

# The oldest record of *Polypodium interjectum* in Sweden, with notes on the variability of *P. vulgare*

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The variability of *Polypodium vulgare* s. lato (Polypodiaceae) in the Nordic countries was studied. An unpublished herbarium record of *P. interjectum* from Sweden (province Scania) was confirmed. The respective specimen, originally classified as *P. vulgare*, was later re-identified as *P. interjectum* but that remained unnoticed in the literature. Here, the first find of the species in Sweden is reported. Another Swedish specimen with a similar identification history was found to be originally correctly identified. Field work and screening of herbarium material did not yield specimens of *P. interjectum* from Finland. Instead, the Finnish *P. vulgare* displayed unexpectedly much previously unknown variability, including four types of rhizome taste and the presence, even of two kinds, of sporangiasters in part of the material. Sensory observations also suggest that the rhizome of *P. vulgare* emits the volatile compound 2-nonenal. As some of the variability noted is often diagnostic at the species level in the *P. vulgare* aggregate elsewhere, the existence of cryptic species within the Nordic *P. vulgare* cannot be totally ruled out. No hybrid plants with heterogeneous spores were detected.

## Introduction

Since long ago, I have occasionally made observations on the morphological variability of the tetraploid *Polypodium vulgare*, the only representative of the genus *Polypodium* in Finland. Primarily, the aim of the present study was to discover possible occurrences of the closely related hexaploid *P. interjectum*, but at the same time I also paid attention to the overall variability of *P. vulgare*.

Surprisingly, I found two unpublished Swedish specimens that were renamed as *P. interjectum* correctly filed in the collections of the Botanical Museum (H) of the Finnish Museum of Natural

History. As the flora works and internet resources did not know *P. interjectum* from Sweden, and as *P. interjectum* is so closely related and similar to the common *P. vulgare*, I also decided to check the identification of both specimens under the dissecting microscope, and I studied the spores and stomata under the light microscope.

The present contribution firstly presents revisions of these specimens identified as *P. interjectum*. Secondly, notes on previously unknown characters and variability detected in Finnish *P. vulgare* are given; the variability of *P. vulgare* (excluding the cultivated leaf variants) seems quite unexplored (but see e.g. Øllgaard & Tind 1993, Nielsen & Johnsen 2000).

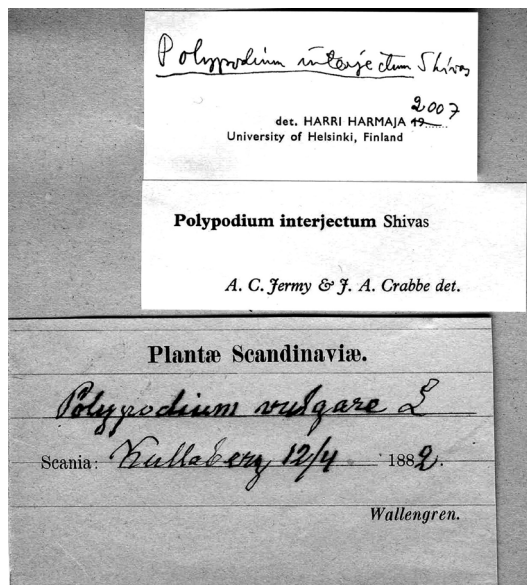


Fig. 1. The labels on the *Polypodium interjectum* sheet from Sweden in H.

Recently, I have also come across an article (Ljungstrand 2010) that reports two finds of *P. interjectum* from SW Sweden in October 2010: (i) in Kullaberg rocky hill (i.e., in the same locality as the old herbarium specimen reported in the present paper), and (ii) in a locality in the province Bohuslän.

## Material and methods

I observed and collected the plants in the southernmost Finland from sites differing in the type of rock, exposition and lightness. Collecting deliberately covered all the phenotypic variation that was readily observable. The field studies were carried out mainly in 2007.

The taste characteristics of the fresh stem (rhizome) of 51 plants were examined. A juvenile portion at the growing point and a somewhat older part were included in the test. All the tested plants were collected as voucher specimens.

First, I examined the Nordic herbarium material of *Polypodium* in H by eye, and then studied the specimens deviating morphologically and made spot checks among all material under the dissecting microscope. Many specimens were further studied under the light micro-

scope: the characters of the spores and stomata were observed in mounts of Melzer's reagent (a common mountant used by mycologists; see Leonard 2006).

