On flower anatomy and embryology of Lophiocarpus polystachyus (Lophiocarpaceae)

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Source: Willdenowia, 43(1) : 185-194
Published By: Botanic Garden and Botanical Museum Berlin (BGBM)
URL: https://doi.org/10.3372/wi.43.43120
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Abstract

Stable URL: http://dx.doi.org/10.3372/wi.43.43120

Flower anatomy and embryology of Lophiocarpus polystachyus was studied, based on over one thousand slides left by Theo Eckardt and prepared from material of a wild source in South Africa chiefly in the 1970s. The all bisexual flowers of the polytelic inflorescences possess a well-developed nectary, are promoted on the abaxial side, have a perianth of five tepals, a 4-merous or, more rarely, by fusion of two adaxial stamens, 3-merous androecium, a somewhat stipitate, unilocular gynoecium of two fused carpels with one basally inserted ovule. Wall formation of the introrse and tetrasporangiate anther conforms to the Monocotyledonous type. Meiosis of the microsporocytes during which 9 bivalents are visible leads after simultaneous delimitation of the microspores to tetrahedral and decussate pollen tetrads. The tricolpate pollen is 3-celled when shed. The ovule is campylotropous, bitegmic and crassinucellate, as is common in the Centrospermae. Embryo sac development is of the monosporic, Polygonum type. Embryogenesis follows the Caryophyllad type of Johansen. The fruit is a 1-seeded drupe. The flower anatomy of Lophiocarpus confirms an affinity to the Phytolaccaceae s.l. and its distance from the Chenopodiaceae because of the missing gynophore in the latter. A comparison with the embryological data for Corbichonia does not corroborate an intimate relationship of the two genera.

Additional key words: Caryophyllales, Phytolaccaceae, Corbichonia, systematics

Introduction

The small southern African genus Lophiocarpus (comprising four species), originally placed in the Chenopodiaceae, was transferred by Brown (1909) into the Phytolaccaceae, and there even merged with the Caribbean-South American genus Microtea. Its placement in the Phytolaccaceae, but again as a separate genus, was maintained by later authors such as Heimerl (1934), Eckardt (1964), Nowicke (1968), Takhtajan (1969), Behnke (1974) and Rohwer (1993), mostly in a separate subfamily ly Rivinoideae or Microteoideae, while authors splitting up Phytolaccaceae, such as Hutchinson (1959, 1973) and Brown & Varadarajan (1985), placed it with Microtea in the segregate family Petiviaceae. In all classifications, Lophiocarpus, however, is considered as an anomalous genus and usually included therefore with reservations only. Bortenschlager (1973), based on his palynological investigations, was the first who separated Lophiocarpus in a family of its own, but a valid publication of the family name Lophiocarpaceae was only provided by Doweld & Reveal (2008). Molecular phylogenetic analyses

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by Cuénoud & al. (2002), Brockington & al. (2009) and Schäferhoff & al. (2009) unambiguously confirm Phytolaccaceae to be polyphylectic and support the separation of Lophiocarpus from both Phytolaccaceae and Pentavieae. Lophiocarpaceae are recognized as a separate family also in the latest update of the Angiosperm Phylogeny Group classification (APG III 2009). Schäferhoff & al. (2009), moreover, show that Lophiocarpus and the Caribbean-South American Microtea, are by no means closely related; while Microtea constitutes a separate family in an isolated position of the caryophyllid clade, Lophiocarpus is sister to the Aizoaceae-Nyctaginaceae-Phytolaccaceae clade. A relationship of Lophiocarpus with Corbichonia (the latter formerly misplaced in Molluginaceae) as revealed in the molecular analyses is also supported by both genera sharing betacyanins (Cuénoud & al. 2002). Both genera are accepted to constitute the family Lophiocarpaceae by Stevens (2001+).

