TAXONOMY AND POLLEN MORPHOLOGY OF THE
CALTHA LEPTOSEPALA COMPLEX

BY

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I. INTRODUCTION

Contrary to Europe, with only one Caltha species, North America has at
least three species of this genus. These are the polymorphic C. palustris
L., also widely distributed in Europe, the floating aquatic C. natans Pall.
and the polymorphic C. leptosepala-biflora group.

Two previous papers (SMIT 1967, 1968) dealt with taxonomic aspects
of C. palustris, that in North America were not essentially different from
those of European material.

C. natans does not provide great taxonomical problems. It is a rare
aquatic with small white flowers with many follicles in each flower that
together form a globular capitule, as it were. This species occurs in
relatively few places in Minnesota, Northern Wisconsin, Canada, Alaska
and Northern Asia. DAHL and ROWLEY (1956) examined cytology and
pollen morphology of C. natans. As in C. palustris, the pollen grains are
tricolpate, but noticeably smaller than in that species. The chromosome
number is 32, also one of the cytotypes of C. palustris has 32 chromosomes,
but the chromosomes of C. natans are much smaller and thinner.

The group C. leptosepala-biflora has posed many problems to taxonomists.
Both C. leptosepala DC. and C. biflora DC. were published in 1818. HOOKER,
in his Flora of Boreal America (1833), repeated De Candolle's short
descriptions, but adding to C. biflora: "I have seen no specimens which
exactly accord with this, but it seems to me too nearly allied to C. lepto-
sepala." TORREY and GRAY (1838-1840) followed Hooker with regard to
C. biflora. LAWSON (1884) distinguished only C. leptosepala with 2 varieties,
viz. C. leptosepala var. leptosepala and C. leptosepala var. biflora. HUTH
(1892) combined C. biflora with C. leptosepala. GREENE (1899), however,
distinguished 8 species in this complex, viz. C. biflora, C. malvacea, C.
confinis, C. leptosepala, C. macounii, C. chelidonii, C. howellii, C. rotundi-
folia and C. chionophila [thus creating 6 new species of his own in addition
to those of De Candolle]. Most modern flora's such as those of HULTÉN
(1941), ABRAMS (1944), ANDERSON (1959), HITCHCOCK (1964), recognize
C. leptosepala and C. biflora, sometimes with subspecies or varieties.

The distribution area of C. leptosepala is not as large as that of the
circumboreal *C. palustris*, being limited to Western North America: Cascade Range, Sierra Nevada and Rocky Mountains, to Southwestern Alaska. The problems encountered in trying to define this species have led to the present study.

The results published here are mainly based on investigation of herbarium material, but further biosystematical research is necessary, such as cultivation and crossing experiments, as well as combined cytological, ecological and morphological studies.

The taxonomical part of this study was carried out by the first author, the pollen morphological part by the second author.

II. MATERIAL AND METHODS

446 Herbarium sheets of North American *Caltha* material were obtained on loan by courtesy of the U.S. National Herbarium, Washington D.C.

The complex *C. leptosepala-C. biflora* will henceforth be referred to as *C. leptosepala* coll.

The total number of specimens belonging to *C. leptosepala* coll. amounted to 177 herbarium sheets; the following morphological characters were recorded for each specimen in a table: locality, altitude, leaf shape, leaf margin, inflorescence, stipitate vs. sessile follicles, shape of the tepals (linear or oblong), pollen characters, and shape of the stamens. Pollen grains of the following 8 herbarium sheets considered representative for the variation within the material of *C. leptosepala* coll. were acetolysed: Baker 227, Colorado; Barber s.n., Colorado; Jones 5743a, Utah; Griggs s.n., Alaska; Gale 265, Oregon; Coville et all. 1168, Alaska; Coville et all. 384, Oregon; Howell s.n., Oregon.

The pollen grains were acetolysed according to the method described by Erdtman in 1960, and which is revised by Reitsma (1969). The grains were mounted in glycerine-jelly and sealed with paraffin.

From the remaining specimens one or two anthers were dissected which were then immersed in a droplet of an alcohol, water and safranin mixture. After some stirring with a needle to separate the pollen grains from the

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**PLATE I**

Variation in pollen grains of the *Caltha leptosepala* complex.

1. Baker 227; optical section, nexine without endocracks; $\times 1700$
2. Baker 227; spinules along margins of the colpi; $\times 1700$
3. Baker 227; surface view; $\times 1700$
4. Coville et all. 1168; colpi; $\times 1400$
5. Coville et all. 1168; surface view; $\times 1400$
6. Howell s.n.; optical section, nexine with endocracks; $\times 1700$
7. Howell s.n.; circular porus with granulate porus membrane; $\times 1700$
8. Griggs s.n.; endocracks pattern; $\times 1700$
9. Griggs s.n.; short colpus or elongated porus; $\times 1700$
10. Coville et all. 384; slightly elongated porus; $\times 1700$
PETRA G. SMIT AND W. PUNT: Taxonomy and pollen morphology of the Caltha leptosepala complex.

