OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

Floral and Inflorescence Morphology and Ontogeny in *Beta vulgaris*, with Special Emphasis on the Ovary Position

HILDA FLORES OLVERA[1], ERIK SMETS[2] and ALEXANDER VRIJDAGHS[3,*]

[1]*Instituto de Biología, Universidad Nacional Autónoma de México, Apdo. Postal 70-367, México D.F. CP 04510, México,*
[2]*National Herbarium of the Netherlands, Leiden University Branch, Leiden, The Netherlands and* [3]*K.U. Leuven,*
Biology–Institute for Botany and Microbiology, Kasteelpark Arenberg 31, Heverlee (Leuven), Belgium

• *Background and Aims* In spite of recent phylogenetic analyses for the Chenopodiaceae–Amaranthaceae complex, some morphological characters are not unambiguously interpreted, which raises homology questions. Therefore, ontogenetic investigations, emphasizing on 'bracteoles' in Atripliceae and flowers in Chenopodioideae, were conducted. This first paper presents original ontogenetic observations in *Beta vulgaris*, which was chosen as a reference species for further comparative investigation because of its unclarified phylogenetic position and its flowers with a (semi-)inferior ovary, whereas all other Chenopodiaceae–Amaranthaceae have hypogynous flowers.
• *Methods* Inflorescences and flowers were examined using scanning electron microscopy and light microscopy.
• *Key Results* Floral development starts from an inflorescence unit primordium subtended by a lateral bract. This primordium develops into a determinate axis on which two opposite lateral flowers originate, each subtended by a bracteole. On a flower primordium, first five tepal primordia appear, followed by five opposite stamen primordia. Simultaneously, a convex floral apex appears, which differentiates into an annular ovary primordium with three stigma primordia, surrounding a central, single ovule. A floral tube, which raises the outer floral whorls, envelops the ovary, resulting in a semi-inferior ovary at mature stage. Similarly, a stamen tube is formed, raising the insertion points of the stamens, and forming a staminal ring, which does not contain stomata. During floral development, the calyces of the terminal flower and of one of the lateral flowers often fuse, forming a compound fruit structure.
• *Conclusions* In *Beta vulgaris*, the inflorescence is compound, consisting of an indeterminate main axis with many elementary dichasia as inflorescence units, of which the terminal flower and one lateral flower fuse at a later stage. Floral parts develop starting from the outer whorl towards the gynoecium. Because of the formation of an epigynous hypanthium, the ovary becomes semi-inferior in the course of floral development.

Key words: *Beta vulgaris*, Chenopodiaceae, floral ontogeny, gynoecial development, epigynous hypanthium, semi-inferior ovary, inflorescence ontogeny, LM, SEM.

INTRODUCTION

Preliminary floral ontogenetic investigations in Chenopodiaceae raised several homology questions and doubts about the interpretation in literature of inflorescence and floral parts, particularly in the genera *Atriplex*, *Beta*, *Chenopodium* and *Spinacia*. Therefore, a morphological project, focusing on the inflorescence and floral ontogeny in these genera, was started. Within Chenopodiaceae *Beta* draws attention because of its flowers with an unique ovary position, which has been described as inferior (e.g. Lawrence, 1951; Eichler, 1954) or semi-inferior (e.g. Tutin, 1964; Heywood *et al.*, 2007), whereas the other members of the family have a superior ovary. Consequently, floral ontogenetic data are needed in order to clarify the ovary position in *Beta*.

Beta, a herbaceous genus of 11–13 species of the Mediterranean region and west and central Asia, is one of the five genera of subfamily Betoideae (treated as a tribe under subfamily Chenopodioideae by Kühn *et al.*, 1993). Cuénoud *et al.* (2002) found that *Beta* and *Hablitzia* (subfamily Betoideae) form a monophyletic group distinct from a clade comprising genera of subfamily Chenopodioideae. However, using a more representative sampling of Amaranthaceae and Chenopodiaceae, Kadereit *et al.* (2003) found that none of the five genera classified within Betoideae (*Acroglochin*, *Aphanisma*, *Beta*, *Hablitzia*, *Oreobliton*) form a clade and their relationships with respect to Amaranthaceae *s.s.* and Chenopodiaceae (Cheno–Am) are uncertain (Fig. 1). According to Judd *et al.* (1999) as well as Stevens (2001 onwards), Amaranthaceae and Chenopodiaceae are merged into one family (Amaranthaceae). Nevertheless, Cuénoud *et al.* (2002) recuperated a well-supported clade with genera formerly included in Amaranthaceae *s.s.*, whereas genera formerly included in Chenopodiaceae form a monophyletic group only in some of the shortest trees. In this paper a simplified phylogenetic tree (Fig. 1) based on Kadereit *et al.* (2003, figure 2) was used, because it contains the most complete and recent sampling for the Cheno–Am alliance.

Inflorescences in Amaranthaceae and Chenopodiaceae show considerable variation. The inflorescence is compound, with an indeterminate main axis carrying many cymosely branched partial inflorescences with flowers in clusters of three or more, or which are reduced to a single flower. The flowers are hermaphroditic to unisexual (plants monoecious, gynomonoecious or dioecious). In most species, the flower is usually bibracteolate, actinomorphic, characterized by a uniseriate perianth of five tepals, five stamens, and a unilocular,

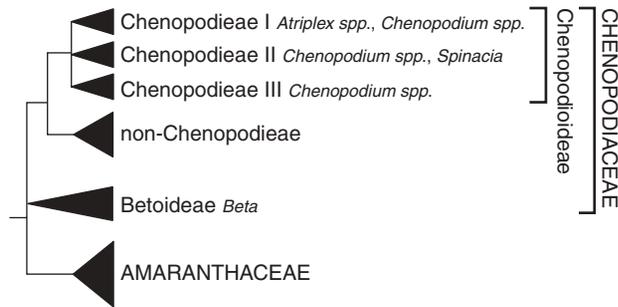* For correspondence. E-mail alexander.vrijdaghs@bio.kuleuven.be

Fig 1. Simplified cladogram for Amaranthaceae and Chenopodiaceae, based on Kadereit *et al.* (2003).

superior to semi-inferior (in *Beta*) ovary with a single style (e.g. Kühn *et al.*, 1993).

