

## Pollen studies in British *Hieracium* sect. *Alpina* (Asteraceae)

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### ABSTRACT

A survey of pollen production in British *Hieracium* sect. *Alpina* (Asteraceae) taxa has been undertaken using Alexander's Stain as a test for potential pollen viability. Diploid *H. alpinum* s.s. from Europe produces abundant, well-formed, stainable pollen. No pollen was observed in British *H. alpinum* s.s. Potentially viable pollen was observed in 14 of the 39 sect. *Alpina* taxa investigated, including *H. holosericeum* (triploid), *H. leptodon* (tetraploid) and *H. pentaploideum* (pentaploid), sometimes in abundance. Cultivated plants were more likely to have pollen than wild plants, suggesting pollen production may be under both environmental and genetic control. The involvement of pollen should be considered in theories of evolution of *Hieracium* sect. *Alpina*.

KEYWORDS: *Hieracium alpinum*, pollen viability, Alexander's Stain, Britain.

### INTRODUCTION

As far as it is known, all the British taxa of *Hieracium* sect. *Alpina* (Griseb.) Grelli are polyploid, obligate apomicts (Stace *et al.* 1997). In Britain, the taxa are triploid ( $3n = 27$ ), tetraploid ( $4n = 36$ ), or rarely pentaploid ( $5n = 45$ ) (Stace *et al.* 1995). The apomictic reproductive system is by diplospory (as has been shown for *H. alpinum* sensu stricto by Skawińska (1962)), in which the embryo is derived directly from an unreduced archesporial cell, with no male gamete input either to the embryo, or for formation of the endosperm (i.e. they are not pseudogamous). Thus reproduction in these British apomictic polyploids is essentially clonal, with progeny genetically identical to the mother and to each other. In Europe, both diploids and polyploids occur in sect. *Alpina*. Chrtek (1997) and Štrochová *et al.* (2002) showed that *H. alpinum* in the eastern Carpathians is a diploid, sexual, self-incompatible taxon, with apomictic triploid clones elsewhere (including Britain) which did not produce pollen.

The main theory to explain the evolution of polyploid apomictic groups such as *Hieracium* sect. *Alpina* is that they have evolved through hybridisation. Hybridisation may have occurred between two sexual taxa, with the progeny subsequently becoming apomictic and polyploid independently. Alternatively, hybrids might have arisen from pollination of a sexual species by an apomictic polyploid whose apomictic genes are then expressed in subsequent generations. A combination of both mechanisms may have occurred. Other possible speciation mechanisms include point mutations, 'pseudo-sexual' recombination among different copies of the same chromosomes within the mother, and chromosome structural mutations which simultaneously affect numerous genes and traits (Tyler 2006).

Of the British sect. *Alpina* 34 of the 35 species are endemic, but it is not known where they originated or what their parentages are (Stace *et al.* 1997). As diploid *H. alpinum* is currently only known from the Carpathians, and is also the only known diploid in sect. *Alpina*, it is possible that some of our endemics originated in the Carpathians and then migrated to Britain. For the taxa to then become endemic to Britain, they would need to evolve independently in Britain and/or Europe, and diverge so much that they are recognised as distinct taxa. Alternatively, it is possible that diploid *H. alpinum* migrated to Britain after the last glacial period, gave rise to some of our endemics and then died out. The British endemics could also have arisen from some closely related sexual taxa, not necessarily diploids, which had migrated to Britain.

Stace *et al.* (1997) investigated the cytological and molecular variation in British sect. *Alpina*, and found that despite being apomictic, there is genetic variation both between and within taxa. The origin of the variation could be explained in parts by mutations, polyphyletic origin and relics of sexual hybridisations. As the amount of genetic variation

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observed was low, they suggested that the same or similar sexual parents, both diploid and probably tetraploid, gave rise repeatedly to a series of different diploid, triploid, tetraploid and pentaploid hybrids in more than one locality. They suggested that if diploid *H. alpinum* had been present in Britain, it may have died out due to low seed set due to its requirement for cross-pollination which would have been unpredictable in our suboptimal climate. The low levels of genetic diversity within the sect. *Alpina* species also suggested recent origins (presumably post-glacial), with some subsequent mutations.

