Floral organogenesis of *Helleborus thibetanus* and *Nigella damascena* (Ranunculaceae) and its systematic significance

LIANG ZHAO¹, PING LIU¹, XIAO-FEN CHE¹, WEI WANG² and YI REN¹*

¹College of Life Sciences, Shaanxi Normal University, Xi’an 710062, China
²State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, China

Received 18 December 2010; revised 5 March 2011; accepted for publication 25 March 2011

Floral organogenesis in *Helleborus thibetanus* and *Nigella damascena* was examined and compared using scanning electron microscopy and light microscopy, and the putative relationships of *Helleborus* and *Nigella* were analysed. *H. thibetanus* and *N. damascena* share some features of floral phyllotaxis and development of the sepals, petals, stamens and carpels, which are also found in other members of Ranunculaceae. However, they differ strongly in the number and degree of fusion of the carpels: in *H. thibetanus*, the two carpels are slightly united at the base, whereas, in *N. damascena*, the gynoecium is syncarpous and the five carpels are united throughout the ovary. Differences are also noted in petal development. The blade of the young petal of *H. thibetanus* develops two bulges which become connate and then fuse with the blade at the sides, developing more quickly than the blade and forming a tubular petal. In *N. damascena*, a single ridge is formed on the petal blade which develops into the smaller adaxial labium of the bilabiate petal, whereas the blade itself develops into the larger abaxial labium bearing two pseudonectaries. The outermost stamens are delayed in development in *Helleborus*, but not in *Nigella*. Although the results from our investigation are preliminary, differences in floral development characters suggest that *Helleborus* and *Nigella* may not be closely related and possibly support placement into separate tribes.


INTRODUCTION

The genus *Helleborus* L. consists of 20 species and is distributed throughout southern Europe, northern Africa, southwestern Asia and western China (Tamura, 1995). *Nigella* L. comprises 16 species and occurs throughout the Mediterranean region and southwestern Asia, extending westwards to central Europe and eastwards to central Asia (Zohary, 1983; Tamura, 1995).

*Helleborus* and *Nigella* exhibit some characteristics which are uncommon in Ranunculaceae. The carpels are connate only at the base in *Helleborus* (and also in *Dichocarpum* W.T.Wang & Hsiao), but are connate to the apex of the ovaries in *Nigella*. The petals are tubular or cupular in *Helleborus*, but bilabiate in *Nigella* (and also in *Eranthis* Salisb.). The sepals are persistent in *Helleborus*, but caducous in *Nigella* (caducous sepals are common in the family) (Wang, 1979; Tamura, 1995). Both genera have R-type chromosomes. The basic chromosome number is \( N = 8 \) \((2n = 32)\) in *Helleborus*, which is common in the family, and \( N = 6 \) \((2n = 12)\) in *Nigella*, the lowest number in the family (Langlet, 1932).

Because of the special features of *Helleborus* and *Nigella*, their systematic position was, and still is, uncertain. Hutchinson (1959, 1969) treated *Helleborus* and *Nigella* as members of Helleboraceae. Most authors, however, have agreed that they belong to Ranunculaceae, but some have placed them in different subfamilies (Helleboroideae and Trollioideae; see

*Corresponding author. E-mail: renyi@snnu.edu.cn*
Takhtajan, 1997) or, more commonly, both in Ranunculoideae, either in the same tribe (Helleboreae; Tamura, 1966; Wang, 1979; Locente, Campbell & Stevenson, 1995) or in two tribes (Helleboreae and Nigelleae; Langlet, 1932; Duncan & Keener, 1991; Tamura, 1993, 1995). Jensen et al. (1995) placed Helleborus in its own tribe Helleboreae and placed Nigella in Delphinieae, based on the integration of four independent molecular datasets (Hoot, 1995; Jensen, 1995; Johansson, 1995; Kosuge et al., 1995). In contrast, Wang et al. (2009) assigned both Helleborus and Nigella tribal rank. Therefore, the relationships and affinities of these genera in Ranunculoideae remain unclear (Sun, McLewin & Fay, 2001).