Few inflorescence and floral ontogenetic studies have been done for these families. Urmi-König (1981, summary) considered 'the flowering unit of florigenous lateral shoots' in the species of Chenopodiaceae she investigated, to consist of 'one to numerous thyrses ... or their derivates'. Consequently, the inflorescence units in the species studied by her have 'cymose branching' (Weberling, 1992, p. 211). Unfortunately, *Beta* was not included in this study. In his 'Blütendiagramme', Eichler (1954, p. 82) published a

most interesting diagram of an inflorescence unit in *B. longespicata* Moq., stating about the inflorescence unit in Chenopodiaceae that it branches from the axils of the prophylls, where each branch'... kan dabei aber sowohl Schraubel- [helicoid cyme, in *Beta*] als Wickelwuchs regieren'. Also according to Kühn *et al.* (1993), particularly among others in *Beta*, the dichasial partial inflorescences can be reduced to helicoid or scorpioid cymes. Weberling (1992, p. 211) defined helicoid and scorpioid cymes as cymosely branched (partial) inflorescence, hence a 'branching exclusively from the axils of the prophylls, which are developed as the only leaf organs preceding the individual flowers'. These leaf organs, in dicotyledons also referred to as 'bracteoles' (small bracts on a pedicel; Hickey and King, 2002), usually occur in pairs, inserted oppositely or transversely. According to Weberling (1992), a helicoid cyme or bostryx is a monochasially branching structure, which always proceeds from the axil of a prophyl at the same side as in the preceding branching. In a scorpioid cyme or cincinnus, the branches develop from each other in an alternating way. Flowers are rarely solitary, and usually occur in clusters of two or three. According to Kühn *et al.* (1993), this is because of a fusion by their basally indurate perianths.

In *Beta*, a flower consists of an outer whorl of five tepals, a whorl of five stamens basally united into a fleshy ring
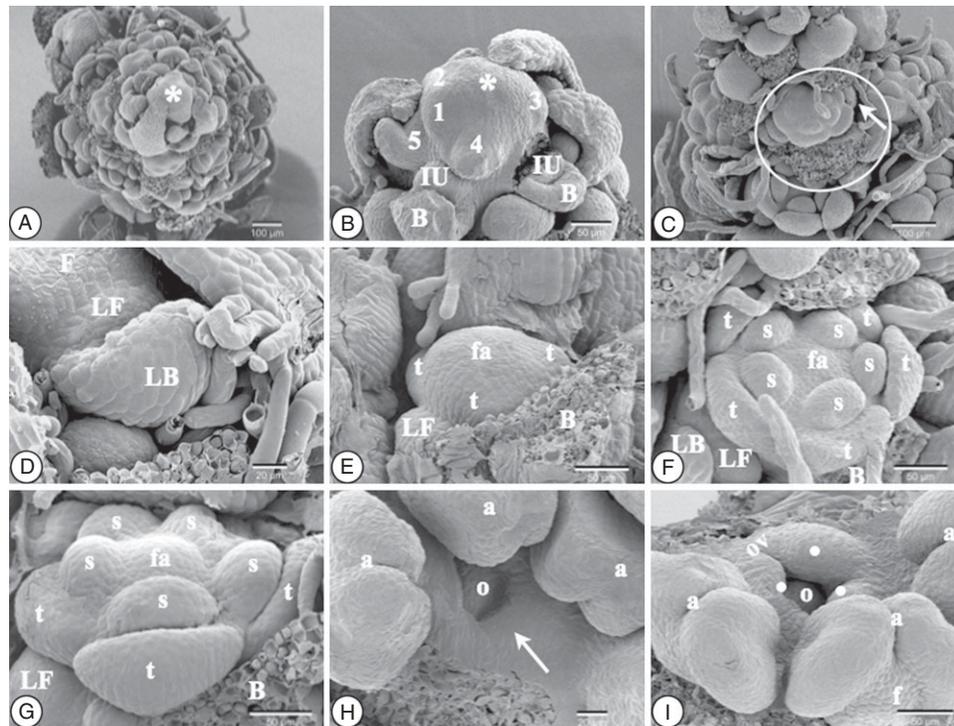

Fig. 2. SEM images of early floral development in *Beta vulgaris*. Abbreviations: a, anther; B, bract; F, terminal flower; f, filament; fa, floral apex; IU, inflorescence unit; LB, lateral bract, LF, lateral flower primordium; o, ovule; ov, ovary wall; s, stamen primordium; t, tepal primordium; white dot, stigma primordium; *, apex of main axis. (A) Apical view on main inflorescence axis with many spirally placed lateral inflorescence units. (B) Indeterminate apex of the main axis and inflorescence unit primordia at different developmental stages (1–5), each subtended by a bract. (C) Inflorescence unit (encircled) with an already developing terminal flower, a lateral flower primordium in the axil of a bracteole (left) and the opposite bracteole (arrowed). (D) Undifferentiated inflorescence unit primordium and trichomes. (E) Differentiating inflorescence unit primordium with the primordium of the terminal flower with three already visible tepal primordia, and a lateral flower primordium. (F, G) Terminal flower primordium with five tepal primordia, five stamen primordia and a convex floral apex. (H) Differentiation of the floral apex into an annular ovary primordium (arrowed) surrounding a central ovule primordium in a concave depression. Each stamen primordium develops into filament and anther. (I) On the ovary primordium, three stigma primordia (white dots) appear.

(Kühn *et al.*, 1993), and a tri-carpellate unilocular ovary, which has been described as inferior (Lawrence, 1951; Eichler, 1954) or semi-inferior (e.g. Tutin, 1964; Heywood *et al.*, 2007). Engler (1964, p. 96) called the perianth 'halb epigyn' whereas Kühn *et al.* (1993) called the perianth perigynous. About the perianth, Payer (1857, p. 309) stated that he never saw petals, and consequently, he called all perianth parts 'sépales'. According to his observations in *Suaeda fruticosa*, his model species for Chenopodiaceae, each 'sepal' primordium originates individually with a quincuncial aestivation. One adaxial and two abaxial sepal primordia originate first, followed by the two lateral ones. Similar observations were done in *B. vulgaris* and *B. trigyna* by Hofmann (1994), though she interprets the initiation of the perianth and androecium as an 'ontogenetic spiral' (p. 149). Ronse Decraene and Smets (1991) mentioned that perianths of five tepals often result from a reduction of two trimerous perianth whorls. According to Payer (1857), the stamen primordia originate immediately after the appearance of the 'sepals', each stamen primordium oppositely to a 'sepal'.

