Another garden escaped fern in Luxembourg – *Adiantum aleuticum* (Rupr.) C.A.Paris (Pteridaceae)

Yves Krippel[1,2], Gil Gallego[1,2], Sylvie Hermant[2] & Guy Colling[2]

[1] Naturpark Öewersauer, 15, rue de Lultzhausen, L-9650 Esch-sur-Sûre · yves.krippel@naturpark-sure.lu https://orcid.org/0000-0003-2700-8347 · gil.gallego@naturpark-sure.lu https://orcid.org/0009-0005-6151-4114

[2] Musée national d'histoire naturelle, 25, rue Münster, L-2160 Luxembourg · yves.krippel@mnhn.lu · sylvie.hermant@mnhn.lu https://orcid.org/0000-0001-7633-1584 · guy.colling@mnhn.lu https://orcid.org/0000-0002-8994-9540

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Abstract. *Adiantum aleuticum* (Rupr.) C.A.Paris, the western maidenhair fern, was discovered in June 2022 in a basement light shaft in Ettelbruck, Grand Duchy of Luxembourg. The present paper relates the first discovery of this exotic garden escaped fern in Luxembourg. Identification was based on morphological traits and DNA barcoding.

Keywords. *Adiantum aleuticum*, *Adiantum pedatum* complex, pteridophytes, Polypodiopsida, alien species, garden ferns, garden escapees, DNA barcoding, Luxembourg.

1. Introduction

As already related in a previous paper (Krippel & Thommes 2021), the discovery of new fern taxa in Luxembourg is rather unlikely, except for garden escapees or for invasive alien species. After the discovery of *Cyrtomium fortunei* J.Sm. in 2020 near Esch-sur-Sûre (Krippel & Thommes op. cit.), representatives of the *Adiantum pedatum* complex have been discovered in 2022, well sheltered in two brick-built basement light shafts, in Ettelbruck, Grand Duchy of Luxembourg.

The genus *Adiantum* L. (Pteridaceae), found worldwide, is primarily a tropical genus, containing about 200 species (Paris 1993, Prelli & Boudrie 2021). The *A. pedatum* complex – the northernmost element of the genus – is widely distributed in temperate regions of North America, eastern Asia, and the Himalayas (Paris 1991); three species are nowadays distinguished (*A. pedatum* L. s.str., *A. aleuticum* (Rupr.) C.A.Paris and *A. viridimontanum* Paris).

The discovery of the maidenhair fern in Ettelbruck was made fortuitously, on 21st June 2022, on the premises of the former site of the Lycée technique agricole (75,028/101,930). During a guided visit of the arboretum and the so-called "Klimabaumpfad", a young *Paulownia* tree growing out of a basement shaft (Fig. 1) caught the attention of the first author. A closer inspection showed that the cavity not only hosted the invasive *Paulownia tomentosa* (Thunb.) Steud., but also some *Spiraea japonica* L. f. saplings, *Dryopteris filix-mas* (L.) Schott and – to the delight of the first author – the *Adiantum*; the latter one was also found in a second light shaft nearby.

The alien fern turned out to be *Adiantum aleuticum* (Rupr.) C.A.Paris (the western maidenhair fern), which had escaped from a nearby garden, where the fern was grown for didactic purposes by the nurserymen of the Lycée technique agricole (LTA), a school training farmers, landscape gardeners and foresters.

As the representatives of the *Adiantum pedatum* complex are quite difficult to differentiate based on morphological traits, the identification of the maidenhair fern was confirmed by DNA barcoding.



Fig. 1. Basement light shaft with *Paulownia tomentosa*, *Spiraea japonica*, *Dryopteris filix-mas* and *Adiantum aleuticum* [hidden]. Photo: Y. Krippel, 21.06.2022.

2. Material and methods

The discovery of the *Adiantum aleuticum* was made fortuitously. A first identification was made based on morphological characters (using above all Paris 1991, Paris 1993 and Chadde 2013).

A herbarium specimen was collected (herb. YK-20220621-001) and some plant material – dried in silica gel – was handed over to the Natural History Museum of Luxembourg (MNHNL) for DNA barcoding.