The general floral organogenesis of Helleborus and Nigella was first addressed by Hirmer (1931) and Schöffel (1932). Detailed descriptions of the mature flowers of Helleborus and Nigella have been provided, especially for petals and carpels (Troll, 1933; Hiepko, 1965; Tamura, 1965, 1984; Rohweder, 1967; Kaussmann & Neitzel, 1972; Lang, 1977; van Heel, 1981; Kosuge & Tamura, 1989; Weber, 1993; Ronse De Craene & Smets, 1995; Erbar, Kusma & Leins, 1998; Vesprini, Nepi & Pascini, 1999; Endress & Matthews, 2006; Jabbour et al., 2009). Studies of the floral morphology and development of other genera and infrafamilial taxa of Ranunculaceae have also been conducted (Lehmann & Sattler, 1994; Chang, Ren & Lu, 2005; Tucker & Hodges, 2005; Song, Tian & Ren, 2007; Jabbour et al., 2009; Ren et al., 2009; Ren, Chang & Endress, 2010) and have contributed significantly to a better understanding of the evolution of Ranunculaceae. A comprehensive comparative study of the floral organogenesis of Helleborus and Nigella is still lacking. Here, we present our comparative study of the floral organogenesis of Helleborus thibetanus Franch. and Nigella damascena L.

MATERIAL AND METHODS

Flower buds of H. thibetanus were collected at all stages of development between 2003 and 2005 at Haopingsi, Mt. Taibaishan, Meixian County, Shaanxi Province, China (altitude, 1100 m; voucher: Bai Gen-lu 2004036, SANU). Flowers buds of N. damascena were collected between 2007 and 2008 in a glasshouse at the College of Life Sciences, Shaanxi Normal University, Xi’an, China (voucher: Wang Zi-fen 2008001, SANU). The material was fixed in formalin–acetic acid–ethanol–water (10 : 5 : 50 : 35). The material was dehydrated in an ethanol and iso-amyl acetate series, critical point dried in liquid CO₂, mounted on aluminium stubs and viewed in a scanning electron microscope (Hitachi S-4800). For light microscopy, the samples were dehydrated in an ethanol series, infiltrated with xylene and embedded in paraffin wax. The embedded samples were sectioned at 8 μm using a Leica RM2235 rotary microtome. Mounted sections were stained with safranin and fast green, and viewed under a Leica DM5000B optical microscope. Photographs of mature flowers were taken with a Nikon Coolpix 990 digital camera (Fig. 1A, B). The description of the floral morphology is based on 15 mature flowers. The symbols used in the floral diagrams and floral formulae follow Ronse De Craene (2010) and Prenner, Bateman & Rudall (2010).

RESULTS

Helleborus thibetanus (Figs 1A, C, 2–4)

The mature flower is 3.5–6.0 cm in diameter, terminal and solitary in position, bisexual and polysymmetric. The calyx consists of five (rarely six) sepals which are narrow-elliptic, persistent, petaloid and white with pink–red venation at anthesis, but green when fruiting. The corolla consists of eight to ten petals, which are small, yellowish-green, tubular, shortly stalked and nectariferous. There are 15–35 stamens and two (rarely one) carpels (Fig. 1A, C). Each carpel contains approximately 10–15 ovules.

The flower bud is subtended by two bracteoles (Fig. 2A). The sepals, petals and stamens are initiated clockwise or counterclockwise in a spiral sequence, with an average divergence angle of 137° between two consecutively initiated primordia (Fig. 2A–C). The five (rarely six) sepal primordia are crescent-shaped and truncate (Fig. 2A, B). There is a relatively long plastochron between the initiation of the last sepal and the first petal primordium (Fig. 2C). The petal and stamen primordia are narrow and hemispherical during early development (Fig. 2D). Therefore, distinguishing between the last petal and the first stamen in early development is difficult (Fig. 2D, E). Two carpel primordia are initiated one after another in an almost opposite position (Fig. 2G, H). The carpels do not occupy the entire floral apex, so a small residual floral apex remains (Figs 2H, 3E, F). This is later hidden by the accrescent carpels. If only one carpel primordium emerges, the residual floral apex remains larger (not shown).

In later development, the sepals enlarge and enclose all the following floral organs (Fig. 2I). The development of the petals is delayed and they remain smaller than the stamens until anthesis (Fig. 2H–J). Each young petal differentiates into a short stalk and a large lamina, which is slightly concave (Fig. 2K). During this stage of development, a depression appears on the ventral side of the lower part of the lamina. Then, two median bulges arise in a short succession at the base of the depression (Fig. 2L). The bulges expand laterally (Fig. 2M), which results in
them fusing with each other and with the lamina at the margin (Fig. 3A). The fused part grows more quickly than the lamina and the petal is initially obliquely cup-shaped (Fig. 3B) and then cup-shaped (Fig. 3C). Finally, because of the faster growth of the cupular part, the petal becomes tubular with a short stalk (Fig. 3D).