In his general description of Chenopodiaceae, Payer (1857, p. 309) wrote about the ontogeny of the gynoecium:'... Ce sont trois mamelons carpellaires, distincts d'abord, mais qui deviennent promptement connés et enveloppent comme une cupule à trois crénelures le sommet du réceptacle.' Therefore, according to Payer, three carpel primordia originate and fuse postgenitally. Concerning the ovary position, Payer (1857, p. 309) stated:'... dans les *Beta maritima*, au contraire, l'ovaire, d'abord supère, devient infère par suite d'un développement inégal des trois portions du réceptacle qui supportent, la première les sépales et les étamines, la deuxième les parois de l'ovaire, et la troisième l'ovule'. Therefore, according to him, the ovary in *B. maritima* is originally superior, becoming semi-inferior in the course of the floral development. According to Weberling (1992, p. 173), a (semi-)inferior gynoecium can in many cases be explained as the result of a fusion 'from the very beginning' of the 'dorsal faces [of the carpels] with the inner wall' of an 'apical depression of variable depth'. This corresponds to the hypothesis of congenital fusion of the carpels with the floral axis, going back to Celakovsky (1874) and von Goebel (1884). However, to understand some (semi-)inferior ovaries, Weberling (1992, p. 174) also referred to the 'appendix theory' of de Candolle (1813) and van Tieghem (e.g. 1875). According to this hypothesis, the receptacle protrudes the gynoecium because of a congenital fusion of calyx, corolla, and stamens. Consequently, the ovary wall consists of a fusion product of all floral organs, called a 'hypanthium' (Weberling, 1992, p. 175). Takhtajan (1991, p. 110) distinguished between phyllome and receptacular inferior ovaries, which originated in different evolutionary lines. Phyllome inferior ovaries can be explained as resulting 'from the coalescence of the gynoecium with the floral tube'. Takhtajan (1991) rejected the use of the word 'hypanthium' and replaced it by 'floral tube'. Kuzoff *et al.* (2001) and Soltis *et al.* (2003) merged several hypotheses to understand hypogynous flowers, as well as epigynous flowers, into three basic patterns of floral ontogenesis: (1) hypogynous development, in which a convex floral apex is maintained throughout the floral ontogenesis, results in flowers with a superior ovary; (2) receptacular-epigynous development results in flowers with an inferior ovary – after the initiation of the gynoecium development, the periphery of the floral apex rises, enveloping the gynoecium; and (3) in appendicular-epigynous flowers, the convex floral apex flattens, and the receptacle becomes cup-shaped, forming a concave depression in the centre, where also the gynoecium primordia are formed. However, according to Leins (2000), the position of a gynoecium as well as the formation of various floral structures, such as a stamen-corolla tube or calyx-corolla tube, depends on the relative development of annular intercalary meristems present in the floral axis. A perigynous floral tube can be formed if annular intercalary meristems below all floral whorls, except for the gynoecium itself, grow up together (Leins, 2000, figure 100, 6). An inferior ovary results from the common development of annular intercalary meristems below all floral whorls, including the upper part of the carpels (Leins, 2000, figure 100, 7). Greyson (1994, p. 19) defined perigynous flowers as 'those in which the joining of sepals, petals and stamens forms a cup or tube around the gynoecium. The ovary in these two situations [hypogynous and perigynous flowers] is described as superior, for the attachment of the other organs to the receptacle is proximal to the attachment of the gynoecium. Epigynous flowers are those in which the joining of the sepals, petals and stamens are adnate to the gynoecium.'

The aim of this paper is to clarify the ovary position in *Beta vulgaris* L. and to present, for the first time, elaborate inflorescence and floral ontogenetical data based on scanning electron microscopy (SEM) and light microscopy (LM) observations. This species was chosen as a first study object and a comparative reference species for further investigation in the Cheno–Am alliance, because of its unclarified phylogenetic position in between Chenopodiaceae and Amaranthaceae, and because it has been considered to have flowers with a semi-inferior ovary, whereas all other Chenopodiaceae–Amaranthaceae have hypogynous flowers. In subsequent papers, the inflorescence and floral morphology and ontogeny for *Chenopodium* (usually with bisexual flowers), *Atriplex* and *Spinacia* (considered to have bracteolate perianthless female flowers) will be presented in terms of homology assessments within Chenopodiaceae. Similar investigations within Amaranthaceae are also in progress, in order to obtain a detailed morphological description of the Cheno–Am allicance as a whole. Combined with molecular phylogenetic hypotheses, this will clarify the evolution of the entire complex.

MATERIALS AND METHODS

Young floral shoots of *Beta vulgaris* (Belgium, H. Flores and A. Vrijdaghs 1536) and mature floral shoots (Germany, Botanical Garden, Bonn University, H. Flores 1540) were freshly collected and immediately fixed in 70 % ethanol. Voucher specimens are kept at the Herbario Nacional de México (MEXU).

Floral buds were dissected in 70 % ethanol under a Wild M3 stereo microscope (Leica Microsystems AG, Wetzlar,

Germany) equipped with a cold-light source (Schott KL1500; Schott-Fostec LLC, Auburn, NY, USA). The material was washed twice with 70 % ethanol for 5 min and then placed in a mixture (1:1) of 70 % ethanol and DMM (dimethoxymethane) for 5 min. Subsequently, the material was transferred to 100 % DMM for 20 min, before it was critical point dried using liquid CO_2 with a CPD 030 critical-point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using Leit-C and coated with gold with a SPI-Module[TM] Sputter Coater (SPI Supplies, West-Chester, PA, USA). SEM images were obtained with a JEOL JSM-6360 (JEOL Ltd, Tokyo) at the Laboratory of Plant Systematics (K.U. Leuven). Reference material is kept at the same laboratory.