Theo Eckardt, when he was working at the end of the 1950s on the familial classification of the Centrospermae for the 12th edition of Engler’s “Syllabus” (Eckardt 1964), developed an interest in the then still little known Phytolaccaceae, especially in Phytolaca, in those days the so-called “genus primordioides” of the Centrospermae (Bittrich 1993), and subsequently also in Lophiocarpus. When Eckardt in 1977 unexpectedly passed away, he had been studying Lophiocarpus for some years (Eckardt 1974), without having, however, investigated its embryology, but he left behind more than one thousand anatomical slides of Lophiocarpus polystachyus. Since an embryological study of Lophiocarpus, when compared to the available data on other Centrospermae taxa (Robert 1927; Mauritzon 1934; Johanson 1950; Kajale 1954; Narayana & Lodha 1963; Davis 1966; Hakki 1972; Johri & al. 1992) might help to clarify the phylogeny and systematic position of this odd genus, I worked up this material during the last years. The results of these flower anatomical and embryological studies are presented here.

Material and methods

This study is based on more than one thousand slides of Lophiocarpus polystachyus left by Theo Eckardt that had been sectioned by Maria Gerstenberger, Monika Lüchow (née Schröder, Berlin) as well as by various students of him during the years 1969 to 1977. Much of the material had been collected in South Africa near Vioolsdrif (28°54’S, 17°44’E) and fixed in FPA (formaldehyde, proprionic acid and ethyl alcohol) and CRAF V (chromic acid, ethyl alcohol and formaldehyde) by Hans-Dieter Illenfeldt and Heidrun Hartmann (Institut für Allgemeine Botanik und Botanischer Garten Hamburg) in 1969 and 1971, respectively (for details see Eckardt 1974: 16).

Some plants were grown from seed in the Botanic Garden Berlin-Dahlem and their inflorescences were fixed in CRAF V and serial sections were made by Monika Lüchow. Prior to embedding in Malinol the serial sections of 6 to 10µm thickness were stained either with Heidenhain’s haematoxylin and counterstained with Fast Green FCF or, for the sake of rendering the vascular system more clearly, with Fuchsin and Fast Green.

For an embryological study of Lophiocarpus, haematoxylin is definitely better but older pollen is to such an extent overstained that it is not possible to detect the nuclei contained therein. For clear pictures of the contents of the pollen grains as well as for critical anatomical work Fuchsin has proved to be more suitable. Both stains have kept perfectly well. This is perhaps explained by the fact that the slides were constantly kept in darkness, being no more viewed since 1977. I used a Leitz-Dialux microscope for the study of the sections and in addition I also studied the herbarium collections at B. All figures have been drawn by the present author.

Results

Flower morphology

All flowers of the polytelic inflorescences are bisexual. They possess a well-developed nectary and are promoted on the abaxial side. Five tepals make up the perianth, with the adaxial member being a little wider than the others. 5-merous androecium, showing the vascular bundles.
The androecium is either 4-merous (Fig. 1–3) or to a lesser extent 3-merous (Fig. 4). The latter condition is due to fusion of the two adaxial stamens. Also the somewhat stipitate and unilocular gynoecium with one basally inserted ovule is oligomerous, showing two intimately fused carpels, the hairy sutures of which form two narrow bands of inner stigmatic tissue, leading down to the foot of the funicle and acting as obturator (Fig. 39). Only the abaxial carpel produces the ovule, the adaxial one being sterile, see Fig. 1–4.

The nectarium

The nectarium is situated on the filaments of the stamens, right at the foot of the androecium (Fig. 5), building a conspicuous ring of densely staining tissue around the base of the somewhat stipitate ovary. Viewed in the early stages of flower development, it originates from a small group of cells of the filaments. In longitudinal sections of the earliest stage about 8 such cells destined to build the nectarium are discernible (Fig. 6) at the base of each filament. At the time when the ovule has become hemitropous (Fig. 5, 5a) about 18 such cells can be seen. When the ovule is completely bent just after RT II with the fertile macrospore (Fig. 25, 25a) the number of the future secretory cells is almost four times that of the earliest stage, showing many cells with 2 smaller and other cells with single but much larger nuclei. This perhaps indicates nuclear fusion and polyploidization. The already enlarged nectarium tightly embraces the base of the ovary showing no gap between the two. The flower is still a closed bud and I noticed no sign of nectar secretion. During double fertilization (Fig. 36) the flower is open, the nuclei of the nectarium cells have become still bigger and the cytoplasm of the cells is granular and very dense. There is a small gap between the nectarium and ovary wall. When the embryo sac shows a 2- or 3-celled embryo and 4 endosperm nuclei (Fig. 41, 41a), the nectarium has reached the peak of its development consisting of about 100 cells and the gap between nectarium and ovary is large. After that, the number of secretory cells does not increase; on the contrary the nectarium declines gradually, showing signs of degeneration due to lysis and absorption of its cells. Finally, at the 9-celled embryo stage, it is almost completely lost, showing vacuolated cells with very little cytoplasm.