PLATE I
anthers, a cover slip was placed on the droplet and the slide was sealed with nail polish. This simple method was sufficient for a quick classification of the pollen grains into one of the groups found in the acetolysed material.

The photographs were taken with a Leitz Ortholux microscope, combined with a Leitz Orthomat, using an obj. plan apo öl 90/1.40 and a Leitz interference green filter AL 546.

Plants obtained from seeds of C. leptosepala coll. that were received so far, were grown in the experimental garden. Root tips from these plants provided the material for chromosome counts. Like C. palustris (Smit 1967: 501), C. leptosepala has many and long chromosomes and, due to this, microtome slides again proved unsatisfactory so that the squash technique had to be used. In order to shorten the chromosomes, living root tips of 2 cm long were first treated in a colchicine 0,1 solution during 4 hours. Next, the root tips were fixed in Carnoy, hydrolysed in 1 N HCl at 58-60° C. during 20 minutes, and then stained for one hour in leuco-fuchsin (Feulgen). Plastic cover glasses were used for squashing which were removed in acetone after one hour. After going through the acetone-acetone/xylol-xylol sequence, the preparations were mounted in Canada balsam under cover glasses.

III. Results

III.1. Description of the pollen grains

Pollen grains either colpate or porate.

Apertures: the number of the apertures varies from 3 colpi via 6 colpi to 12 pori. The apertures are only slightly sunken and rather broad. The margins of the apertures are diffuse. There is a colpus membrane with distinct granules (membrana granulata).

Exine: the outer part (sexine) is thicker than the inner part (nexine). The sexine consists of short, rather thick columellae which support a tectum provided with small spinules. These spinules are smaller than 1 μ (microechinate) and rather regularly distributed. The nexine in species with more than 3 apertures have narrow slits ("endocracks"; Oldfield, 1959). These endocracks form irregular, reticulate patterns.

Shape: specimens with 3- and 4-colpate grains have a polar axis which is slightly longer than the equatorial diameter (prolate sphaeroidal). All specimens with more than 4 apertures are sphaeroidal.

It was possible to distinguish 4 groups of different pollen grains. These groups are:
1. Pollen grains tricolpate (Baker 227; Barber s.n.; Jones 5743a).
2. Pollen grains 4- to 6-colpate (Gale 265; Jones 5743a).
3. Pollen grains with 6- or more apertures (pantocolpate, pantoporate); apertures are short colpi or elongated pori (Griggs s.n.; Coville et all. 1168).
4. Pantoporate pollen grains, 12 circular pori (Coville et al. 384; Howell s.n.).

N.B.: These groups are not strictly delimited. Transitions occur from one group to another.

III.2. Chromosome counts

Only few chromosome counts have been published for *C. leptosepala* coll. Langlet (1932) counted 2n = 48 in *C. leptosephala* (sic!); Wiens and Halleck (1962) counted n = 22 in *C. leptosepala* from Boulder, Colorado; Mulligan and Taylor (1968, pers. comm.) found 2n = 48 in plants from 3 different populations on the Queen Charlotte Islands, B.C., which they considered as *C. biflora*.

The present author, so far, only had plants from 3 populations of *C. leptosepala* coll. at her disposal from which the chromosome number could be determined. The results are given in table 1.

**TABLE 1**

List of specimens examined cytologically, with the number of chromosomes, reference to the collection number, and the origin of the material.

<table>
<thead>
<tr>
<th>Coll. no.</th>
<th>no. of plants studied</th>
<th>2n</th>
<th>Origin of the material</th>
</tr>
</thead>
<tbody>
<tr>
<td>D504-509</td>
<td>6</td>
<td>96</td>
<td>France, botanical garden Grenoble</td>
</tr>
<tr>
<td>D484-493</td>
<td>5</td>
<td>96</td>
<td>U.S.A., Winnemucca Lake California</td>
</tr>
<tr>
<td>D494-503</td>
<td>6</td>
<td>48</td>
<td>U.S.A., Kangaroo Lake California</td>
</tr>
</tbody>
</table>