For LM, inflorescence apices were dehydrated through a graded ethanol series and subsequently embedded in paraffin. Longitudinal sections, 5 μm thick, were made with a rotation microtome HM 360 (Walldorf, Germany) and stained with 1 % saffranine and 1 % aniline-blue. Observations were made with an Olympus BX51 microscope equipped with an Evolution LC digital camera.

RESULTS

The inflorescence of *Beta vulgaris* consists of an indeterminate main axis (asterisk in Fig. 2A and B) with many spirally arranged lateral inflorescence units (Fig. 2A, B). Each lateral inflorescence unit originates from a primordium in the axil of a bract (Fig. 2B). The primordium grows, forming an irregular inflorescence unit primordium (Fig. 2B). This develops into a dichasial partial inflorescence with a terminal flower primordium, and two lateral bracts below it, each with a flower primordium (Figs 2B–D and 5A–C). In Fig. 6C, an LM section through an inflorescence unit shows the subtending bract, the terminal flower and a less-developed lateral flower subtended by a bracteole. The development of one of the lateral flower primordia is delayed compared with the opposite one (Figs 2C, 5A–C and 6C). A flower primordium first forms three tepal primordia (two abaxially and one adaxially) and a floral apex (Fig. 2E). Subsequently, two other (lateral) tepal primordia and five distinct stamen primordia originate, the stamen primordia opposite to the tepal primordia (Figs 2F, G and 5A). At this stage, the floral apex is convex (Figs 2G and 5A). With the stamen primordia developing into filament and anther, the floral apex flattens (Fig. 5B) and differentiates into an annular ovary primordium surrounding an ovule primordium (Fig. 2H). Meanwhile, the flower receptacle is becoming cup-shaped, causing the ovule primordium to be situated below the annular ovary primordium (Figs 2H, I and 5A). Subsequently, three distinct stigma primordia become visible on the ovary wall, which grows up from the base (Figs 2I, 3A and 5C). At this stage, the gynoecium is superior (Figs 3B–D and 5C). Meanwhile, a perigynous hypanthium is formed, which envelops the proximal part of the gynoecium and raises the insertion points of the perianth and staminal whorl (Figs 5A–C and 6A, C). Simultaneously, an (intra-)staminal ring is formed, raising the insertion points of the filaments (Figs 3B–D, 5C and

6A, C). The ovary wall rises and the stigma primordia grow out, enveloping the ovule (Figs 3A–D, 5A, C and 6A). At this stage, the ovule primordium develops into a bitegmic campylotropous ovule (Fig. 3E and F), and the inner surface of the stigmas becomes papillose (Fig. 3D). The anthers and filaments elongate, resulting in dorsifix, introrse stamens standing on a conspicuous ring at the inner side of the filaments, linking the bases of the stamens (Figs 3G, I, 5C and 6B). The staminal ring in (semi-)mature flowers bears no stomata (Figs 3D and 4C). The aestivation of the perianth in a developing flower is imbricate, quincuncial (Fig. 5D). The development of the lateral floral primordia is delayed compared with the development of the terminal flowers (Figs 3H, 4A, B and 5A–C). The (developing) flowers form clusters on the main axis. In between the flowers, there are many thread-like multicellular trichomes (Fig. 3H, J). Each cluster is organized in determinate, dichasial, cymosely branched inflorescence units, of which one of the branches has a delayed development (Figs 2C, 4A, B and 5A, B). Often a terminal flower is fused with one of its lateral flowers. At maturity, this results in a structure consisting of two combined fruits, each with one seed, on which, at two opposite sides, remnants of the stigmas are visible, as well as of the original peduncle (Fig. 4D). In the seeds, the embryo is curved, lying in a transverse plane (Fig. 4D).

DISCUSSION

Our ontogenetic observations in *Beta vulgaris* concur only partially with the literature description of the inflorescence in Chenopodiaceae (Eichler, 1954; Kühn *et al.* 1993). A typical inflorescence unit *in Beta vulgaris* consists of clusters of three flowers in an elementary dichasium (not a bostryx or cincinnus, as suggested by Kühn *et al.*, 1993) composed of a very short pedicel of a terminal flower with two lateral bracteoles, each subtending a flower; the terminal flower is usually fused with one of the two lateral flowers, which develops with some delay as compared with the terminal flower. Usually, the development of the other lateral flower primordium is delayed or stopped. This can explain why the inflorescence unit has been considered to be monochasially branching.