The stamens differentiate into filaments and anthers after the carpel primordia have appeared (Fig. 2H, I). Histological observations show that the stamen maturation is in a centripetal direction (Fig. 4A–E), even though the outermost stamen (St₁) is shorter than the other stamens (Fig. 2I, J).
After the initiation of the carpels, a longitudinal concavity appears on the ventral side of each carpel (Fig. 3E). With carpel enlargement, the concavity becomes deeper (Fig. 3F), and then the sides of the carpel fuse gradually from the base to the apex, except for the uppermost part (Fig. 3H). After closure of the carpels, the growth of the ovary is faster than that of the upper part of the carpel (Fig. 3I). Subsequently, the style elongates (Fig. 3J). During the development of the carpels, the residual floral apex remains and unites the two carpels basally to a slight degree (Fig. 3G–I). The style becomes slightly reflexed backwards and everted distally. A short recurrent stigma differentiates along the ventral slit and at the tip of the carpel. In the mature flower, it appears covered with unicellular papillae (Fig. 3K).

**Nigella damascena** (Figs 1B, D, 5, 6)
The mature flower is 2.5–3.0 cm in diameter, terminal and solitary in position, bisexual and polysymmetric. There are five (to eight), petaloid, blue sepals. The petals are small, blue, numbering five (rarely up to eight), or absent in some cultivated individuals. Each petal is stalked and has an abaxial bilobed lamina with a conspicuous pseudonectary on each lobe and an adaxial scale. The androecium consists of 30–45 stamens which differentiate into filament and anther. The carpel number ranges from three to five, with fused ovaries and free styles (Fig. 1B, D).

The five sepal primordia are initiated spirally and are crescent-shaped and truncate initially (Fig. 5A). Some of them then become rounded at the apex (Fig. 5A–C). Apparently, there is a relatively long plastochron between the initiation of the last sepal and the first petal, because initially the last sepal is much larger than the first petal (Fig. 5A). The petal primordia are initiated in the same sequence as the sepal and are rounded (Fig. 5A, B). The stamen primordia follow the same sequence of the perianth. They closely resemble petal primordia, and so distinction between the petals and the stamens in early flower development is difficult (Fig. 5C, D), and the prediction of whether or not the flower will have petals is impossible. The divergence angles in the perianth and stamens fluctuate at approximately 137°, which indicates spiral phyllotaxis according to the Fibonacci pattern. The carpel primordia are initiated spirally, but their sizes differ between the individual primordia. The carpel primordia are wider than the stamen primordia (Fig. 5F). They soon form a whorl and become equal in size (Fig. 6G). Sets of five, eight and 13 contact parastichies were recorded in all flowers, despite the occasional intercalation of isolated stamens between the contact parastichies (Fig. 5G). After the carpel primordia are initiated, a small residual floral apex remains (Figs 5F, G, 6F).

In later developmental stages, the young sepals enlarge and enclose all the other floral organs. The young petals start to flatten after the appearance of the carpels and can be clearly distinguished from the stamens (Fig. 5H). However, the petals show distinctly delayed development. They remain smaller than the stamens until anthesis (Fig. 5I). The petals differentiate into a wide lamina and a narrow base; a depression appears near the base of the lamina on the ventral side, and soon a bulge appears at the base of the depression (Fig. 6A). In subsequent development, the bulge expands (Fig. 6B) to form a scale (Fig. 6C) and finally develops into the adaxial labium (Fig. 6D). The narrow base of the young petal elongates and finally forms the stalk of the petal (Fig. 6B–E). Meanwhile, the lamina expands, becomes bilobate (Fig. 6B, C) and forms the abaxial labium (Fig. 6D). A bulge appears at the base of each lobe (Fig. 6B) and then develops into the pseudonectary (Fig. 6C, D). Hairs are present around the two pseudonectaries and the lower edge of the abaxial labium (Fig. 6C–E).

Each young stamen quickly differentiates into a filament and anther (Fig. 5H). Histological observations indicate that anther maturation is in a centripetal direction (not documented here).

After the carpels have formed a whorl, a depression appears at the base of each carpel (Fig. 6G). Subsequently, the flanks of two neighbouring carpels fuse gradually (Fig. 6H, I). Each of the carpels becomes horseshoe-shaped (Fig. 6I). The carpels enlarge...
(Fig. 6J) and the remaining free part of each carpel elongates to form a style (Fig. 6K, L). Before anthesis, unicellular papillae appear along the margin of the style to form the stigmatic tissue, and the upper parts of the styles are twisted (Fig. 6M, N).