III.3. Herbarium studies

According to De Candolle's description, *C. leptosepala* has stems with 1 or 2 flowers, leaves rosulate, petiolate, ovate-cordate, crenate, tepals 10, linear, and follicles 8–10, substipitate; *C. biflora* has stems with 2 flowers and one leaf, other leaves rosulate, petiolate, reniform, crenate, and oblong tepals. The flowers of *C. leptosepala* coll. are white and have more tepals than *C. palustris* which has only 5 or 6, yellow tepals. The mature seeds of *C. palustris* are different from those of *C. leptosepala* coll. The dark brown seeds of *C. palustris* have a light brown, swollen, persistent raphe and chalaza which form a floating organ. Experiments by the first author with seeds from 20 populations of *C. palustris* showed that these seeds could remain floating for about 10–15 days on the average. This applied both to cytotype 2n = 32 and to cytotype 2n = 56. This ability to float was much less in seeds that had been kept one year or longer, all these had sunk after one week. Contrary to the asymmetrical seeds of *C. palustris*, the mature seeds of *C. leptosepala* coll. are bilaterally symmetrical, probably due to the lack of a swollen raphe and chalaza, and they are also much darker, almost black. These seeds cannot float and sink immediately. The shape of the seed of each species is shown in fig. 1.
As mentioned under "Material and methods", the characters upon which De Candolle based his division, and all other possible ones, have been tabulated. Localities where material was collected, as far as they could be found in an atlas, have been mapped in fig. 2. A number of characters could also be indicated on the map of fig. 2 by means of different symbols.

Fig. 2 shows that all material collected in the Rocky Mountains of Utah, Colorado, Wyoming, the S.E. part of Idaho, S. Montana, N.E. Nevada and N.E. Arizona had one-flowered stems, leaves with a length/width ratio of 3:2 and tricolpate pollen grains. These plants strongly agreed with the description of *C. leptosepala* DC. The plants collected in the Sierra Nevada and the Cascade Range constitute a somewhat less homogeneous group. This concerns material from W. Nevada, California, Oregon, W. Washington, to Vancouver Island. These plants mostly had round peltate-like leaves with a length/width ratio of 1:1, but also leaves slightly longer than broad occurred. One-flowered stems occurred in about 70% of the cases, two-flowered stems in the remaining 30%. The pollen grains were mostly pantoporate, but in a number of cases pantocolpate. These plants in general showed most resemblance to *C. biflora* DC.

Even more heterogeneous was the material from N. Idaho, the greater
Fig. 2. Distributional map of the investigated herbarium material showing the intergradation and variability in *C. leptosepala* coll. in N.W. America.
part of Washington, British Columbia, Alberta and S. Alaska. Often the leaves were reniform, but also plants with ovate-cordate leaves occurred. About 70% of the plants had two-flowered stems and only 30% had one-flowered stems. The microscope slides of the pollen showed a continuous transition from tricolpate to pantoporate grains. This material partly more or less corresponded with the description of *C. biflora*, partly so with that of *C. leptosepala*, but an important part had characters of both species.

The following characters showed little constancy and varied even within the two regions with habitually and pollen morphologically fairly homogeneous populations, i.e. the Rocky Mountains on one side and the Cascade Range and Sierra Nevada on the other:

A. **Leaf margin:**

All plants from Washington had a clearly crenate leaf margin, those from California and Utah had an entire margin. In the other states where *C. leptosepala* coll. occurs part of the plants had a dentate leaf margin, other plants had entire, crenate or crenate-dentate leaves.

B. **Height of the fruiting plants:**

This varied from 35 cm to less than 10 cm, the average height for each state varying from 23 to 14 cm. The height of the plants seemed to depend upon the altitude of the localities where the plants grew: 10 plants which had been collected at an altitude of about 2000 m averaged about 23 cm in height, but this average was only 13 cm for 14 plants collected at about 3000 m.

Unfortunately, the altitude has been recorded for a part of the material only. This phenomenon of plants becoming progressively smaller the higher the vegetations in which they grow are situated, was also observed with *C. palustris* (Smit 1968). Plants in a population of this species at an altitude of 1650 m never became higher than 15 cm, whereas plants in a population 700 m lower could attain a height of 80 cm. The chromosome number was the same for both populations. After one year's cultivation in the experimental garden, there was no difference in height any more between plants from the two populations.

C. **Follicles:**

The follicles of *C. leptosepala* have been described as substipitate, but this has not been confirmed by the author's own investigations. Both in *C. leptosepala* and *C. biflora* follicles varied from shortly stipitate to sessile.

D. **Shape of the tepals:**

This did not prove to be a very stable character either, though regional shifts occurred: about 60% of the plants from the Rocky Mountains had
linear tepals and 40% oblong tepals, the reverse being the case in the Cascade Range and the Sierra Nevada with 40% of the plants having linear and 60% having oblong tepals. In the area comprising Northern Washington and Idaho to Alaska, 50% of the plants had more or less linear tepals and 50% had oblong, rather than linear, tepals.