Floral primordia are formed from a common inflorescence unit primordium in the axil of a main bract, which is actually also the bract of the terminal flower. Usually, only one lateral floral primordium develops, which, from the very beginning of its development, is fused with the terminal flower by the floral receptacles (Fig. 5A–C) and results in a compound structure containing two fruits, each with one seed (Fig. 4D). Consequently, we do not agree with Kühn *et al.* (1993), where they state that the flowers are fused by their basally indurated perianths. A floral primordium develops from the outside to the inside, beginning with the formation of tepal primordia, followed by the staminal primordia, which originate simultaneously, and eventually by the ovary wall primordium (Figs 2, 3 and 5A–C). We follow Payer (1857) in his approach to use the floral whorl concept to describe the floral ontogeny in flowers of Chenopodiaceae, also avoiding the suggestion
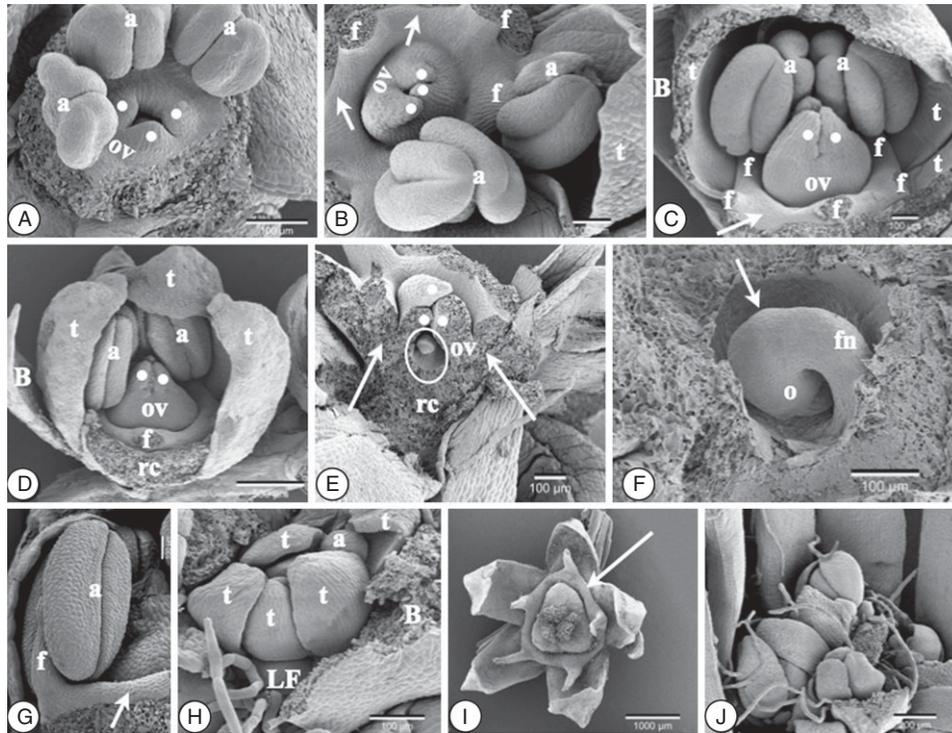
FIG. 3. SEM images of floral development in *Beta vulgaris*. Abbreviations: a, anther; B, bract; f, filament; fn, funiculus; LF, lateral flower primordium; o, ovule; ov, ovary wall; rc, receptacle; t, tepal; white dot, stigma primordium. (A) Growing ovary wall with stigma primordia (white dots). The anthers are well developed. (B, C) The ovary wall grows up enveloping the ovule. Meanwhile, the stigma primordia also develop. The bases of the filaments are raised by the formation of a staminal ring (arrowed). The insertion of the filaments is at the outside of the staminal ring. (D) The inner side of each stigma becomes papillose. The ovary wall grows faster than the perigynous hypanthium, giving the ovary a superior aspect. (E) Longitudinal section through a developing flower. At this stage, the perigynous hypanthium (arrowed) is growing very slowly, so that the ovule (encircled) stands more or less at the same height as the insertion points of the filaments (arrowed). The second integument of the ovule is being formed. The receptacle is slightly cup-shaped. (F) Campylotropous (arrow) and bended ovule. (G) Lateral view of a dorsifixed and introrse, semi-mature stamen, and part of the staminal ring (arrowed). The staminal ring does not have stomata. (H) Apical-lateral view of a developing terminal flower. The inner floral whorls are protected by the tepals. Below the terminal flower, the yet undifferentiated primordium of a lateral flower is visible. (I) Apical view of a mature flower with conspicuous staminal ring (arrowed). The perigynous hypanthium now gives the ovary a semi-inferior aspect. (J) Cluster of developing flowers.

that the single whorled pentamerous perianth in *Beta* is a result of the reduction of two perianth whorls of each three members. In that sense, we do not agree with Hofmann (1994, p. 149), who described the origin of the stamens as a continuation of the 'ontogenetic spiral'. Like Payer (1857), we observed that the stamen primordia originate individually on the flower receptacle. If, at later developmental stages the stamens seem to be epitepalous, as Kühn *et al.* (1993) considered, this is because of the simultaneous formation of a staminal ring and a floral tube. Moreover, the filaments remain free from each other, as illustrated in Figs 3I and 4C. According to Endress (2001), in angiosperms the ovary wall usually closes before the ovules are formed, so that the ovules are never exposed to the open air. However, in some groups like Plumbaginaceae, Chenopodiaceae (De Laet *et al.*, 1995; Endress, 2001) and Cyperaceae (e.g. Vrijdaghs *et al.*, 2006), the ovule(s) are formed before the ovary closes.

The uncertain relationships of *Beta* with respect to the Cheno–Am alliance in the phylogenetic hypothesis of Kadereit *et al.* (2003), impose further research about the evolution of the gynoecium within this group, since it is generally accepted that (semi-)inferior ovaries are derived from superior ones (e.g. Gustafsson, 1999). The cladogram