A Maxwell® RSC 48 Instrument (Promega, Madison, Wisconsin) was used to extract the DNA from the collected leaf material. The *rbcL* (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial coding sequence, chloroplast) and *rpoA* (RNA polymerase alpha subunit, partial sequence, plastid) gene fragments were amplified by Polymerase Chain Reaction (PCR) using newly designed forward and reverse primers (Table 1). The 10 µl PCR reaction consisted of 5 µl GoTaq® G2 Hot Start Master Mixes (Promega), 0.25 µl of each primer (10 µM), 1 µl of template DNA (10 ng/µl), and 3.5 µl of nuclease free water. PCR cycling condition was as follows: 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 sec, annealing at 60 °C for 15 sec, and extension at 72 °C for 30 sec; and a final extension for 5 min at 72 °C. The PCR products were then purified with AMPure XP Reagents (Beckman Coulter).

The sequencing reaction consisted of 0.5 µl ProDye™ Terminator Sequencing System (Promega), 2 µl of 5X sequencing Buffer (Promega), 3.75 µl nuclease free water, 0.25 µl Primer (10µM), and 4 µl of purified PCR products. Cycling condition was as follows: 96 °C for 2 min, followed by 25 cycles of denaturation at 96 °C for 10 sec, annealing at

Table 1. Forward (F) and reverse (R) primer sequences used for the amplification of the *rbcL* and *rpoA* gene fragments.

Primer name	Sequence (5'->3')	Length [mer]
rpoA-F	CCATCTCTTCGGAAACGGTAG	21
rpoA-R	TCCACTTCATTCGCGTTACC	20
rbcL-F	TTAAGGCTCTACGCGCTCTG	20
rbcL-R	ACGCAAGCCTCTAATGCAAC	20

Table 2. Parsimony informative sites for the *rbcL* (partial cds, chloroplast). Total number of segregating sites: 6; number of parsimony informative sites: 3. The Luxembourg specimen is highlighted in bold.

Accession	Parsimony informative sites			Taxon
KP898424.1	C	G	C	*Adiantum pedatum*
KP898426.1	C	G	C	
KU147276.1	C	G	C	
MH019612.1	C	G	C	
JF935362.1	T	T	A	*Adiantum aleuticum*
JN052802.1	T	T	A	
JN052806.1	T	T	A	
MH019535.1	T	T	A	
OR659634	T	T	A	

Table 3. Parsimony informative sites for the *rpoA* gene (partial sequence, plastid). Total number of segregating sites: 4; number of parsimony informative sites: 3. The Luxembourg specimen is highlighted in bold.

Accession	Parsimony informative sites			Taxon
KU147285.1	G	G	G	*Adiantum pedatum*
MH019732.1	G	G	G	
MH019657.1	A	A	A	*Adiantum aleuticum*
OR659634	A	A	A	

50 °C for 5 sec, and extension at 60 °C for 4 min. The sequencing products were purified with CleanSEQ Dye-Terminator Removal Kit (Beckman Coulter) and were analysed with an automated sequencer (3730xl DNA Analyzer, Applied Biosystems, Warrington, UK). The sequence data were visualized and assembled in *Geneious Prime* version 2022.2.2 (Biomatters).

The amplified DNA consensus sequences of the specimen from Luxembourg for the *rbcL* and *rpoA* gene fragments were aligned using the Geneious alignment algorithm of *Geneious Prime* with sequences from *A. aleuticum* and *A. pedatum* downloaded from GenBank (for accession numbers, see Tables 2 and 3).

The taxonomy and nomenclature followed Lambinon & Verloove (2015), and for non-included taxa, World Flora Online (WFO 2023).

3. Results: *Adiantum aleuticum* (Rupr.) C.A.Paris new to Luxembourg

The fern observed in Ettelbruck showed blue-green, arching, pseudo-pedately branched, fan-shaped fronds with 5 to 6 main pinnae. The pinnules were oblong (± 3 times as long as broad) with quite acute tips, the segment stalks were ca. 5 mm long and the lobes separated by deep (3-4 mm) sinuses (Fig. 2). A single pinnule was observed on the rachis between the fork and the first pinnae. It was identified morphologically as *Adiantum aleuticum* (Rupr.) C.A.Paris.