Fig. 2a–i. Transverse sections of a flower with five tepals and a 4-merous androecium, showing the vascular bundles; 3a–f: Transverse sections of a triplet of flowers, the middle flower has a 3-merous androecium due to fusion of the two adaxial stamens.

Fig. 4a–j. Transverse sections of a flower with five tepals and a 3-merous androecium. Study of the vasculature; 5: Longitudinal section of a young flower, showing the nectariferous tissue (Fig. 5a) on the base of the anther filaments; 6: Longitudinal section of the nectariferous tissue, the youngest stage observed.
Anther wall formation, microsporogenesis and microgametogenesis

The introrse anther is dithecous and tetrasporangiate (Fig. 4a). Its wall develops after the monocotyledonous type of Davis (1966), comprising 4 layers, i.e. epidermis, endothecium, one middle layer and a single-layered tapetum (Fig. 9). Immediately after their completion all wall layers show uninucleate cells. The tapetal layer is of dual origin and although the nuclei of its cells multiply to 4 or 5 during meiosis I of the pollen mother cells, yet these nuclei fuse later and mostly 2 large nuclei remain in each cell until maturity and thereafter (Fig. 11, 14). The mature tapetum is thus binucleate. After meiosis and simultaneous cytokinesis the microsporocytes (Fig. 14) develop into tetrahedral and decussate tetrads of microspores (Fig. 15) that are nourished by the glandular tapetum. Ubisch bodies (oribicules) of differing sizes are always produced. They are most abundant when the tapetum has largely declined and the uninucleate pollen grains are highly vacuolated, being in the so called “signet-ring” stage (Fig. 17). After the first mitosis, a large vegetative and a small lenticular generative cell are produced in the pollen grain. At first the small lenticular cell is placed on the inner wall of the pollen grain (Fig. 18);
later it is engulfed by the vegetative cell and comes to lie right in the middle of the pollen grain (Fig. 19). Already before anther dehiscence, at the stage of the young 8-nucleate embryo sac, the generative nucleus divides in the pollen grain, producing two vermiform nuclei (gametes). The mature pollen grains are 3-nucleate (Fig. 20), showing also three conspicuous colpi. The endothecium (fibrous layer) develops the usual thickenings and anther dehiscence is longicidal. The pollen grains germinate monosiphonously on the ripe stigma, during which the two vermiform gametes move into the developing pollen tube. I could not detect the tube nucleus.

**Megasporogenesis and gametophytogenesis**

In *Lophiocarpus*, like in all other members of the *Cen-trospermae*, ovules are orthotropous when initiated. They bend in the course of further development, becoming campylotropous. At maturity they are bitemic and crassinucellate with the inner integument forming the micro-

... pyle and also showing a distinct air space between the two integuments at the chalazal side of the ovule (Fig. 21–25).

Fig. 22 shows the longitudinal section of a fairly young ovule a short time after both integuments have been initiated. The nucellus of the ovule already displays one to two macrosporocytes and one layer of parietal cells. The pollen mother cells in the anthers of the same flower are in the meiosis I stage of development. Fig. 23, 24 and 26–34 show the development of the monosporic *Polygonum* type embryo sac.

