



MICROMORPHOLOGICAL EVIDENCE FOR ANDROECIUM ORIGIN OF *CLAYTONIA* (MONTIACEAE) PETALOIDS

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Abstract. Caryophyllales is an order distinguished for having flowers with only one perianth whorl – the perigone. The perigone is a calyx derived structure that can have either petaloid or sepaloid appearance. Members of the Portulacinae suborder have tendency to have a false bipartite perianth, forming a petaloid perigone and an epicalyx with the subtending bracts of the flower. Although *Claytonia* belongs to the Portulacinae suborder, previous studies have suggested a different origin for its petaloid organs other than the sepals. In this study we investigated the floral development of *Claytonia sibirica* and *Claytonia perfoliata* using Scanning Electron Microscopy (SEM) to understand the origin of the petaloid organs in the genus. Our results show that petaloid organs in *Claytonia* are of androecium origin and can be interpreted as the expression of the typical Caryophyllales' perigone growing in androecium tissue.

Key words: *Claytonia*, Caryophyllales, Portulacinae, Floral development, perigone, perianth

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Introduction

Caryophyllales have a fascinating evolutionary history, which is deeply related with the evolution of the perianth of their flowers. The perianth is usually formed by two series or whorls, a protective whorl formed by sepals (the calyx) and a pollinator-attractive whorl formed by petals (the corolla). In Caryophyllales the perianth is formed by one single whorl (the perigone) due to the loss of the corolla, which is thought to be related with a wind-pollinated ancestor (BROCKINGTON *et al.*, 2009). Even though the perigone might have sepaloid or petaloid appearance it is of calyx origin, having a typical 2/5 initiation pattern and imbricate aestivation (RONSE DE CRAENE 2008). Another important feature found in Caryophyllales that might be associated with the loss and reinvention of petaloid organs is the centrifugal stamen initiation (BROCKINGTON *et al.* 2009; RONSE DE CRAENE 2008).

The typical flower of the Portulacinae suborder has a pentamerous petaloid calyx (NYFFELER & EGGLI 2010). As the sepals have gained petal function, the protection role was taken by the two bracteoles acting as an epicalyx. Even though this has been largely assumed, previous studies on *Claytonia* L. (Montiaceae) have suggested that petaloids might have a different origin from the traditionally assumed for Portulacinae. The anatomical studies of MILBY (1980) suggest that petaloids and stamens are

originated from the same primordium, suggesting an androecium origin for the petaloids in *Claytonia*. As the typical Portulacinae, flowers of *Claytonia* also have five petal-like organs and two bracteoles (NYFFELER & EGGLI 2010), however the petaloids in *Claytonia* are fused at the base with the stamens, and both organs appear to share the same vascular bundle (MILBY 1980).

In this study a micromorphological analysis was conducted using Scanning Electron Microscopy (SEM) to investigate the origin, development and evolution of the petaloids of *Claytonia*. This study is part of an MSc dissertation.

Material and methods

Several floral buds in different stages of development of *Claytonia sibirica* L. and *Claytonia perfoliata* Donn ex Willd. were collected and fixed in FAA (90% ethanol; 5% formaldehyde; 5% acetic acid). Samples were previously stored in 70% ethanol and analyzed under a ZEISS Stemi SV6 Dissecting Microscope. The material was critical point dried, mounted on SEM pin stubs and coated with platinum. The specimens were observed and photographed using a LEO Supra 55VP Scanning Electron Microscope. SEM images were processed using Adobe Photoshop CS2.