The identification based on morphological traits was confirmed by DNA barcoding. Parsimony informative sites (PIS) were used to identify the taxon with the use of reference sequences. For both markers we identified three informative sites each, where the nucleotides clearly differed between the two



Fig. 2. *Adiantum aleuticum* (Rupr.) C.A.Paris, Ettelbruck. Photo: Y. Krippel, 07.07.2022.

taxa. PIS can be used to infer evolutionary relationships, as PIS are important in constructing phylogenetic trees by the maximum parsimony method (Ma et al. 2018). For aligned DNA sequences, this is interpreted as the tree that requires the fewest nucleotide substitutions to explain the data. The results of the molecular study for both genetic markers clearly indicated that the specimen from Luxembourg belonged to the taxon *Adiantum aleuticum* (Tables 2 and 3).

To our knowledge, this is the first documented naturalised occurrence in Luxembourg for *Adiantum aleuticum* (western maidenhair fern).

4. Discussion

Adiantum aleuticum is a member of the *A. pedatum* complex and its differentiation from true *A. pedatum* (northern maidenhair fern) is not easy, as morphological differences are subtle. Long considered as a variety or a subspecies of *Adiantum pedatum* L., the western maidenhair fern shows furthermore great phenotypic plasticity, superposed on high genetic variation (Paris 1991, Paris 1993). This complexity became also evident when consulting relevant literature and in discussions with other fern specialists. Before species splitting, *A. aleuticum* used to be listed in the floras as *A. pedatum*; a fact that still has repercussions in horticulture, where – to make matters worse – numerous cultivars are grown (Mickel 1994, Olsen 2007). Furthermore, the origin, identification and labelling of many ferns sold in nurseries are often abstruse, as we already noticed for *Cyrtomium fortunei* J.Sm. and *C. falcatum* (L. f.) C.Presl (Krippel & Thommes 2021). According to Rickard (2000), true *A. pedatum* is very uncommon in cultivation in Europe, and plants supplied as *A. pedatum* usually correspond to *A. aleuticum*. This also proved to be true for the recent observation of *A. aleuticum* in Luxembourg; the ferns, which are grown for educational purposes by the landscape gardeners of the Lycée technique agricole, were bought around the year 2000 from a nursery in Luxembourg under the label "*A. pedatum*" (Jablonski, pers. comm.).

Observations of *A. aleuticum* and of *A. pedatum* in other parts of Europe show that both species are obviously capable of self-propagation via spores (Verloove et al. 2007, Merryweather 2020, Rumsey 2021), which favours escaping from horticulture. As the western maidenhair fern seems to be the most frequent "Five Finger Fern" in cultivation, at least in Great Britain, it is consequently also the most likely to escape (Edgington 2006). In the Netherlands and

Belgium too, all known garden escaped ferns of the *A. pedatum* complex seem to be *A. aleuticum* (Simons 2021).

Pursuant to Dostál (1984), besides the European native *Adiantum capillus-veneris* L., *A. pedatum* s.l. [so *A. pedatum* and its related species], a popular North American and East Asian fern grown in gardens, is the only other hardy *Adiantum* in our temperate climate. Nowadays, another trendy fern species, often held indoors as container plant, namely *Adiantum raddianum* C.Presl (a South American species), can locally naturalise after having escaped from horticulture (Rumsey 1998, Prelli & Boudrie 2021). The Asiatic *Adiantum venustum* D.Don., which is completely hardy too and a fine garden plant, is also widely grown and now offered for sale by many nurseries. Interestingly it has never been recorded as an escapee (Rumsey, pers. comm.).

Even if water wells and light shafts offer well-sheltered conditions for escaped subtropical or less hardy fern taxa (Keil et al. 2009), the increasing proliferation of exotic ornamental ferns might also be explained to some extent by the climatic variations in recent years, which have been particularly accentuated in urban areas (Middleton 2005, Robinson 2009, Steinlein 2019).

At present date, the list of ferns capable of "jumping the garden wall" is quite large (Rumsey 2021). Besides *A. raddianum*, other adventive garden runaways, such as *Pteris cretica* L. and *P. vittata* L., are already known from neighbouring countries (Verloove 2006, Verloove et al. 2007, Keil et al. 2009, Crouch & Rumsey 2010, Sarazin et al. 2013, Prelli & Boudrie 2021). Other popular garden escapees – i.e. *Azolla filiculoides* Lam., *Cyrtomium fortunei* [var. *clivicola*] and *Matteuccia struthiopteris* (L.) Tod. – were already observed in Luxembourg (Krippel & Thommes 2021, Krippel & Helminger 2022). It is probably only a matter of time until other new taxa will follow.

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