As part of work trying to understand evolution of the British sect. *Alpina* endemics, a survey of pollen production was undertaken using Alexander's Stain (Alexander 1969). Alexander's Stain stains the outside of the pollen grain greenish-blue and cytoplasm inside red, so well-formed pollen grains with cytoplasm (which are potentially viable) can be picked out quickly. It is a quick method to screen many plants, including historical herbarium specimens, for potential fertility, but does not prove that the grains are viable. It is generally reported that the polyploid *Hieracium* apomicts produce no pollen or only small amounts of inviable pollen (e.g. Stace *et al.* 1997), but we have recently observed reasonable quantities of apparently viable pollen in both herbarium specimens and living material. The involvement of pollen in sexual reproduction might help to explain evolution in the genus and the concentrations of endemic species. *Hieracium alpinum* was investigated in detail due to the potential role of sexual diploids in the origin of the endemics, with a selection of material of the remaining British species of sect. *Alpina* for comparison.

#### METHODS

Pollen samples of British *H. alpinum* were taken from herbarium specimens in **BM**, **E** and **NMW**, aiming to cover as much of the geographic range as possible. Diploid material from Europe was provided by P. Mráz. Specimens of the remaining British sect. *Alpina* species were sampled in **NMW**. The accession numbers of all herbarium sheets examined are listed in Appendix 1.

Capitula were examined under a microscope and the ring of anthers removed from florets about to open, or where not available from recently opened florets with emerging styles,

using tweezers and a low-power binocular microscope. At least two florets from each specimen were examined, sometimes more. The anthers were placed onto a slide with a drop of Alexander's Stain, warmed briefly on the hotplate, then broken up with the tweezers to release the pollen, and the tissue 'stroked' to free the pollen grains stuck inside the anthers. The preparation was then covered with a cover slip and either placed onto a hotplate to improve the uptake of the stain for at least five minutes or left overnight.

The slides were then examined under a high-power compound binocular microscope. The areas on the slide with high numbers of pollen grains were sampled to estimate the percentage of fertile grains. Grains present on the stigmas were ignored as they may have come from another source. Numbers of potentially fertile and infertile grains were recorded, and the percentage fertility calculated.

#### RESULTS

Two main types of pollen grains were found. First, spherical grains with the cytoplasm inside which stained bright red and thick papillate cell walls which stained green. The grains could vary in size, sometimes more so in one species than another. These pollen grains were taken as potentially viable. Second, spherical grains staining green only, with no red staining inside (i.e. no cytoplasm). These were sometimes smaller in size than those with cytoplasm, but very few were deformed. These grains were considered infertile.

Potential pollen viability in European material of diploid *H. alpinum* is shown in Table 1. The specimens examined generally produced abundant, well-formed pollen of uniform size which had a high stainability.

In contrast, no pollen was found in the 85 British *H. alpinum* herbarium specimens, apart from in two specimens of doubtful origin or identification. The locations of *H. alpinum* specimens from which we have examined for pollen are shown in Figure 1.

Surprisingly, 14 of the 39 British *Hieracium* sect. *Alpina* taxa produced large amounts of pollen (Table 2). Where pollen was present within a taxon, it was not necessarily present in all individuals examined, and it varied in quantity between individuals. Furthermore, potential viable pollen was observed across the three higher ploidy levels (Table 3) including *H. holosericeum* (triploid), *H. leptodon* (tetraploid) and *H. pentaploideum* (pentaploid).

TABLE 1. POTENTIAL POLLEN VIABILITY IN DIPLOID *H. ALPINUM*. (TOTAL NUMBER OF POLLEN GRAINS COUNTED ARE GIVEN IN BRACKETS)

Origin	No. florets examined	Potential pollen viability
Chornohora Mountains, Ukraine, plant 1	2	99% (n = 50)
Chornohora Mountains, Ukraine, plant 2	2	99% (n = 50)
Chornohora Mountains, Ukraine, plant 3	2	41% (n = 86)
Rodna Mountains, Romania	2	99% (n = 50)
Bucegi Mountains, Romania	2	96% (n = 50)