Results

The inflorescence type in *Claytonia* is a monochasium – each axillary bud originates lateral branches in alternation that give rise to other younger flower buds (Fig 1 A-B). From each undifferentiated flower bud, two opposite bracts are formed; the first one arises at the apex and the second one at the base of the flower (Fig 1 A-C). An invariable number of 5 stamens initiate more or less simultaneously (Fig 1 C-E). Only after this stage, the initiation of the petaloids is visible, arising from the base of each stamen (Fig 1 E-G). Petaloids grow simultaneously and centrifugally from the base of each stamen apparently in an imbricate aestivation (Fig 1 H-I). In the mature flowers it is possible to see the 5 stamens opposite to the 5 petaloids, all fused at the base forming a ring surrounding the gynoecium (Fig 1 I). The analysis of the developmental stages suggests a congenital fusion of stamen – petaloid and a postgenital fusion of the androecium, originating the ring that surrounds the gynoecium. The gynoecium is usually 3-loculate (rarely 2 or 4; Fig 1 I) and differentiate more or less simultaneously with the androecium (Fig 1 C-E).

Discussion

In this study we suggest two hypotheses for the origin of petaloids in *Claytonia*. There is evidence for an androecium origin of the petaloids, although the differentiation of these organs can also be interpreted as sepal-derived petaloids.

The timing and pattern of initiation of the perianth and the petaloids' position relatively to the stamens are evidence for petaloids with androecium origin. The petaloid initiation is much delayed, always arising after the stamens initiation and development. The results from the SEM analysis suggest that petaloids arise as part of the androecium from the base of the filaments, going in accordance with MILBY's (1980) results. The position of the petaloids relatively to the stamens also suggest that the perianth is not a second whorl of staminodes as they are not alternating with the stamens, arising opposite to the stamens. This is also evidence for the theory that petaloids in *Claytonia* are outgrowths of the stamen filaments, and thus part of the androecium. This point of view assumes that the perianth is totally lost in this group and new organs are formed previously from the filaments.

The theory of sepal-derived petaloids relies on the delay of calyx initiation, which in an extreme situation could lead to the incorporation of calyx parts in the androecium, maybe due to lack of space as the androecium initiation is very fast and occurs centrifugally. A similar situation has been recorded for *Portulaca* L., where there is also a delayed growth of perianth parts (DOS SANTOS 2011 – unpublished MSc thesis), showing that this situation is likely to happen also in *Claytonia*. Another evidence for this theory is the quincuncial aestivation pattern found in *Claytonia*. Although there is no evidence of a typical 2/5 calyx initiation due to the simultaneous arising of the petaloids, these show a quincuncial aestivation in maturity. This suggests that the calyx (or the perigone) is being expressed in androecium tissue due to a delay of growth of the petaloids. This can lead to a misinterpretation of the identity of perianth parts, appearing as stamen appendages.

Further evidence could be given by arguing that, for example, in *Montia fontana* L., a species belonging to a sister genus of *Claytonia*, there is sometimes a reduction in the number of stamens (3 instead of 5) but not in the number of petaloids (HOFMANN 1993), however rudimentary stamens in the single petaloids were not reported to be found. This might give evidence for the theory that the petaloids are not part of the androecium in *Claytonia*, although further investigation of these species should be carried out. HOFMANN (1993) described these organs as petals (stapetals) with a delayed growth, assuming also the total loss of sepals in this genus but not giving an explanation for the emergence of petals, lacking in the rest of the group. A new gain of petals in *Claytonia*, as assumed by HOFMANN (1993) is very unlikely and these are rather other misinterpreted organs of the flower that should be worth of a detailed morphological study in future.

The delay of calyx growth and subsequent incorporation in the androecium tissue seems to be the most suitable interpretation of our results; however molecular studies are essential to support this hypothesis.