**Fertilization, endosperm and embryo development**

The ripe 3-celled pollen grain germinates monosiphonously on the stigma. Stained with haematoxylin, the two sperm cells are well visible, whereas I could not localize the tube nucleus in the pollen tube. The latter traverses at first the short style, reaching the inner surface at the top

Fig. 26–31. Megagametogenesis and development up to 4-nucleate embryo sac stage. Longitudinal sections of micropylar part of the nucellus (Fig. 26, 27, 29–31) and overview of whole ovule (Fig. 28). – 26: Uninucleate embryo sac. – 27: First mitosis leading to 2-nucleate stage. – 28: A not completely bent ovule carrying a 2-nucleate embryo sac, which is rather unusual. – 29: Two-nucleate embryo sac from a normally bent ovule. – 30: The normal-sized 4-nucleate embryo sac. – 31: A rather smaller 4-nucleate embryo sac.

... later it is engulfed by the vegetative cell and comes to lie right in the middle of the pollen grain (Fig. 19). Already before anther dehiscence, at the stage of the young 8-nucleate embryo sac, the generative nucleus divides in the pollen grain, producing two vermiform nuclei (gametes). The mature pollen grains are 3-nucleate (Fig. 20), showing also three conspicuous colpi. The endothecium (fibrous layer) develops the usual thickenings and anther dehiscence is longicidal. The pollen grains germinate monosiphonously on the ripe stigma, during which the two vermiform gametes move into the developing pollen tube. I could not detect the tube nucleus.

Fig. 32–36. Development of 8-nucleate embryo sac up to early double fertilization. Longitudinal sections of micropylar part of nucellus. – 32: The not-yet organized 8-nucleate stage with a small deposit of starch granules. – 33–34: The semi-organized (Fig. 33) and completely organized (Fig. 34) 8-nucleate embryo sac. – 35: The 7-nucleate ready-to-fertilize embryo sac with ample starch granules around the fused polar nuclei. – 36: Embryo sac during double fertilization. Right above the burst pollen tube on the right are the 2 synergids, the nucleus of one of which is degenerating. The sperm cell on the left has not yet reached the larger egg nucleus and above these are the huge central nucleus and the second sperm cell in the process of fusion. The 3 antipodals are still present. Note the multitude of starch grains, some of which have been delivered by the pollen tube.
of the ovary. From there it makes its way down to the micropyle of the single basal ovule. At the inside of the ovary the pollen tube is guided by one of the two narrow bands of inner stigmatic tissue (obturator, Fig. 25, 39) that run on the suture of the two intimately fused carpels, right from the very top of the ovary up to the base of the funicle of the ovule. Fig. 39a shows the inner stigmatic tissue which is made up of a tuft of loosely interwoven 2- to 3-celled trichomes, the end cells of which are multinucleate and rich in cytoplasm. Fig. 35 and 36 show double fertilization. Fig. 37, 38, 41 and 44 present the early and Fig. 50 and 53 the later endosperm development. Finally, Fig. 54–64 document the development of the embryo.

Development of the seed coat

Seen in longitudinal sections of ovules at the time when the tetrad of megaspores is formed, the outer integument situated in the height of the fertile megaspore consists of two layers of cells, whereas at the chalazal end three layers are present. During further development the number of layers in the outer integument may increase on the chalazal side and especially at the tip in the vicinity of the micropyle. The inner integument consists of two layers of cells except at the tip where it is much thicker (5–6 layers).

Fig. 37–40. Late stages of double fertilization, young zygote and early endosperm development, pollen tube transmitting tissue and trichomes. Longitudinal sections of parts of nucelli (Fig. 37, 38). – 37: Late double fertilization, fusion of male gamete with central nucleus is already completed, syngamy of egg cell nucleus and second male gamete is not yet finished. – 38: Embryo sac showing the young zygote, rest of the still visible pollen tube and first division of the endosperm nucleus. – 39: Longitudinal section of the middle flower of a triplet, the gynoecium is cut transversely right at the plane of fusion of the two carpels, showing both the external as well as the internal pollen tube transmitting tissue. Deep below the stigmatic crests of the style, concealed in the gynoecium, two narrow bands of hairy tissue extend along the margins of the two intimately fused carpels from the top of the ovary downward up to the base of the funicle of the ovule, forming an efficient transmitting tissue (obturator) for the pollen tubes. – 39a: Detail of pollen-tube-transmitting tissue comprising 2- or 3-celled hairs the long-end cells of which are multinucleate and rich in cytoplasm. – 40: Trichome (epidermal vesicle) swollen above the level of the other epidermal cells on the tip of a young tepal.