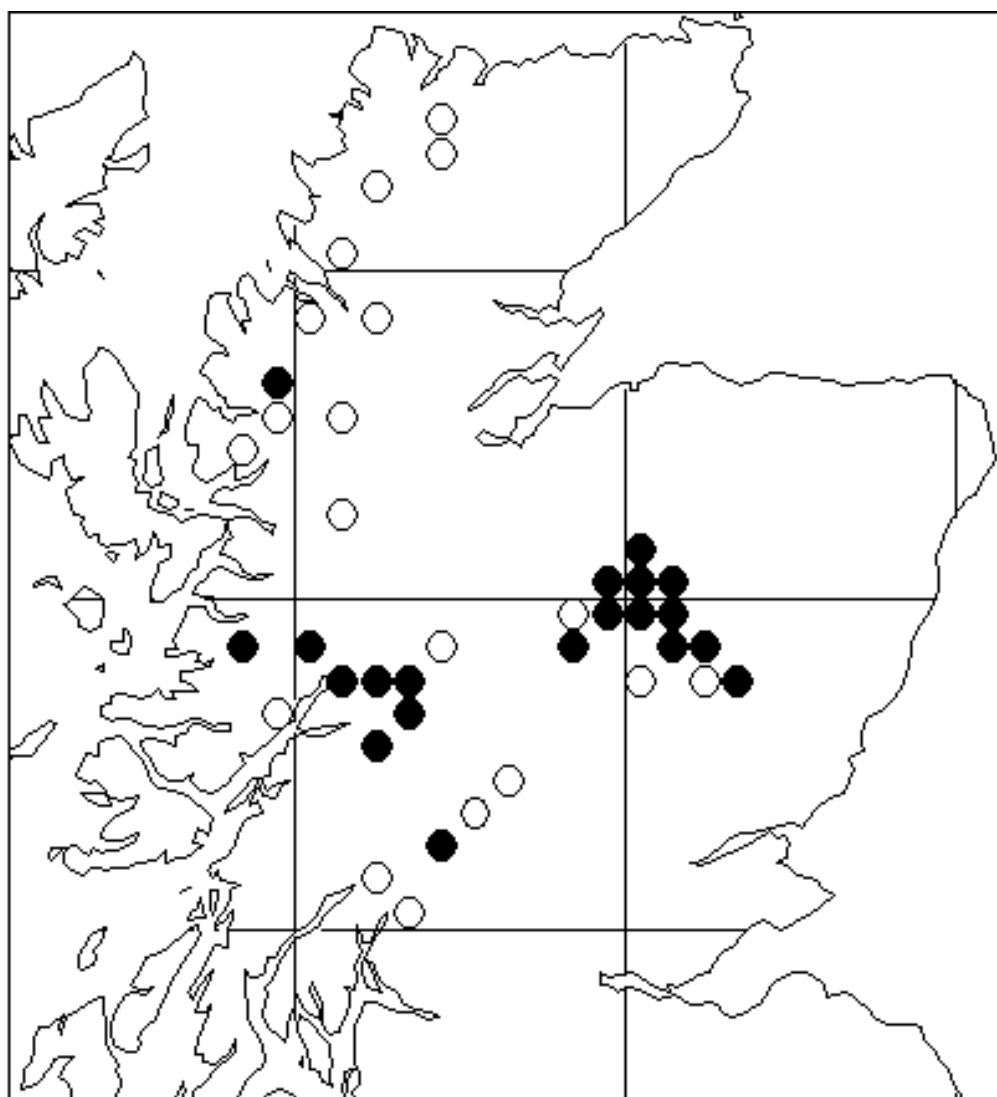


FIGURE 1. Localities from which no pollen was found in the 85 *Hieracium alpinum* herbarium specimens examined (●). Other *H. alpinum* localities from which no material has been examined (○). All records are shown irrespective of date.

TABLE 2. POLLEN PRODUCTION IN BRITISH SECT. *ALPINA* SPECIES. PLOIDY LEVELS ARE TAKEN FROM STACE *ET AL.* (1995, 1997). TOTAL NUMBER OF POLLEN GRAINS COUNTED ARE GIVEN IN BRACKETS