DISCUSSION

ASPECTS OF FLORAL DEVELOPMENT

The floral phyllotaxis of Helleborus and Nigella was previously considered to be spiral throughout the
flower (Schöffel, 1932). However, from our observations of *H. thibetanus* and *N. damascena*, the floral phyllotaxis of sepals, petals and stamens is spiral, but that of the carpels is whorled, although they are initiated asynchronously.

Erbar et al. (1998) found that the divergence angle between the primordia of the last sepal and the first petal is approximately 50°, instead of 137°, in *H. foetidus* L. However, we found an equal 137° divergence angle between the primordia of the last sepal and the first petal in *H. thibetanus*. When we compared our findings with the images of *H. foetidus* of Erbar et al. (1998: fig. 1), we found that the initial two petals were hidden by the developing sepals. This may suggest a possible explanation for the difference between the two species in the same genus.

Anther maturation follows the stamen initiation sequence and is thus centripetal in *H. thibetanus* and *N. damascena*, as in most Ranunculaceae, e.g. *Caltha* L and *Trollius* L. (Song et al., 2007), *Adonis* L. and *Callianthemum* C.A.Mey. (Ren et al., 2009) and *Clematis* L. (Ren et al., 2010). However, in *Helleborus*,

---

**Figure 3.** Floral development of *Helleborus thibetanus*. A–D, Petal development. A, Two bulges fuse with each other and with the lamina at the sides. B, The fused bulges grow faster than the lamina, and the petal becomes obliquely cup-shaped. C, Petal becomes cup-shaped. D, Petal becomes tubular. E–K, Carpel development. E, Horseshoe-shaped carpels; a longitudinal cavity appears. F, Carpels become conduplicate. G, Same developmental stage as E, side view; star indicates residual floral apex between the two carpels. H, Carpel enlarges and closes. I, Same developmental stage as H, side view; star indicates residual floral apex between the two carpels. J, Carpels before anthesis. K, Close-up of distal part of a carpel shown in J, with slightly decurrent and unicellular papillate stigma. C, carpel; P, petal; S, sepal; St, stamen. Scale bars: A, G, K, 150 μm; I, 200 μm, E, F, 200 μm; B, C, H, 250 μm; D, 1.4 mm; J, 1.5 mm.

**Figure 4.** *Helleborus thibetanus*, longitudinal sections of stamens, showing that the stamen maturation is in a centripetal direction. A, Longitudinal section of flower. B–E, Close-up of different stamens shown in A. B, The outermost stamen (St₁) comprising mature pollen. C, The second stamen (St₂), showing microspores in tetrad before microspore release from tetrad. D, The third stamen (St₃), showing microsporogenesis in tetrad. E, The innermost stamen (St₄), showing microsporogenesis in stages of meiosis. C, carpel; S, sepal; St, stamen. Scale bars: A, 1.5 mm; B–E, 5 μm.
the delayed elongation of the filament in the outermost stamens gives the impression that the maturation of the stamens is opposite to the direction of their initiation, and is thus centrifugal (see *Aquilegia* L.; Tepfer, 1953; Feng et al., 1995). Some reports of *Anemone* indicate that anther maturation can be bidirectional (Chang et al., 2005; Ren et al., 2010). The developmental pattern of the androecium in *H. thi-
**betanus** is thus so far unique in Ranunculaceae. Further comparative research in other genera of the family is necessary to gain a better understanding of this process and of the discordance between the direction of stamen initiation and maturation.

Syncarp is rare in Ranunculaceae (Endress & Igersheim, 1999). The carpels have been reported to be basally connate in Helleborus (Rohwedder, 1967; Wang, 1979; Tamura, 1995) or connate in Nigella (Toxopéus, 1928; Eames, 1931; Troll, 1933; Kaussmann & Neitzel, 1972; Lang, 1977; Tamura, 1995). The carpels in *H. thibetanus* are slightly united at the base. The present observations in *N. damascena* are in agreement with previous studies.