E. Shape and size of the filaments:

Plants from the Rocky Mountains generally had filaments as broad as or slightly broader than the anthers. The plants from the other regions partly had filaments narrower (to filiform) than the anthers, partly had filaments broader than the anthers. As in C. palustris, the length of the filaments strongly depended upon the development stage of the flower.

The material examined above also included plants seen by Greene and cited by him in his paper: Segregates of Caltha leptosepala (1899). They may be commented upon as follows:

C. malvacea Greene. Wilkes expedition 484, Cascade Mts. eastward and Vasey 162, Mts. of Eastern Washington. It seems likely that Greene misread the locality, the writing on the label being difficult to read, for he cites: “near the Cascades in Eastern Oregon”. The leaf sinus of this species should be narrow or closed, but this on the Vasey sheet appears to be very variable even among the leaves of the same plant, going from closed to wider than 90°. This species is also supposed to have light green to yellow-green leaves, which is very difficult to ascertain in dried material.

C. confinis Greene. White s.n., Reindeer station at Port Clarence Alaska. Greene based this species on this single incomplete specimen in the U.S. herbarium only. All characters of this plant agreed with the description of C. palustris [alternative: all characters of this plant well match the concept of C. palustris], so that it cannot be considered a distinct species.

C. macounii Greene. Piper 2001, Mt. Rainier California; Coville and Leiberg 217, Cougar Peak Oregon; Henderson 3139, Florence Idaho; Sandberg and Leiberg 723, Stevens Pass Washington. Of the last collection Greene states that the flowering stem bears 4 flowers and he regards 1–4 flowers as a diagnostic character of this species. The herbarium sheet, with two mounted plants, indeed suggests inflorescences with 3 or 4 flowers respectively when glanced at superficially. A closer examination reveals that 3 (1 and 2 respectively) flowers are loose and do not belong to these plants at all, only having been mounted in such a way as to deceive the casual visitor. Henderson 3139 indeed shows a plant with a stem bearing two leaves and three flowers, but this suggests abnormal, rather than normal, growth. The four collections cited form a heterogeneous lot. Piper 2001 has dentate-crenate leaves with an open sinus, one-flowered stems and pantocolpate pollen grains, and there is a clear resemblance with the two specimens cited as C. malvacea. Henderson 3139 resembles Piper 2001 in its more or less orbicular dentate-crenate leaves, but the sinus is closed, the pollen grains are porate and most stems bear two
flowers. The two plants on the sheet Sandberg and Leiberg 723 have leaves which are longer than broad, with an almost entire margin and an open sinus; the pollen under the microscope showed a transition from grains with many short colpi to porate grains. The plants of Coville and Leiberg 217 have entire, oblong leaves with a closed sinus, and two-flowered stems.

*C. howellii* Greene. Howel s.n., base of Mt. Hood Oregon; Brown 388, Mt. Shasta California; Austin 181, Butte Co. California; Sonne s.n., Mt. Stanford California. The last sheet bears four specimens, 1 large and 3 smaller ones. The large plant much resembles the plants of the other 3 collections, such as in its long-petioled, orbicular leaves with overlapping basal lobes. The 3 smaller plants, on the other hand, also show clear resemblance with, among others, *C. chionophila*—Jones 5743a—, such as, for example, in the short-petioled leaves that often are slightly longer than broad.

*C. rotundifolia* (Huth) Greene. Greene does not cite any specimen here; it is supposed to be a widely distributed alpine species from Colorado to Montana.

*C. chionophila* Greene. Jones 5743a, 5822a, and 5779a; Fishlake Utah; Jones 5893a, Marysvale Utah. This “species” is supposed to be acaulescent with leaves having a distinct constriction below the middle. None of the specimens examined was more clearly acaulescent, however, than a part of the other specimens of Greene’s 8 species and there was no constriction of the lamina either.

IV. DISCUSSION

A study of the pollen grains of the species *Caltha leptosepala* coll. has given important additional data for the classification of the specimens. Structure and ornamentation of the pollen wall (exine) is essentially the same in all investigated specimens, but there was a large variation in the number and shape of the apertures. It is possible to make a perfect morphological series from 3-colpate pollen grains to 12-porate ones.

As is suggested in other publications (e.g. Punt 1967) such a morphological series might be called an “evolutionary trend”. In *C. leptosepala* coll. two major trends (1. and 2.) and two minor trends (3. and 4.) could be distinguished.