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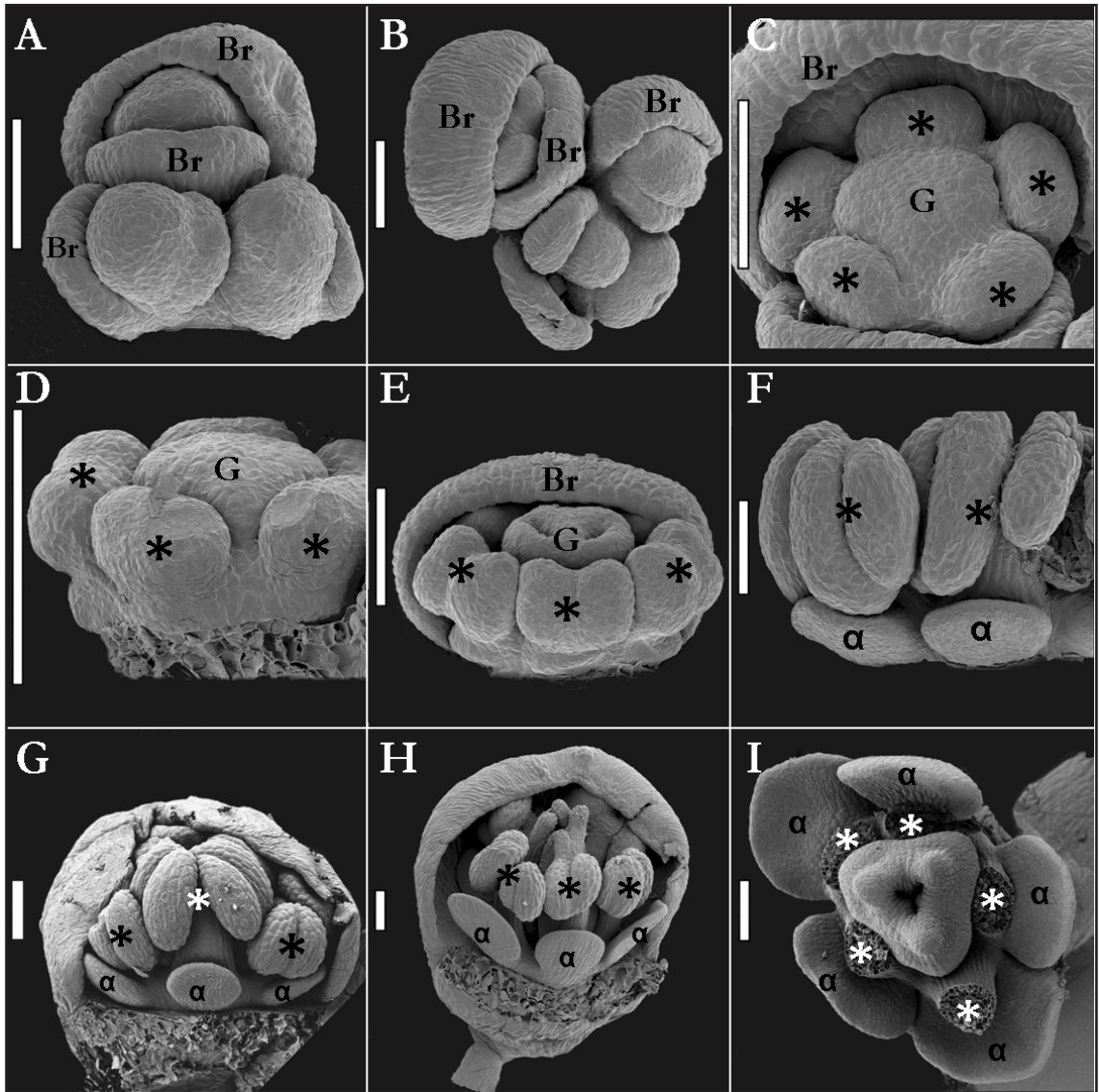


Fig. 1. *Claytonia sibirica* (A-F and I) and *Claytonia perfoliata* (G-H) SEM figure plate. A-B – inflorescence; C-D – stamen and carpel early development; E-G – petaloid initiation from the base of the stamens; H-I – growth of petaloids and full developed flower. (White bar A-I = 100µm). Br = bracteoles; α = petaloids; * = stamens and stamen primordia; G = gynoecium and gynoecium primordium.

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