Fig. 41–43. Flowers of Lophiocarpus polystachyus, Phytolacca acinosa and Chenopodium bonus-henricus. Longitudinal sections. Overview of ovule, ovary and dwindling nectary; 41: Lophiocarpus polystachyus. Somewhat stipitate (gynophore) ovary after fertilization with the dwindling nectary (stippled), pollen-tube-transmitting hairs (obturator) at the foot of the funicle and embryo sac with 2-celled embryo and 4 endosperm nuclei; 41a: Lophiocarpus polystachyus. Nectary in higher magnification showing the vacuolated, already degenerating cells; 42: Anthetic flower of Phytolacca acinosa (stamens and tepals not drawn) showing gynoecium, nectary and gynophore; 43: Flower of Chenopodium bonus-henricus shortly before anthesis, without gynophore.
Both outer as well as the inner integument participate in the formation of the seed coat, which comprises all the layers of the outer integument plus the innermost layer of the inner integument in the still not fully mature seed (oldest examined stage). The outer layer of the inner integument becomes crushed, is resorbed (except at the tip) and disappears during development of the testa (Fig. 45–48). The cells of the outer layer of the outer integument become very conspicuous by excessive radial elongation. Also cells of the remaining (extant) inner layer of the inner integument elongate radially and in both of these cells dark brown deposits occlude (fill in) the cell lumina. Cells of the two inner cell layers of the outer integument have some starch-like deposits and healthy-looking nuclei but no brown deposits. Especially the outer tangential cell walls (“roof” of the cells, building the surface of the seed) of the outer layer of the outer integument show a tremendous increase in thickness.

The innermost layer of the testa is derived from the inner layer of the inner integument.

**Conclusions and discussion**

*Lophiocarpus polystachyus* has polytelic inflorescences with all flowers being bisexual. The flowers possess a well developed nectary and are promoted on the abaxial side. Five tepals make up the perianth with the adaxial member being a little wider than the others. The androecium is either 4-merous or, more rarely, due to fusion of the two adaxial stamens, 3-merous. Also the somewhat stipitate and unilocular gynoecium with one basally inserted ovule is oligomerosus, showing two intimately fused carpels the hairy sutures of which form two narrow bands of inner stigmatic tissue leading down to the foot of the funicle and acting as obturator. Only the abaxial carpel produces the ovule, the adaxial one being sterile. Wall formation of the introrse and tetrasporangiate anther conforms to the Monocotyledonous type and four wall layers including a glandular tapetum with binucleate cells are built. Meiosis of the microsporocytes during which 9 bivalents are visible leads after simultaneous delimitation of the microspores to tetrahedral and deccussate pollen tetrads. The tricolpate pollen is 3-celled when shed. The ovule is campylotropous, bitegmic and crassinucellate. Early in its ontogeny a parietal cell is cut, later also a many-layered nucellus epidermis (nucellar cap) develops. The inner integument alone forms the micropyle (endostome) and there is an air space between the inner and outer integument at the chalazal side. Embryo sac development is of the monosporic, *Polygonum* type and at maturity the megagametophyte is 7-celled. Soon after double fertilization the 3 antipodals and the intact synergid degenerate. During early embryogenesis endosperm development is free nuclear and an aggregate of larger nuclei and cytoplasm with coarse-grained contents functions as chalazal haustorium, digesting the tissue around the massive core of the nucellus. After starch deposition, the latter forms the major food reserve (perisperm) of the circular embryo. In late embryogenesis the endosperm becomes cellular and in addition to the perisperm also a scanty residue of cellular endosperm remains clothing the radicle of the embryo. The seed coat comprises all the layers of the outer integument and the inner layer of the inner integument. The outer layer of the inner integument disappears during development of the testa. The fruit is a 1-seeded drupe.