The specimens collected are deposited in H.

## Results

### *Polypodium interjectum* in Sweden

One of the specimens in H with this renaming was collected from the province of Scania (Skåne) and the other from Värmland. Both of them were originally identified as *P. vulgare*. The annotation labels with the renaming were signed "A. C. Jermy & J. A. Crabbe" (Fig. 1). The slips did not contain any dates but, judging from the loan data of the specimens, they were added in 1989 or 1990.

### The Scania specimen: a correct re-identification

The Scania specimen comprises one mature, fairly tall rhizomatous plant with two leaves. All the diagnostic characters of the sporangial annulus (overall colour, the number of the indurated, or thick-walled cells, the number of the thin-walled basal cells) and the spore length measured by the author, ca. 75–87  $\mu\text{m}$ , correspond to the descriptions of *P. interjectum* in the literature, e.g. in the thorough contribution by Ivanova (2006), who also provided good colour microphotographs of the annuli of *P. interjectum* and *P. vulgare*. According to the literature (e.g., Ivanova 2006) and my measurements on the Finnish material, the spores of *P. vulgare* are shorter, being roughly 55–75  $\mu\text{m}$  long. The length of the stomata was found to be ca. 60–73  $\mu\text{m}$ . This length corresponds to that given for *P. interjectum* in the literature (e.g., Bureš *et al.* 2003), i.e. it is greater than that reported for *P. vulgare* (see the description of the Värmland specimen below). Figure 1 shows the labels attached to the specimen.

SPECIMEN EXAMINED. **Sweden**. Scania (Skåne). Höganäs commune, Brunnby parish, Kullaberg, 12.IV.1882 *Wallengren* (H-1053261).

## The Värmland specimen: an incorrect re-identification

The specimen comprises three separate rhizomatous leaves that represent a morph with a rather triangular leaf lamina. It was identified as *P. vulgare*  $\beta$  [= var.] *auritum* originally. All the leaves are immature so the characters of the sporangia and spores could not be ascertained. However, of the diagnostic features, I studied the length of the stomata in the right-hand leaf. The stomata measured ca. 40–60  $\mu\text{m}$ ; a few were rotund. Their length is thus that of *P. vulgare* (e.g., Bureš *et al.* 2003; my own unpublished data on Finnish material), i.e. smaller than that of *P. interjectum* (see the description of the Scania specimen above). My conclusion is that the Värmland specimen is *P. vulgare*.

SPECIMEN EXAMINED. **Sweden.** Värmland. [Säffle commune], Tveta parish, Mossvik, 14.VI.1900 E. Berggren (H-1053260).

## Previously unreported characters and variability in *Polypodium vulgare*

### Rhizome taste

#### Taste types

I found four different tastes: (i) both a sweet, licorice-like component and a bitter one present, (ii) taste sweet, licorice-like only, (iii) taste bitter only, and (iv) taste inconspicuous, neither sweet nor bitter. The juvenile portion, or the tip, of the rhizome and a somewhat older part did not appear to differ in taste. The strength of both the sweet and the bitter component was variable. The first taste type was the most common, the fourth one the rarest. The distribution of the taste types among the plants studied ( $n = 51$ ) was as follows:

1. Sweet, licorice-like + bitter: 33 (66%),
2. Bitter only: 10 (20%),
3. Sweet, licorice-like only: 2 (or 7, see Discussion) (4/14%),
4. Inconspicuous: 1 (2%).

I did not find clear-cut correlations among

the variation in taste and other characters (morphology, habitat, etc.).

### Previously unknown, common component of taste

I noted one more taste, that of cucumber (*Cucumis sativus*), in the rhizome of fresh south-Finnish plants of *P. vulgare*. Apparently, its presence is independent of the taste types described above. The cucumber taste may be always present; at least all the plants that I tasted in late summer 2007 after the discovery of the presence of this taste, possessed it. A cucumber smell was always observed simultaneously. This smell strongly suggests that the rhizome emits a well-known volatile aldehyde named 2-nonenal (Maarse 1991, Wood *et al.* 1994).