The styles of *Nigella* are functionally elaborate structures, first described by Sprengel (1793), and later documented cinematographically by Weber (1992, 1993, 1995). At the final developmental stages of the androecium (pollen release), the styles curve downward and twist in a corkscrew fashion. This ensures that, during stigma receptivity, the stigmatic depression appears at the ventral base of the blade, and two bulges in *H. thibetanus* and a transversal ridge in *N. damascena* appear at the base of the depression. This pattern of development was observed in the genera with petals (Kosuge & Tamura, 1988, 1989; Erbar et al., 1998; Tucker & Hodges, 2005; Ren et al., 2009), with the exception of Adonis (Kosuge & Tamura, 1989; Ren et al., 2009), Anemonopsis Siebold & Zucc. (Kosuge & Tamura, 1989), Coptis Salisb. and Xanthorrhiza Marshall (Kosuge & Tamura, 1989). The depression and the appendage on the ventral side of the petal add to the morphological variability found in the petals of Ranunculaceae.

Based on ontogenetic observations of the petals of Aconitum napellus L., Aquilegia vulgaris L., *H. foetidus* and *Ranunculus ficaria* L., Erbar et al. (1998) presumed that the two ventral bulges (the appendage) could be considered to be rudimentary (sterile) adaxial pollen sacs. However, this presumption cannot explain the single transverse ridge that appears on the petals of *Nigella* (this study), *Cimicifuga Wernisch* [= *Actaea L.*], *Delphinium L.*, *Consolida Gray*, *Ranunculus L.* and some species of *Dichocarpum* (Kosuge & Tamura, 1989). If the presumption is correct, the blade portion of the petal primordium may result from the development of the two sterile abaxial pollen sacs and/or the connective tissue. However, this pattern of development was not found in our examination of *Helleborus, Nigella* or in previous investigations of other genera (Kosuge & Tamura, 1988, 1989; Lehmann & Sattler, 1994; Tucker & Hodges, 2005; Song et al., 2007; Jabbour et al., 2009; Ren et al., 2009; this study). On the basis of the later development of the two bulges and the transverse ridge in the flowers of *Helleborus* and *Nigella*, we suggest that the developmental result of the appendage may be a highly modified structural and functional feature for concealing the nectar and controlling the access of the visitors (see Erbar et al., 1998).

**PETALS**

The most variable organs in the flowers of Ranunculaceae are the so-called petals, which are inserted between the petaloid sepals and stamens and are one of the most important diagnostic characters within the family (Tamura, 1995). As the petals of most genera are nectariferous, they were called in the German literature ‘Honigblätter’ (honey-phyllaries) (Prantl, 1887), ‘Nektarblätter’ (nectar-phyllaries) (Jarchen, 1949) or nectary organs (Erbar et al., 1998). Prantl (1887) presumed that the petals in Ranunculaceae are staminal in origin, and this interpretation was widely adopted (Hiepko, 1965; Tamura, 1984, 1993; Kosuge & Tamura, 1989; Weber, 1993; Kosuge, 1994; Lehmann & Sattler, 1994; Endress, 1995; Feng et al., 1995; Erbar et al., 1998; Kramer, Di Stilio & Schlüter, 2003; Tucker & Hodges, 2005; Gu & Ren, 2007; Song et al., 2007; Ren et al., 2009, 2010). The present observations of the floral organogenesis of *H. thibetanus* and *N. damascena* and scanning electron microscopy studies in some other genera of the family (Kosuge & Tamura, 1988, 1989; Kosuge, 1994; Lehmann & Sattler, 1994; Endress, 1995; Feng et al., 1995; Erbar et al., 1998; Tucker & Hodges, 2005; Gu & Ren, 2007; Song et al., 2007; Ren et al., 2009, 2010) clearly show a relatively long plastochron between the last initiated sepal and the first petal, but shorter and equal plastochrons between the primordia of the petals and stamen. In addition, the sepal primordia are crescent-shaped and truncated, whereas those of the petals and stamens are hemispherical and rounded and similar in the early stages of development. These features support the possible evolutionary relationship between the petals and stamens in Ranunculaceae.

During early developmental stages, each of the petal primordia of *H. thibetanus* and *N. damascena* differentiates into a short basal stalk and a larger, emarginate blade. With the enlargement of the blade, a depression appears at the ventral base of the blade, and two bulges in *H. thibetanus* and a transversal ridge in *N. damascena* appear at the base of the depression. This pattern of development was observed in the genera with petals (Kosuge & Tamura, 1988, 1989; Erbar et al., 1998; Tucker & Hodges, 2005; Ren et al., 2009), with the exception of Adonis (Kosuge & Tamura, 1989; Ren et al., 2009), Anemonopsis Siebold & Zucc. (Kosuge & Tamura, 1989), Coptis Salisb. and Xanthorrhiza Marshall (Kosuge & Tamura, 1989). The depression and the appendage on the ventral side of the petal add to the morphological variability found in the petals of Ranunculaceae.

which differs from petals are absent and the carpels are free in the lack of descriptions of floral development. The borus with tribe Cimicifugeae is difficult because of Helle-

Alternatively, Jensen et al. (1995) and Wang et al. (2009) have suggested that Helleborus forms no close relationships with other taxa. Comparison of Helleborus with tribe Cimicifugeae is difficult because of the lack of descriptions of floral development. The petals are absent and the carpels are free in Caltha, which differs from Helleborus (Song et al., 2007).