1. Number of aperture increasing from 3, via 4 and 6 to 12
2. Shape of apertures changing from a distinct colpus, via short colpus and elongated porus to circular porus
3. Shape of the pollen grains changing from prolate sphaeroidal to sphaeroidal
4. Nexine without endocracks to nexine with distinct endocracks

A morphological series starting with 3-colpate pollen grains and ending with pantoporate grains is found in several other genera of the *Ranun-
culaceae; e.g. in *Anemone* (SI I-Tsjen and Tsjan Tsin-Tan 1964) and *Ranunculus* (Bot and v. d. Spoel-Walvius 1968). However, it is striking, that such a series occurs within one species.

Nair (1961) also found pantoporate pollen grains and 3-colpate ones within one species of *Caltha*. However, it is not quite certain if *C. palustris* var. *alba* (Cambess.) Hook. f. et Thoms. [= *C. alba* Cambess. in Jacqem. Voy. dans l'Inde: 6 (1844)], with its pantoporate pollen grains and white flowers actually belongs to the polymorphic species *C. palustris* with yellow flowers. It would be interesting to investigate more material from this particular variety *alba*; especially as regards its taxonomical features.

It has become evident, from these investigations, that a clear definition of species within the *C. leptosepala-biflora* complex is not possible on morphological grounds. In the literature, too, there is much confusion regarding this complex probably due to the variability being insufficiently known to the authors. Lack of knowledge of the variability must have led Greene to distinguish as many as 8 species; for this reason, and also in view of a number contradictions and inaccuracies in Greene's work, the present author cannot agree with Greene. Most other authors disagree among each other on whether one or two species, whether or not with subspecies and/or varieties, are to be distinguished within the *C. leptosepala-biflora* complex.

The leaf shape is always given as the most important character to differentiate between *C. leptosepala* and *C. biflora*, i.e. lamina longer than broad vs. lamina orbicular respectively. This difference was clearly observed, with few exceptions, in the material from the southern part of the Rocky Mountains on the one hand and that from the Cascade Range and the Sierra Nevada on the other. In the remaining part of the distribution area, however, there appeared to be a continuous intergradation between the two extremes. The presence of one vs. two flowers on the stem could hardly be considered as a character of diagnostig value at all, both regards data from literature and in the authors own investigations. Only plants with one flower would have to be regarded as *C. leptosepala* accordingly, but often there appeared to be no correlation with other characters: *C. leptosepala* should have oblong leaves, but plants combining orbicular leaves with one flower proved no exception. There was even less correlation among other characters studied (see table of the results). A good correlation could only be found between leaf shape and pollen morphology, sometimes in combination with the number of flowers in the inflorescence. Therefore these characters were selected for the map of fig. 2. On this map one can discern two groups of plants within *C. leptosepala* coll. that are quite distinct from each other; the remaining plants constitute a third group which is intermediate between the other two and which has plants totally intermediate of resembling plants from one or the other group from most, but not all, characters.

In the extensive area of the Rocky Mountains, reaching up to the
Yukon River, many montane plants which had been able to persist south of the Pleistocene ice cover could migrate again towards the North and thus re-establish themselves in that area. The geographical distribution pattern of the present *C. leptosepala* coll. strongly suggests that the ancestral forms were such montane plants which during the Pleistocene occurred in two geographically isolated populations in the southern parts in the Sierra Nevada and the Rocky Mountains respectively. After the retreat of the ice, the plants of the populations came into contact with each other on their northbound migration in the region of the Snake and Columbia Rivers, giving rise to a swarm of hybrids which continued to expand in a northerly direction. This hypothesis would get a strong support if it could be demonstrated that crossing between plants from the two well-defined populations would result in fertile hybrids with intermediate characters.

After examining all available herbarium specimens, the author found it impossible to delimit or define more than one species within *C. leptosepala* coll. by means of constant characters of significant value. Since, however, the two populations in the Rocky Mountains and in the Sierra Nevada and Cascade Range respectively are well separable in a number of morphological characters, and the third group has the appearance of a hybrid swarm between these two, the author has arrived at the conclusion (which, however, needs confirmation by future experiments) that *C. leptosepala* coll. can be regarded as one species with two subspecies which may be defined as follows:

*C. leptosepala* DC. ssp. *leptosepala*. Stem with one flower, rosulate leaves ovate-cordate, tepals ± linear, pollen grains tricolpate.

*C. leptosepala* DC. ssp. *biflora* (DC.) P. G. Smit, comb. nov. (*C. biflora* DC.). Stems with one or two flowers, rosulate leaves mostly reniform, tepals more tending to be oblong, pollen grains pantoporate (sometimes pantocolpate).

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