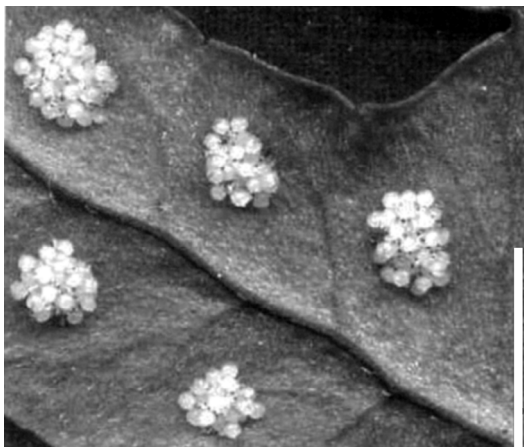
## Sporangiasters

### Eglandular sporangiasters

Sporangiasters of this kind (Fig. 2) develop synchronously with sporangia, almost reaching the length of submature sporangia. The sporangiasters are stalked and possess elongated, somewhat curved heads that are much smaller than the heads of the sporangia. They are pale brown for the most part, but their very tips are medium or dark brown. The sporangiasters are often numerous in a sorus. In the same plant individual, the number of sporangiasters per sorus may vary greatly (the sporangiasters possibly tending to be more numerous towards the bases of the segments and the laminae). The sporangiasters of this type are often conspicuous, especially in immature sori (Fig. 2).

The occurrence of these eglandular sporangiasters in Finnish *P. vulgare* is infrequent. In general, there is no other type of sporangiasters present, but very rarely I observed also solitary glanduliferous ones (see below) in the same sorus.

I found no clear correlation between the occurrence of the eglandular sporangiasters and any other trait (however, the plants with eglandular sporangiasters may tend to grow in fairly



**Fig. 2.** Immature sori on a fresh current season's leaf of *Polypodium vulgare*: sporangiasters are present; their small, brown, extreme tips appear as dark spots among the whitish immature sporangia (Finland, Siuntio, Holstens, 29.VIII.2007 Harmaja). Scale bar = 3 mm.

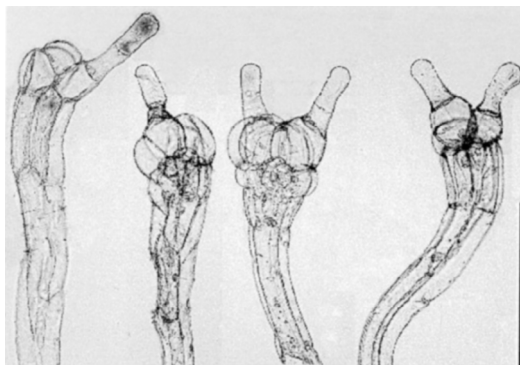
shaded habitats, may have fairly tall leaves, and may have a retarded maturing of the sori/sporangia).

### *Glanduliferous sporangiasters*

These sporangiasters (Fig. 3) are inconspicuous, and a thorough inspection under the dissecting microscope is needed to detect them. They are distinctly shorter than the sporangia. Their stalks end in a head that is much smaller than the heads of the sporangia. At first, the sporangiasters are hyaline but eventually become pale brown. Apically they are beset by 1–3, short, elongated, sessile glands that are one-celled or have two cells. Most heads possess two glands. The sporangiasters of this kind are always very scanty in a sorus. Sometimes, I observed only very occasional ones in a whole leaf, but I also noted a fairly constant occurrence in the sori of a leaf. The number of the sporangiasters in a sorus is possibly somewhat dependent on the position of the sorus in the lamina (the sporangiasters may be commoner upwards).

The glanduliferous sporangiasters are sometimes present in Finnish *P. vulgare*. Those of the eglandular type are then usually absent.

I found no clear correlation between the occurrence of the glanduliferous sporangiasters



**Fig. 3.** Glanduliferous sporangiasters from a dried current season's leaf of *Polypodium vulgare* (taste of fresh rhizome sweet + bitter; Finland, Tuusula, by the church, on/between superficial spruce roots, 1.VIII.2007 Harmaja). Scale bar = 200  $\mu$ m.

and any other trait (however, the plants possessing them would generally appear to have “average” leaves and to occur in fairly sunny habitats).