The flower anatomy of *Lophiocarpus* confirms an affinity to the *Phytolaccaceae* s.l. and its distance from the *Chenopodiaceae* because of the missing gynophore in the latter (Fig. 41–43). The ovule in *Lophiocarpus* is built as usual in *Centrospermae*, the embryo sac development is of the common *Polygonum* type, present in all *Centrospereae*, and embryogenesis follows the Caryophyllad type of Johansen (1950). A comparison of the flower morphology and embryology of *Lophiocarpus* with the
Fig. 49–52a. Older stages of endosperm, development of perisperm, of the seed coat and fruit wall. Transverse sections (Fig. 49, 51, 52) and longitudinal section (Fig. 50); 49: Part of the fruit wall and of the integuments, stage between Fig. 47 and 48. – 50: Overview of ovule at the time of the 9-celled embryo with free endosperm nuclei lining the walls of the embryo sac, a basal aggregation of haustorially active coarse granular plasma and larger endosperm nuclei at the chalaza and a circular tissue right in the middle of the ovule that will remain in the ripe seed as perisperm – a starch deposit for the future use of the adult embryo during germination. – 51: Overview of nearly ripe seed showing the massive perisperm with still living cells and nuclei in the middle and the sectioned circular embryo with the two cotyledons above and the radicle below, both surrounded by a ring of scanty cellular endosperm. The seed wall (testa), especially the outer wall of the outer integument is already heavily incrusted with dark brownish black substances. – 52–52a: Transverse section of 150-celled embryo surrounded by cellular endosperm from the micropylar side of an ovule and in Fig. 52a the haustorially active nuclear endosperm and coarse plasma aggregation from the chalaza of the same ovule.
data known for Corbichonia (Narayana & Lodha 1963 under Orygia decumbens), to which Lophiocarpus presumably has a closer relationship (Cuénoud & al. 2002), indicates that they are not intimately related because of the following differences between them:

(1) Flower morphology:

Corbichonia: Flowers with 5 free tepals. Androecium of numerous stamens of which those in the outer 2 or 3 whors are petaloid staminodia. The gynoecium is pentacarpellary with 2 rows of ovules in each locule on axile placenta.

Lophiocarpus: Flowers with 5 free tepals. Androecium of only 4 or 3 stamens. The gynoecium is bicarpellate and unilocular with only one basally inserted ovule.

(2) Ovule:

Corbichonia: The ovule displays the primordium of an arillus.

Lophiocarpus: The ovule shows no arillar primordium.

(3) Obturator:

Corbichonia: Epidermal cells of the placenta serve as a placental obturator.

Lophiocarpus: Epidermal cells of the funicle act as a funicular obturator.

(4) Development of the embryo:

Corbichonia: Embryogenesis conforms to the Linum variation, Solanad type.

Lophiocarpus: Embryogenesis conforms to the Caryophyllad type of Johansen (1950).

Acknowledgements

The author is grateful to the reviewers, Dietmar Behnke (Heidelberg) and Hiroshi Tobe (Tokyo), for their valuable comments on a previous version of the manuscript, and to the editor, Norbert Kilian, who reviewed the manuscript and improved the Introduction substantially.

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Fig. 53–64. Development of the endosperm and embryo. Longitudinal sections; Fig. 53: Overview of ovule with embryo shortly before initiation of the cotyledons surrounded by cellular endosperm reaching to one third of the length of the embryo sac followed by free nuclear endosperm lining the walls of the rest of the embryo sac. The nuclear endosperm at the chalaza is composed of a multitude of bigger nuclei and coarse granular plasma forming an effective haustorial basal aggregation. – 53a–b: micropylar (Fig. 53a) and chalazal (Fig. 53b) part of Fig. 53 in higher magnification; Fig. 54–64: Embryogenesis from zygote to the 31-celled stage. Small size numbers with the prefix (E = endosperm) give the number of endosperm nuclei accompanying that embryo in the embryo sac. – 54: Zygote; E = 2 (accompanied by 2 endosperm nuclei). – 55–56: Embryo, 2-celled, both with E = 4. – 57: Embryo, 3-celled, E = 8 and 3 antipodals. – 58: Embryo, 4-celled, E = 22. – 59: Embryo, 7-celled, E = 34. – 60: Embryo, 7-celled, E = 37. – 61: Embryo, 9-celled, E = 42. – 62: Embryo, 21-celled, E = 88. – 63: Embryo, 19–21-celled, E = 135 free nuclei.
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