of Cuénoud *et al.* (2002) resolved *Beta* in a rather terminal clade among the rest of the members in the Cheno–Am alliance. Therefore, at present it is impossible to formulate a conclusion about the evolution of the semi-inferior ovary within the alliance. Within Caryophyllales, (half-)inferior ovaries occur only in some genera within Aizoaceae and Cactaceae (Cronquist, 1981), which are nested within the 'core Caryophyllales' (Cuénoud *et al.*, 2002). Soltis *et al.* (2003) considered the (half-)inferior ovaries of the genera in these families to be derived from receptacular epigyny (Kuzoff *et al.*, 2001, figure 1), being therefore not homologous with those derived from an appendicular-epigyny. The semi-inferior ovary in *Beta*, if Kuzoff's developmental hypotheses were followed, could also be considered as resulting from a similar receptacular epigynous development. However, to understand the semi-inferior position of the ovary in *B. vulgaris*, Leins's ontogenetic approach of the old 'appendix theory' (Weberling, 1992, p. 174) seems to us the most appropriate. The three basic patterns of Kuzoff *et al.* (2001) and Soltis *et al.* (2003) are not always adequate to understand the diversity of intermediary ovary positions, as the authors themselves mentioned (Kuzoff *et al.*, 2001). Moreover, the present ontogenetic observations show that the origin of the gynoecium
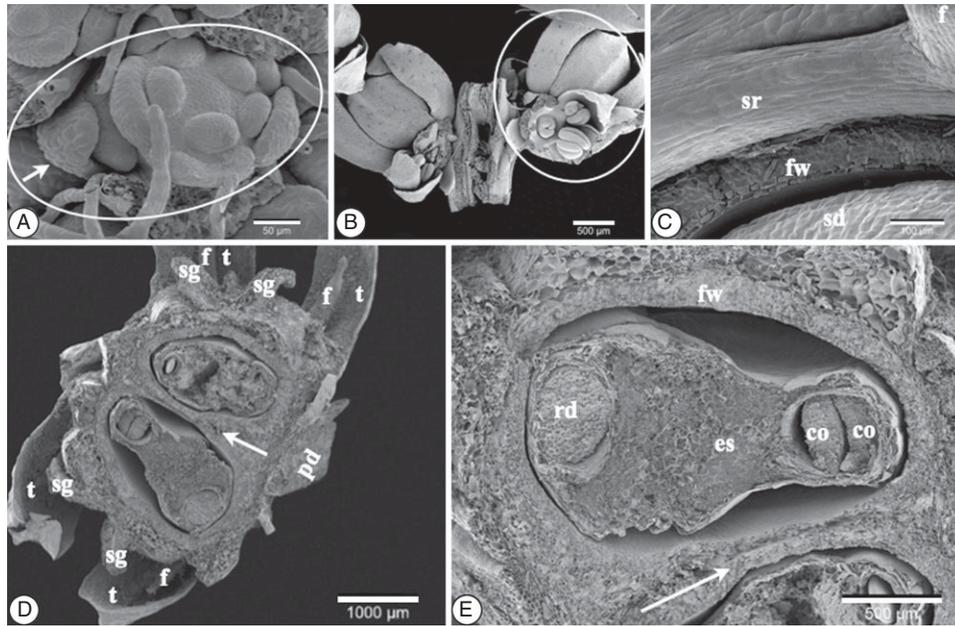
FIG. 4. SEM images of inflorescence and floral morphology in *Beta vulgaris*. Abbreviations: co, cotyledone; es, endosperm; f, filament; fw, fruit wall; pd, peduncle; t, tepal; rd, radicle; sd, seed; sg, stigma; sr, staminal ring. (A) Lateral view of a primordial inflorescence unit (encircled) with an already differentiating terminal flower primordium, and a lateral bracteole subtending a lateral flower primordium (arrowed). (B) Lateral view of a part of the main axis with two inflorescence units (one is encircled). The terminal flower is near maturity, while the lateral flower is developing. (C) Staminal ring without stomata in mature flower. (D) Mature terminal and lateral flower, fused through the receptacles which form a common, septum-like wall (arrowed) between the locules of each flower. (E) Fruit with transverse section through the single seed, showing the radicula and two cotyledones of the curved embryo.
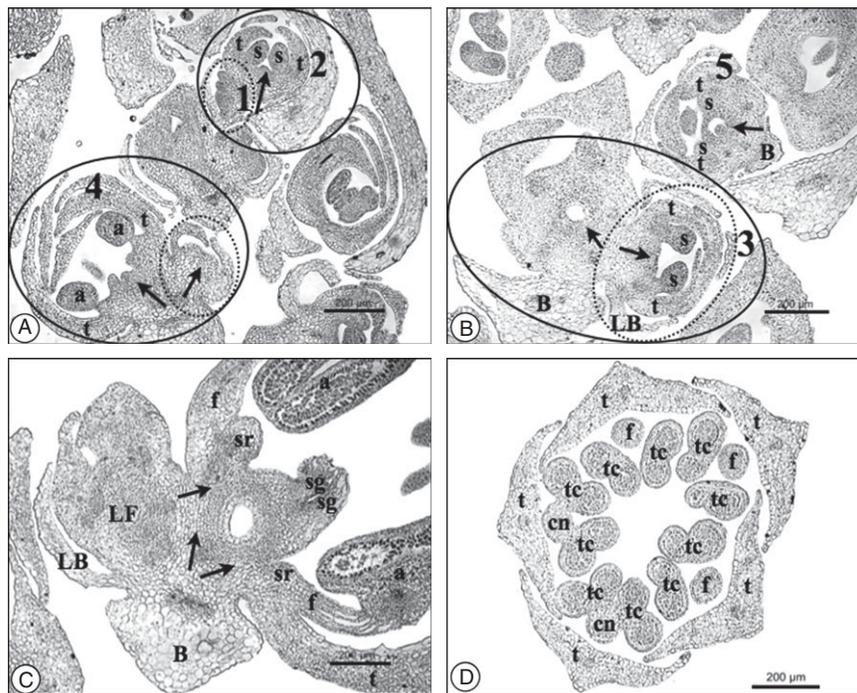

FIG. 5. LM images of a longitudinal section through an apex of a main inflorescence axis in *Beta vulgaris*. Abbreviations: a, anther; B, bract; cn, connective; f, filament; LB, lateral bracteole; LF, lateral flower; s, stamen primordium; sg, stigma; sr, staminal ring; t, tepal; tc, theca. (A, B) Dichasially branched inflorescence units (encircled with continuous lines) at different developmental stages (numbered 1–5 from youngest to older). The orientation of the considered flowers and the floral apex/ovary are indicated by arrows. Lateral flowers are encircled with dotted lines. (C) Longitudinal section through a developing terminal flower, with ovary wall and perigynous hypanthium distinguishable (arrows). (D) Transverse section above the stigmas through semi-mature flower, with imbricate, quincuncial perianth aestivation.
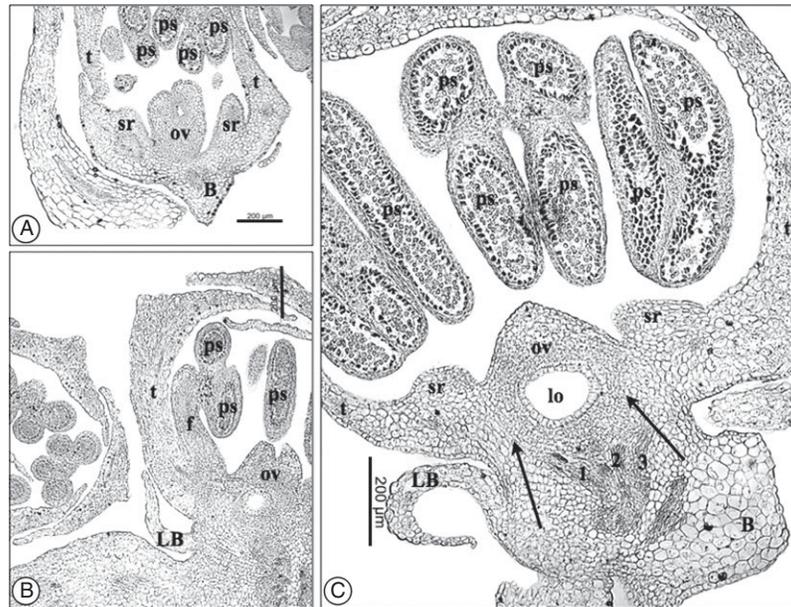
FIG. 6. LM images of longitudinal sections through flowers at different developmental stages in *Beta vulgaris*. Abbreviations: B, bract; LB, lateral bracteole; f, filament; lo, locule; ov, ovary wall; ps, pollen sac; sr, staminal ring; t, tepal. (A) Developing flower with at this stage an apparently superior ovary. (B) Part of a developing flower with dorsifixed stamen and already semi-inferior ovary. (C) Developing flower with semi-inferior ovary. The ovary wall is distinct from the receptacular and hypanthial tissues (arrows). Three vascular bundles enter the ovary (numbered 1–3).