## Discussion

### *Polypodium interjectum*

Apart from the very recent paper by Ljungstrand (2010), no flora works, even those published after 1990, report *P. interjectum* for the Swedish flora. These include floristic treatments of Brunby parish and Kullaberg (Kraft 1982, 1984, 1991, Johansson 2007), the province of Scania (Tyler *et al.* 2007), Sweden (Karlsson 1998), the Nordic countries (Øllgaard & Tind 1993, Nielsen & Johnsen 2000, Mossberg & Stenberg 2003), and Europe (Akeroyd & Jermy 1993). Also the internet resources treating the Swedish vascular plants (<http://linnaeus.nrm.se/flora/orm/polypodia/polyp/welcome.html> [In Swedish], <http://www2.nrm.se/fbo/chk/chk3.htm> [In Swedish]) do not know *P. interjectum* from Sweden.

Probably, the two specimens that were re-identified as *P. interjectum* have been omitted from the literature as being filed in a non-Swedish herbarium. For some reason, one of the re-identifiers (A. C. Jermy) did not consider these specimens when acting as one of the revisers of the treatment of *Polypodium* in the second edi-

tion of *Flora Europaea* vol. 1 (Akeroyd & Jermy 1993). The printing of the volume may have had progressed too far to allow for more updates. Hints or rumours may have been released, though: Øllgaard and Tind (1993) mention that *P. interjectum* may have occurrences in southern Sweden, and Nielsen and Johnsen (2000) wrote “Reports from southern S[Sweden] have, so far, not been verified”.

Erik Ljungstrand (Sweden) kindly informed me that the collector “Wallengren” of the *P. interjectum* specimen from Scania is very probably H. D. J. Wallengren. Kullaberg is a very famous rocky hill area. Unlike most of Scania, it is composed of primary rocks. Within Sweden, Kullaberg is a most plausible locality for *P. interjectum* as it lies (i) in the southernmost part of the country, (ii) in the northern temperate bioclimatic zone (Ahti *et al.* 1968), (iii) in the suboceanic section of that zone (Ahti *et al.* 1968), and (iv) hardly 100 km away from the nearest localities of the species in Denmark (central Zealand). Kullaberg is also famous for its rich flora, for example housing one of the few Swedish localities of the oceanic fern *Asplenium adiantum-nigrum* (see e.g. Kraft 1982).

The specimen label does not specify from where exactly in the Kullaberg area the *Polypodium* was collected.

In northern Europe, *P. interjectum* was hitherto known from very scattered localities in Denmark, SW Norway, and the Kaliningrad area (Akeroyd & Jermy 1993, Øllgaard & Tind 1993, Nielsen & Johnsen 2000, Mossberg & Stenberg 2003, Elven 2005). The total area of *P. interjectum* — almost exclusively a European species — may entirely lie within the temperate bioclimatic zone(s) in the sense of Ahti *et al.* (1968). The species clearly favours oceanic and suboceanic climate.

The length of the stomata is only infrequently mentioned to separate *P. vulgare* and *P. interjectum* (e.g., by Bureš *et al.* (2003)). The present case with the Swedish specimens proved the usefulness of this difference when treating plants with unripe sporangia.

The sporangia of *P. interjectum* being larger is to be expected: the species is hexaploid and its spores are more voluminous than those of the tetraploid *P. vulgare*. I also compared the

sporangia of herbarium specimens of the diploid *P. sibiricum*: they were even smaller than those of *P. vulgare* (for the characters of *P. sibiricum*, consult Haufler *et al.* 1993 and the literature cited therein). Thus, the spore size, sporangium size and ploidy level are — as could be anticipated — positively correlated in *P. sibiricum*, *P. vulgare* and *P. interjectum*.

The sporangial size and the number of sporangial “apical cells” (the thin-walled cells at the apical end of the annulus) are two characters that apparently have not been used for separating *P. interjectum* and *P. vulgare*. These features can easily be studied and compared under a dissecting microscope. The Swedish specimen as well as other material of the former species was found to possess larger sporangia and a higher number of apical cells as compared with the material of *P. vulgare*. However, the latter difference can be seen in the photographs of Ivanova (2006) and in the drawings of Øllgaard and Tind (1993) and Nielsen and Johnsen (2000).