Taxon	Ploidy	No. plants examined	Potential pollen viability (%)
<i>H. backhousei</i> F. J. Hanb.	×4	2	No pollen
<i>H. calenduliflorum</i> Backh.	×4	2	No pollen
<i>H. calvum</i> P. D. Sell & D. J. Tennant	×4	2	No pollen
<i>H. completum</i> P. D. Sell & C. West	×4	3	45% (n = 115), 2 plants no pollen
<i>H. deargicola</i> P. D. Sell & D. J. Tennant	×4	1	No pollen
<i>H. einichense</i> P. D. Sell & D. J. Tennant	×4	1	No pollen
<i>H. eximium</i> Backh.	×4	3	No pollen
<i>H. eximium</i> Backh. f. <i>eximium</i>	×4	2	No pollen
<i>H. eximium</i> Backh. f. <i>tenellum</i> (Backh.) P. D. Sell & C. West	×4	3	No pollen
<i>H. globosiflorum</i> Pugsf.	×3	3	No pollen
<i>H. graniticola</i> W. R. Linton	×3	2	No pollen
<i>H. grovesii</i> Pugsf.	×4	3	11% (n = 63), 13% (n = 111), 6% (n = 63)
<i>H. hanburyi</i> Pugsf. f. <i>hanburyi</i>	×4	12	No pollen
<i>H. hanburyi</i> Pugsf. f. <i>atraticeps</i> (Pugsf.) P. D. Sell & D. J. Tennant	×4	2	4% (n = 22), 1 plant no pollen
<i>H. hanburyi</i> Pugsf. f. <i>pusillum</i> P. D. Sell & D. J. Tennant	×4	1	No pollen
<i>H. holosericeum</i> Backh.	×3	10	24% (n = 50), 8% (n = 50), 8 plants no pollen
<i>H. insigne</i> Backh. f. <i>insigne</i>	×4	1	Underdeveloped pollen
<i>H. insigne</i> Backh. f. <i>celsum</i> P. D. Sell & C. West	×3 or ×4	5	35% (n = 47), 12% (n = 68), 100% (n = 31), 34% (n = 89), 1 plant no pollen
<i>H. kennethii</i> P. D. Sell & D. J. Tennant	×4	1	8% (n = 23)
<i>H. larigense</i> (Pugsf.) P. D. Sell & C. West	unknown	1	No pollen
<i>H. leptodon</i> P. D. Sell & D. J. Tennant	×4	6	64% (n = 62), 46% (n = 90), 33% (n = 24), 3 plants no pollen
<i>H. macrocarpum</i> Pugsf.	×4, ×4 +1	3	86% (n = 50), 2 plants no pollen
<i>H. marginatum</i> P. D. Sell & C. West	×4	2	94% (n = 72), 61% (n = 103)
<i>H. memorabile</i> P. D. Sell & C. West	×4	4	No pollen
<i>H. milesii</i> P. D. Sell & C. West	×4	3	No pollen
<i>H. mundum</i> P. D. Sell & C. West	×4	2	29% (n = 106), 1 plant no pollen
<i>H. notabile</i> P. D. Sell & C. West	×4	2	No pollen
<i>H. optimum</i> P. D. Sell & C. West	unknown	2	No pollen
<i>H. pensum</i> P. D. Sell & C. West	×4	1	25% (n = 5)
<i>H. pentaploideum</i> P. D. Sell & D. J. Tennant	×5	2	68% (n = 69), 100% (n = 50)
<i>H. perscitum</i> P. D. Sell & C. West	×4	2	24% (n = 148), 1 plant no pollen
<i>H. probum</i> P. D. Sell & C. West	×4	5	No pollen
<i>H. pseudocurvatum</i> (Zahn) Pugsf.	×3	1	No pollen
<i>H. pseudopetiolatum</i> (Zahn) Roffey	×3	3	12% (n = 26), two plants no pollen
<i>H. subglobosum</i> P. D. Sell & C. West	×3	4	1% (n = 50), 3 plants no pollen
<i>H. subgracilentipes</i> (Zahn) Roffey	×4	1	No pollen
<i>H. subtenuifrons</i> P. D. Sell & D. J. Tennant	×3 or ×4	3	No pollen
<i>H. tenuifrons</i> P. D. Sell & C. West	×4	4	36% (n = 112), 68% (n = 105), 2 plants no pollen

TABLE 3. NUMBER OF TAXA IN EACH PLOIDY LEVEL AND POLLEN PRODUCTION IN BRITISH SECT. *ALPINA* SPECIES

Ploidy	Total no. taxa	Pollen absent	Significant pollen production
×3	6	4	2
×3 or ×4	2	1	1
×4*	27	16	11
×5	1	0	1
Total	36	21	15

\*includes *H. macrocarpum* aneuploid

It is unclear why pollen should be present in some individuals of a taxon, but absent in others. We noticed that cultivated plants were more likely to have pollen than plants collected from the wild. For instance, in *H. subglobosum*, the cultivated specimen had masses of pollen (although with only 1% viability) but the wild-collected plants had none. Excluding *H. alpinum*, 13 of the 28 cultivated specimens of sect. *Alpina* species had pollen (46%), compared to only 14 of the 87 wild-collected specimens (24%); this is significantly different ( $\chi^2$ ,  $p < 0.001$ ).