in *B. vulgaris* corresponds to what Takhtajan (1991, pp. 110–112) called a 'phyllome origin' (Figs 3C, D and 6C). Consequently, by applying Troll's principle of variable proportions (e.g. Troll, 1959) on annular intercalary meristems in the floral axis, Leins' 'Spiel mit den Proportionen' (Leins, 2000, pp. 103–106) allows us to understand better the position of the gynoecium in *B. vulgaris*: it is variable, and it depends on the developmental stage of the flower. A perigynous floral tube (also often referred to as 'hypanthium') is formed around the ovary during the floral development. The carpels are fused congenitally, forming an annular ovary primordium (Fig. 2H). Hence, the present results do not confirm the description of Payer (1857), who stated that three carpel primordia fuse postgenitally. At this stage, the floral receptacle has already become cup-shaped, so that the ovule appears to be formed below the annular primordium (Fig. 5A). The ovary primordium grows out from its base, forming an ovary wall, while simultaneously a perigynous floral tube develops from an annular intercalary meristem below both the tepal and the staminal whorl (Fig. 5A–C). The ovary wall is distinct from the receptacular tissue (Figs 5C and 6C). At some stages, the ovary wall develops faster than the perigynous floral tube, providing the ovary with a superior aspect (Figs 3C and 6A). At (semi-)mature stage, the perigynous hypanthium envelops a large part of the ovary (Figs 3I, 5C and 6C), but at all developmental stages, the ovule is partially above the insertion point of the stamens and tepals (Figs 3E, 4D, 5A–C and 6B, C). Consequently, we concur with Payer (1857), who considered the gynoecium to be superior at its origin, as well as with Greyson's definition of a perigynous flower (Greyson, 1994). The present results show a perigynous flower in *B. vulgaris*, with a superior to semi-inferior gynoecium (Fig. 7).

The ovary wall is vascularised by three vessels (Fig. 6C). Since the ovary wall grows up from an annular primordium and not from distinct carpel primordia, the plant obtains the possibility of reorganizing the ovary vascularization. This can explain why only three stigmas are present, where
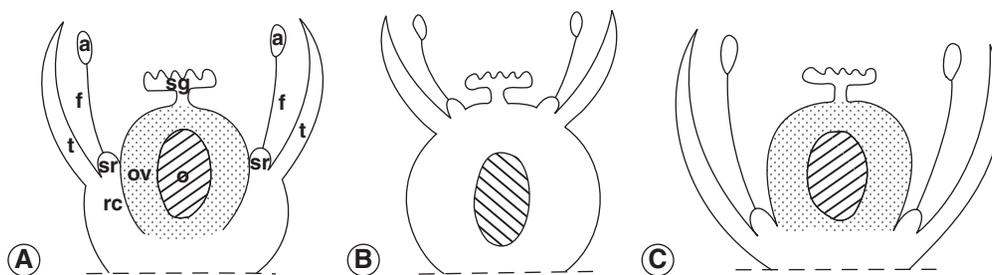

FIG. 7. Schematic representation of the ovary positions based on Greyson (1994, p. 19). Abbreviations: a, anther; f, filament; o, ovule; ov, ovary wall; rc, receptacle; sg, stigma; sr, staminal ring; t, tepal. (A) The ovary in *Beta vulgaris*; (B) a hypothetical inferior ovary in a similar flower; (C) a hypothetical superior ovary of a similar flower.

five can be expected. A similar phenomenon was observed in some genera in Cyperaceae with laterally flattened gynoecia (Vrijdaghs, 2006).

According to Smets (1988), nectar secretion occurs in hermaphroditic flowers in Chenopodiaceae, particularly at the base of the stamens, which are usually described as postgenitally fused, forming a ring. However, according to the present observations in *Beta vulgaris*, the formation of the staminal ring rather develops from an annular inter-calary meristem below the insertion points of the stamens. Although in *Chenopodium polyspermum*, the staminal ring has many stomata (Smets, 1988), in *B. vulgaris* the staminal ring does not have stomata (Fig. 4C), and the staminal ring does not show any other characters indicating nectar secretion (Figs 5C and 6C). Zandonella (1977) stated that a nectar gland is present in *Beta*, but results supporting this statement are not shown. Further research to determine whether or not the staminal ring in *Beta* has lost its nectar-secreting function seems imperative to us.