The frond habit of the Värmland specimen (see above) obviously lead Jermy and Crabbe to their misidentification, which was made without knowledge of the characters of the sporangia or the spores (or, obviously, of the stomata). According to the literature and the experience of the present author, the frond morphology and the size and shape of the sori of *P. vulgare* are so variable that the species cannot be separated on the basis of these features. Moreover, the Värmland locality lies in a more northern bioclimatic zone and in a less oceanic section of the latter than the Scania locality (Ahti *et al.* 1968), which itself can be expected to be at the northeastern border of the distribution area of *P. interjectum*.

### Rhizome taste of *Polypodium vulgare*

In popular and even scientific literature, the taste of the rhizome of *P. vulgare* was quite often given as sweet only, without making a mention of other components. Nowadays, the bitter component is often also noted (at times as ‘acid’, though). For this well-known sweet taste, a compound (saponin and glycoside) named osladin is responsible (Nishizawa & Yamada 1995). For the bitter taste, the flavonoid (+)-afzelechin is,



at least partly, responsible (Kim & Kinghorn 1989). Another flavonoid, polypodine B, is a further important component in the rhizome of *P. vulgare* (Kim & Kinghorn 1989).

The taste should be examined already in the field after picking the plant with its rhizome, or at the latest after the trip on the same day. Five of the seven cases of taste type 3 (sweet, licorice-like only) were namely recorded in plants that had been stored for at least a day after collecting, as their preparation and pressing was delayed. One explanation for this might be that the bitter-tasting compound disintegrates rapidly after the plant has been removed from its natural environment.

*Polypodium vulgare* is an allotetraploid, the parental diploid species most probably being *P. glycyrrhiza* and *P. sibiricum* (Haufler *et al.* 1993, 1995). In the postulated parental *P. glycyrrhiza* (i) the rhizome tastes essentially sweet, however the sweet components do not include osladin but the main compound is polypodoside A (Nishizawa & Yamada 1995), and (ii) the — sometimes unnoticed — bitter component is not (+)-afzelechin but a related flavonoid glycoside (Kim & Kinghorn 1987). The *P. sibiricum* rhizome is reported as “acid-tasting” only (Haufler *et al.* 1993). As stated above, most specimens of *P. vulgare* examined possessed rhizomes that tasted both sweet and bitter quite as could be expected due to the postulated parents (roughly the same situation — a somewhat variable dual taste inherited from one sweet-tasting and one bitter-tasting diploid parental species — is observed in the North American allotetraploids *P. calirhiza* and *P. hesperium*: Windham & Yatskievych 2005). However, the phylogeny of *P. vulgare* may require further analyzing as the chemical compounds of an allotetraploid apparently should be a combination of the *exact* compounds of its parents.

2-nonenal is generated through oxidative degradation from its precursor compounds, some unsaturated fatty acids (Maarse 1991, Haze *et al.* 2001). Thus, 2-nonenal gets developed, for instance, when plant or mushroom tissue is crushed with fingers or teeth, these precursors themselves probably being tasteless and odourless. The cucumber-taste effect that is reported in the present study would appear to be a fallacy

stimulated by the smell of 2-nonenal. These sensory observations strongly suggest that the rhizome of *P. vulgare* emits 2-nonenal (and thus contains the precursors of the compound).

2-nonenal, represented by its different isomers, is widely present in nature. It is one of the main compounds responsible for the characteristic smell and taste of the fruit of cucumber (*Cucumis sativus*; Forss *et al.* 1962), also getting developed, e.g., in parts of other plants, many kinds of foodstuff (Zhou & McFeeters 1998), human skin (Haze *et al.* 2001), and fruit bodies of many mushroom species such as *Clitopilus prunulus* (trans-2-nonenal; Wood *et al.* 1994).

The taste and odour of cucumber are apparently new findings for *P. vulgare*, and probably for the whole genus. So would be the production of 2-nonenal and its precursors supposing that they are responsible for these sensory impressions. In the pteridophytes, 2-nonenal apparently is an infrequently produced compound. Small amounts of (2Z)-nonenal was identified in the essential oil distilled from the above-ground parts of *Matteuccia struthiopteris* (Miyazawa *et al.* 2007).