#### DISCUSSION

The key result is the observation that although no pollen was observed in British *H. alpinum*, significant amounts of potentially viable pollen were observed in over a third of the other sect. *Alpina* taxa. The stain does not prove that pollen is viable, which must be tested by germinating fresh pollen from cultivated plants, but the presence of pollen nonetheless suggests the possibility that it might have a role in evolution of the endemics.

Our survey of pollen on herbarium specimens shows there is no evidence that diploid *H. alpinum* occurs in Britain. Although there are only counts of three plants from two localities published (Stace *et al.* 1995), it is presumably uniformly triploid. Štrochová *et al.* (2002) showed allozyme and RAPD variation occurred within and between populations of the triploid cytotype of *H. alpinum*, and the geographic and genetic distances were found to be correlated, indicating isolation by distance. They interpreted the gradient of genetic variation observed from the variable diploids in the Eastern Carpathians to the uniform triploids elsewhere as resulting from the post-glacial recolonization of Europe from glacial refugia

in the Carpathians. Diploids may therefore never have been present in Britain.

If diploid *H. alpinum* has never been present in Britain, did the endemic taxa evolve here by hybridisation or migrate? The endemics are unlikely to have evolved from hybridisation with diploids in Britain as the only sexual, diploid species of *Hieracium* present is *H. umbellatum* L., which is so morphologically different that it cannot have been involved. The occurrence of pollen in polyploids suggests that the endemics could have evolved by hybridisation between some endemic taxa if the polyploids are partly sexual: this would require both fertile pollen and a diplospory apomictic reproductive system that is not absolute (i.e. occasional fusion of gametes may occur). Detection of such hybrids within sect. *Alpina* would be difficult as the species are generally very similar, differing in few characters and showing a reticulate pattern of morphological variation. *Hieracium* hybrids have only very rarely been observed in the wild (Mráz *et al.* 2003) or cultivation (Pugsley 1948). Migration of at least some parental taxa which subsequently became endemic in addition to *H. alpinum* is still required under this model.

Hybridisation between polyploids could explain how the endemic *H. pentaploideum* arose on An Teallach, Wester Ross (v.c. 105) from the other species recorded on the mountain. It could have resulted from a cross of an unreduced triploid gamete from *H. alpinum* or *H. holosericeum* with a reduced diploid gamete from a tetraploid such as *H. kennethii*, *H. marginatum* or *H. perscitum*. Similarly, Sell & Murrell (2006) note how species within the artificial group sect. *Subalpina* may have arisen as hybrids between sect. *Alpina* species and species within other sections such as sect. *Cerinthoidea*, sect. *Oreadea* and sect. *Vulgata*. Given that 42 of the 45 sect. *Subalpina* species are endemic, this

hybridisation is again likely to have happened between polyploids in Britain.

If the British sect. *Alpina* endemics did migrate from their place of origin in Europe, it is surprising that only *H. alpinum* still occurs there; it is difficult to explain how the 34 other species followed the same or very similar migration pattern.

The involvement of pollen should be considered in theories of evolution of *Hieracium* sect. *Alpina*, and experimental work to test this hypothesis is required. The variation in pollen production between specimens within a species

and the higher pollen production in cultivated specimens suggests that pollen production may be under environmental as well as genetic control.

#### ACKNOWLEDGMENTS

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APPENDIX 1. LIST OF ACCESSION NUMBERS OF HERBARIUM SHEETS OF BRITISH *HIERACIUM* SECT. *ALPINA* EXAMINED. SPECIMENS PREFIXED BM ARE FROM THE NATURAL HISTORY MUSEUM, LONDON, AND PREFIXED E FROM ROYAL BOTANIC GARDENS, EDINBURGH. ALL OTHER SHEETS ARE IN NMW

