of the standard Belgian Flora. This will hopefully allow the discovery of new localities in Belgium and adjacent territories.

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