Molecular systematics studies have suggested that Nigella is sister to Delphinieae (Jensen et al., 1995; Hoot et al., 2008). Jabbour et al. (2009: fig. 8) proposed that, if the Nigella morph (i.e. individuals with eight petals) is ancestral, the corolla of Delphinieae may result from a reduction of four petals (as present in Delphinium, with two spurred, two nonspurred and four rudimentary petals) or six petals (Aconitum, with two spurred and six rudimentary petals), in conjunction with the differentiation of partially new petal identities. Based on our observations of the floral ontogeny, we could not find any relationship between N. damascena and tribe Delphinieae, because the number of petals is inconsistent, with mostly five to six and rarely eight. Nonetheless, the floral development features of Nigella and tribe Delphinieae are more similar than those of other genera (see Feng et al., 1995; Tucker & Hodges, 2005; Gu & Ren, 2007; Song et al., 2007; Ren et al., 2009, 2010). In Nigella and Delphinieae, the sepals, petals and stamens develop spirally, whereas the carpels are whorled. Although Jabbour et al. (2009) did not observe this floral developmental feature, the whorled carpels are clearly apparent in the authors’ images (fig. 3K for Aconitum napellus; fig. 5E for Delphinium grandiflorum L.; fig. S1B for D. staphisagria L.).

Helleborus and Nigella were, at one time, considered to be closely related and were included in the same tribe Helleboreae (Langlet, 1932; Hutchinson, 1959, 1969; Tamura, 1966; Wang, 1979; Loconte et al., 1995). In contrast, other authors have placed them in different tribes (Duncan & Keener, 1991; Tamura, 1993, 1995; Jensen et al., 1995; Wang et al., 2009). Helleborus thibetanus and N. damascena share several floral organogenetic features: (1) spiral initiation of the sepals, petals and stamens; (2) broad, crescent-shaped and truncate sepal primordia; (3) narrow, hemispherical and rounded petal and stamen primordia; (4) a relatively long plastochron between the last sepal and the first petal; (5) delayed development of the petals; (6) centripetal stamen initiation and maturation; and (7) horseshoe-shaped young carpels. However, these features are common in Ranunculaceae (Schöffel, 1932; Hiepko, 1965; Kosuge & Tamura, 1989; Erbar et al., 1998; Chang et al., 2005; Tucker & Hodges, 2005; Gu & Ren, 2007; Song et al., 2007; Jabbour et al., 2009, Ren et al., 2009, 2010). In our examination of the floral organogenesis of H. thibetanus and N. damascena, we note the following floral developmental features that differ between the two species: (1) the carpels are only slightly united at the base in H. thibetanus, whereas, in N. damascena, the gynoecium is syncarpous and the carpels are united throughout the ovary; (2) the appendage at the ventral base of the blade of the young petal is formed by two bulges in H. thibetanus instead of a ridge in N. damascena, and their development also differ; and (3) the outermost stamens are delayed in growth in H. thibetanus, but not in N. damascena. The differences observed in the floral developmental features of these two species support Helleborus and Nigella being placed in separate tribes. However, further investigations of several species within each genus are necessary to support this conclusion.

ACKNOWLEDGEMENTS

We sincerely thank Professor Peter K. Endress (University of Zurich), Dr Julian B. Bachelier and Robert L. Baker (Harvard University), Mare Nazaire (Washington State University), Dr Xin-Wei Li (Wuhan Botanical Garden, Chinese Academy of Sciences), Dr Xiao-Hui Zhang (Shaanxi Normal University) and Dr Jeremy Lundholm (Saint Mary’s University) for helpful comments and for careful reading of the manuscript. We are very grateful to Mr Yin-Hou Xiao (Institute of Botany, Chinese Academy of Sciences) and Mr Yao-Hui Ren (Shaanxi Normal University) for assistance in scanning electron microscopy. This

project was supported by grants from the National Natural Science Foundation of China (Nos. 30870179 and 30800059).

REFERENCES