- H. alpinum*: 25.148.516, 25.149.5156, 27.72.1260, 28.131.4188, 28.131.4242, 49.29.5538, 85.40.2659, V.2005.1.233, BM000916035, BM000916037, BM000916049, BM000916050, BM000916052, BM000916053, BM000916054, BM000916055, BM000916056, BM000916057, BM000916058, BM000916062, BM000916063, BM000916064, BM000916065, BM000916066, BM000916067, BM000916068, BM000916069, BM000916070, BM000916071, BM000916072, BM000916073, BM000916074, BM000916075, BM000916076, BM000916077, BM000916078, BM000916082, BM000916083, BM000916084, BM000916085, BM000916106, BM000916086, BM000916087, BM000916088, BM000916089, BM000916090, BM000916091, BM000916092, BM000916093, BM000916094, BM000916095, BM000916096, BM000916097, BM000916098, BM000916099, BM000916100, BM000916101, BM000916102, BM000916103, BM000916104, BM000916105, BM000916107, BM000916108, E00031564, E00031565, E00031566, E00031567, E00031568, E00031569, E00031571, E00031572, E00031573, E00031574, E00031575, E00031576, E00031577, E00031578, E00031580, E00031581, E00031650, E00031663, E00048447, E00048602, E00171460, E00209980, E00209981.
- H. backhousei*: V.2000.008.6, V87.58.3081.  
*H. calenduliflorum*: 27.72.1265, 28.131.4227.  
*H. calvum*: V.2000.008.28, V.2000.008.30.  
*H. completum*: 25.149.5178, 25.149.5195, V87.58.3080.  
*H. deargicola*: V.2000.008.58.  
*H. einichense*: V.2000.008.59.  
*H. eximium*: 27.72.1268, 28.131.4206, 28.131.4219, 26.553.574, 28.131.4215a.  
*H. eximium* f. *tenellum*: 27.72.1269, 28.131.4217.  
*H. globosiflorum*: 20.347.29, 27.72.1267, 28.131.4244.  
*H. graniticola*: 28.131.4239, V.2000.008.10.  
*H. grovesii*: V.2000.008.12, V.2000.008.14, V.2000.008.15.  
*H. hanburyi*: V.2005.1.234, 25.149.5176, 25.149.5188, 25.149.5192, 25.149.5193, 27.72.1273, 28.131.4303, 28.131.4304, 28.131.4306, 28.131.4307, 28.603.23, V87.58.3084.  
*H. hanburyi* f. *atraticeps*: 85.40.2660, V.2000.008.5.  
*H. hanburyi* f. *pusillum*: V.2000.008.16.  
*H. holosericeum*: 20.7., 26.553.573, 27.72.1262a, 28.131.4192, 28.131.4194, 28.131.4195, 28.131.4196, 28.603.69, 49.29.5536, V87.58.3093.  
*H. insigne* f. *celsum*: V.2000.008.34, V.2000.008.35, V.2000.008.36, V.2000.008.37, V.2000.008.38.  
*H. insigne* f. *insigne*: V.2000.008.39.  
*H. kennethii*: V.2000.008.40.  
*H. larigense*: V.2000.008.41.  
*H. leptodon*: 28.131.4211, 28.131.4212, 28.131.4214, 28.131.4229, V.2000.008.42, V.2000.008.43.  
*H. macrocarpum*: 28.131.4246, V.2000.008.17, V87.58.3095.  
*H. marginatum*: V.2000.008.61, V.2000.008.19.  
*H. memorabile*: 28.131.4257, 28.131.4258, 28.131.4260, V.2000.008.20.  
*H. milesii*: 28.131.4237, 49.29.5534, V.2000.004.27.  
*H. mundum*: 28.131.4259, V.2000.008.51.  
*H. notabile*: 28.131.4225, V.2000.008.21.  
*H. optimum*: 28.131.4213, V.2000.008.52.  
*H. pensum*: V.2000.008.53.  
*H. pentaploideum*: V.2000.008.60, V.2000.008.60.

*H. perscitum*: 28.131.4248, V.2000.008.54.

*H. probum*: 25.149.5179, 28.131.4238, 28.131.4253, 28.131.4319, V.2000.008.55.

*H. pseudocurvatum*: 28.131.4255.

*H. pseudopetiolum*: V.2000.008.22, V87.58.3078.

*H. subglobosum*: 25.149.5173, 25.149.5177, 28.131.4247, V.2000.008.56.

*H. subgracilentipes*: V.2000.008.24.

*H. subtenuifrons*: V.2000.008.57, V.2000.008.63, V.2000.008.63.

*H. tenuifrons*: 28.131.4197a, 25.149.5169, 28.131.4209, 28.131.4231.