### Sporangiasters of *Polypodium vulgare*

Sporangiasters of any kind were previously not known in *P. vulgare*. However, I often observed sporangiasters, even of two kinds (also additional types of them may have been present, but it appears virtually impossible to differentiate between some kinds of possible sporangiasters and poorly developed sporangia). The occurrence of these two kinds of sporangiasters was often inconstant within a leaf, and requires further study.

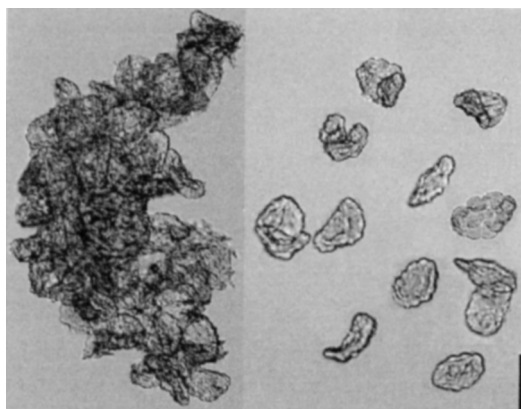
Although fewer per sorus, smaller, and with fewer glands, the glanduliferous sporangiasters observed in several collections of *P. vulgare* are amazingly similar to those illustrated for North American species, like the diploids *P. amorphum* and *P. appalachianum* (Haufler *et al.* 1995: fig. 26), and to a lesser degree the tetraploid *P. saximontanum* (Windham 1993: fig. 4D). In addition, occasional — otherwise quite different — sporangiasters of *P. sibiricum*, one of the supposed parental species of *P. vulgare* (*see above*), of

northern Asia and northern North America, may bear a few glands (Haufler *et al.* 1993).

### Some further variability of *Polypodium vulgare*

Several features of the sori, sporangia and spores displayed variability that is not treated in the present contribution. However, it may be mentioned that, in some specimens, I observed fairly long, non-glandular, adaxial hairs on the leaf rachis. This may prove to have some significance; the kind and position of these hairs resemble the diagnostic trait of one of the supposed parental species *P. glycyrrhiza* (see above).

No plant that unambiguously would possess heterogeneous and partly aborted spores — as those in the photographs of Ivanova (2006) for *P. × mantoniae* — suggesting interspecific hybridization was found by me. However, the plants from a presumably clonal stand from Helsinki possessed curious spores that — when seemingly “ripe” — remained clearly smaller than normal ones, were fairly uniform in appearance, misshapen, apparently empty, seemingly only comprising a collapsed perispore (Fig. 4). Although some of the sporangia got opened, the spores were not forcibly released and, consequently, no spore deposit could be obtained on the pressing paper. These plants were deviating also in other respects: (i) the rhizome regularly seemed to produce two adjacent leaves in an interval of few weeks, (ii) the sori were submarginal, and (iii) the sori (many sporangia) became very dark with age.



**Fig. 4.** A deviating morphotype of *Polypodium vulgare*: vestigial and misshapen “mature” spores from dehiscent sporangia of a dried leaf of current season; on the left, all the contents, or the clustered spore mass, ejaculated by a sporangium (taste of fresh rhizome sweet + bitter; Finland, Helsinki, Pirkkola, 24.X.2007 Harmaja). Scale bar = 50  $\mu$ m.

ally diagnostic at the species level within the *P. vulgare* aggregate elsewhere (see Haufler *et al.* 1993 and the literature cited therein). Therefore, the existence of cryptic species within *P. vulgare* cannot be totally ruled out, even if plants that produce heterogeneous spores to suggest interspecific hybridization were not observed in the present study.

5. The rhizome chemistry (as judged from taste observations and evaluated on the basis of the literature) and the presence of the glanduliferous sporangiasters observed in the present study suggest that the phylogeny of *P. vulgare* may be more complicated than currently outlined.

## Conclusions

1. *Polypodium interjectum* has been collected from one locality in southernmost Sweden.
2. *Polypodium interjectum* was not encountered in the Finnish material included.
3. *Polypodium vulgare* in Finland displayed much more variability than anticipated.
4. The above-described variability in *P. vulgare* appears to be genetically controlled. Variability in the rhizome taste, leaf indumentum and presence and kind of sporangiasters is usu-

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