CONCLUSIONS

In *Beta vulgaris*, the inflorescence is compound and con-sists of an indeterminate main axis with many closed, dichasial and sympodially branched inflorescence units. The terminal flower of each inflorescence unit and one of the lateral flowers fuse at a later developmental stage. Floral parts originate starting from the outer whorl of five asynchronically developed tepals towards the gynoecium. The five stamen primordia originate free from each other, and they are raised in the course of floral development by the formation of an (intra-)staminal ring from an annular intercalary meristem. The present findings provide evidence for considering that the ovary position varies according to the floral developmental stage; at the beginning it is essentially superior, becoming secondarily semi-inferior, as a result of the simultaneous growth of an epigynous floral tube. The ovary wall grows up from a ring primor-dium, surrounding a single central ovule that is initially exposed to the open air. At the same time, a floral tube develops, partially enveloping the ovary, and also raising the insertion points of the outer floral parts. Depending on the relative growth rates of the ovary wall and floral tube, the ovary becomes more or les semi-inferior.

ACKNOWLEDGEMENTS

LITERATURE CITED

Celakovsky LJ. 1874. Über die morphologische Bedeutung der Samenknospen. *Flora* **57**: 113–251.

Cronquist A. 1981. *An integrated system of classification of flowering plants.* New York, NY: Columbia University Press.

Cuénoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ Chase MW. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* **89**: 132–144.

de Candolle C. 1813. *Théorie élémentaire de la botanique.* Paris.

De Laet J, Clinckemaillie D, Jansen S, Smets E. 1995. Floral ontogeny in the Plumbaginaceae. *Journal of Plant Reasearch* **108**: 289–304.

Eichler AW. 1954. *Blüthendiagramme* (reprint of the original text of 1878). Eppenhain: Germany Otto Koeltz.

Endress PK. 2001. Origins of flower morphology. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **291**: 105–115.

Engler A. 1964. *Syllabus der Pflanzenfamilien.* Berlin: Gebrüder Borntraeger.

von Goebel K. 1884. *Vergleichende Entwicklungsgeschichte der Pflanzenorgane.* In: Schenck A, ed. Handbuch der Botanik 3. Breslau.

Greyson RI. 1994. *The development of flowers.* New York, NY: Oxford University Press.

Gustafsson MHG, Albert VH. 1999. *Inferior ovaries and angiosperm diversification.* In : Hollingsworth PM, Balemna RM, Gornall RJ, eds. *Molecular systematics and plant evolution.* London: Taylor and Francis, 403–431.

Heywood VH, Brummit RK, Culham A, Seberg O. 2007. *Flowering plant families of the world.* London: Royal Botanic Gardens, Kew.

Hickey M, King C. 2002. *The Cambridge illustrated glossary of botanical terms.* Cambridge: Cambridge University Press.

Hofmann U. 1994. Flower morphology and ontogeny. In: Behnke H-D, Mabry TJ, eds. *Caryophyllales: evolution and systematics.* Berlin: Springer Verlag, 123–166.

Judd WS, Campbell CS, Kellogg EA, Stevens PF. 1999. *Plant systema-tics: a phylogenetic approach.* Sunderland, MA: Sinauer.

Kadereit G, Borsch T, Weising K, Freitag H. 2003. Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C_4 photo-synthesis. *International Journal of Plant Sciences* **164**: 959–986.

Kühn U, Bittrich V, Carolin R, Freitag H, Hedge IC, Uotila P, *et al*. 1993. *Chenopodiaceae.* In: Kubitzki K, ed. *Families and genera of vascular plants,* Vol. 2. Berlin: Springer.

Kuzoff RK, Hufford L, Soltis DE. 2001. Structural homology and developmental transformations associated with ovary diversification in *Lithophragma* (Saxifragaceae). *American Journal of Botany* **88**: 196–205.

Lawrence GHM. 1951. *Taxonomy of vascular plants.* New York, NY: Macmillan.

Leins P. 2000. *Blüte und Frucht.* Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller).

Payer JB. 1857. *Traité d'organogénie comparée de la fleur.* Paris: Masson.

Ronse Decraene LP, Smets E. 1991. The floral ontogeny of some members of the Phytolaccaceae (subfamily Rivinoideae) with a dis-cussion on the evolution of the androecium in the Rivinoideae. *Biologisch jaarboek Dodonaea* **59**: 77–99.

Smets E. 1988. *Florale nektariën van de Magnioliophytina: karakterizer-ing en systematische betekenis.* PhD Thesis, Instituut voor Plantkunde, K.U. Leuven, Belgium.

Soltis DE, Fishbein M, Kuzoff RK. 2003. Reevaluating the evolution of epigyny: data from phylogenetics and floral ontogeny. *International Journal of Plant Sciences* **164**: S251–S264.

Stevens PF. 2001 onwards. Angiosperm Phylogeny Website. Version 6, May 2005. http://www.mobot.org/MOBOT/Research/APweb/.

Takhtajan A. 1991. *Evolutionary trends in flowering plants.* New York, NY: Columbia University Press.

van Tieghem A. 1875. Recherches sur la structure du pistil. *Mémoires de l'Académie Scientifique* 21. Paris.

Troll W. 1959. *Allgemeine Botanik.* Stuttgart: Ferdinand Enke Verlag.

Tutin TG, ed. **1964.** *Chenopodiaceae.* In: Tutin TG, Heywood NA, Burges NA, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea,* Vol. 1. Cambridge: Cambridge University Press.

Urmi-König K. 1981. Blütentragende Spross-Systeme einiger Chenopodiaceae. *Dissertationes botanicae*, 63.

Vrijdaghs A. 2006. *A floral ontogenetic approach to homology questions in non-mapanioid Cyperaceae/Een bloemontogenetische benadering van homologie-vraagstukken bij niet-mapanioide Cyperaceae.* PhD Thesis, Laboratory of Plant Systematics, K.U. Leuven, Belgium.

Vrijdaghs A, Goetghebeur P, Smets E, Muasya AMM. 2006. The lateral floral scales in *Hellmuthia* (Cyperaceae, Cyperoideae) and *Paramapania* (Cyperaceae, Mapanioideae), a floral ontogenetic study. *Annals of Botany* **98**: 619–630.

Weberling F. 1992. *Morphology of flowers and inflorescences.* Cambridge: Cambridge University Press.

Zandonella P. 1977. Apports de l'étude comparée des nectaires floraux à la conception phylogénétique de l'ordre des Centrospermales. *Berichte der Deutsche Botanischen Gesellschaft* **90**: 105–125.