

## Research Paper

### Aril development in Celastraceae

XIN ZHANG<sup>\*,1,2</sup>; ZHIXIANG ZHANG<sup>2</sup> & THOMAS STÜTZEL<sup>1</sup>

<sup>1</sup>Faculty of Biology and Biotechnology, Ruhr-Universität Bochum, Germany

<sup>2</sup>Faculty of Biology and Biotechnology, Beijing Forestry University Beijing, China

**Keywords:** aril, Celastraceae, outer integument, development, *Euonymus*, *Celastrus*, caruncula

\* Corresponding author: Universitätsstraße 150, Gebäude NDEF 05/770, D-44780 Bochum, Germany,  
E-mail: zhangx48@rub.de, makexyz@163.com

Accepted for publication: June 6<sup>th</sup>, 2012.

DOI 10.1002/fedr.201200007

#### Abstract

To learn more about the evolution of secondarily intercalated seed envelopes, a series of developmental studies of arillate seeds in gymnosperms and angiosperms was undertaken. The goal was to test whether the second (outer) integument could be derived from an aril of gymnospermous ancestors. In our developmental studies of three species of Celastraceae, however, it turned out that the structure termed aril in this family does not originate from the funiculus or the hilum but from the exostomatic micropyle. As a consequence, the micropyle is not inside the aril but at the base of the fleshy structure which is thus better referred to as a caruncula. The fleshy part in seeds of Celastraceae differs thus markedly from those seed appendages usually referred to as an aril.

#### Introduction

Celastraceae are a subcosmopolitan family of 98 genera and about 1211 species with the highest diversity in the tropics and subtropics and with few temperate species (SIMMONS 2004). Many species of Celastraceae are economically important for both traditional medicine and horticulture. For example, “khat” (*Catha edulis*), is used socially as a stimulant in

north-eastern Africa, the Arabian Peninsula, and Madagascar (KRIKORIAN 1985); *Euonymus*, *Celastrus*, and *Paxistima* are widely cultivated as ornamentals; *Kokoona zeylandica* is used as a source of oil, from *Salazia* the pulp of the fruit is eaten, and various species of *Euonymus* are used for latex, medicine, and dyes (HOU 1962; HEYWOOD 1993).

The aril is a very conspicuous and taxonomically important character within Celastraceae. PLANCHON (1845) described the putative arils of *Celastrus scandens* and *Euonymus latifolius* as arillodes – false arils. PLANCHON cited these false arils as derived from the exostome of the outer integument rather than from the funiculus. However, MIERS (1856) disputed PLANCHON’s conclusion and, based on his own investigation of *Euonymus europaeus*, he concluded that the aril is derived from the funiculus and is therefore a true aril. PFEIFFER (1891) described the arils of *Celastrus*, *Euonymus*, and *Gymnosporia cassinoides* as derived from the exostome and the hilum. CORNER (1976) described the aril of *Euonymus glandulosus* as derived entirely from the funiculus, and the aril of other species (*Catha edulis*, *Celastrus paniculatus*, *Sarawakodendron fila-*

*mentosum*) as derived from the exostome and the funiculus. VAN DER PIJL (1972) concluded that *Euonymus* has an arillode, not an aril, but a detailed study on the seed development is still wanting. This study focuses on the different stages of development and intends to show the whole process of aril development in Celastraceae, and thus may help to understand the evolution of this family and the order Celastrales sensu lato which is a loose assemblage of probably not closely related taxa (SHISODE 2011). It may furthermore be helpful and serve towards an evolutionary understanding of the formation of different types of fleshy seed appendages.

### Material and methods

Three species, *Celastrus orbiculatus* THUNB., *Euonymus europaeus* L., and *Euonymus planipes* (KOEHN) KOEHNE, were studied. The material was collected from one individual per species each, all samples were collected from the Botanical Garden of the Ruhr-Universität Bochum. Sampling was done nearly every week from April to July 2010. The fresh floral buds, flowers or young fruits were fixed using FAA (formalin:acetic acid:ethyl alcohol 70% = 5:5:90). In order to optimize infiltration of the fixative, the material was kept in the fixative under moderate vacuum for at least 30 min. For further storage, the FAA was replaced by 70% ethyl alcohol after 2 days.

Dissections were performed in ethyl alcohol under a ZEISS Stemi SV II dissection microscope. Dehydration for SEM studies was performed using FDA (Dimethoxymethane) for 24 hours and subsequent critical point drying with a BALZERS CPD 030. Dried parts were mounted on aluminium stubs and sputter coated (BAL-TEC SCD 050, gold, 200 sec, 42 mA).

### Results

*Celastrus orbiculatus* THUNB. has a pentamerous imbricate perianth. The number of stamens equals the number of petals (Fig. 1A). The gynoecium is trimerous (Fig. 1B) or sometimes tetramerous and starts its formation when the floral bud begins to close. The formation of the two ovules per locule starts prior to the complete closure of the carpel (Fig. 1C). The inner integument is initiated when the incurvation of

the ovule starts (Fig. 1D). When the outer integument is formed the nucellus is positioned at right angle to the funiculus (Fig. 1E, F). During further development the ovule gradually turns into an anatropous position (Fig. 1F). When finally the ovule is completely anatropous, the ovule is slightly exostomatic (Fig. 2A). At this stage the exostome becomes thicker, and the thickening meristem at first encircles the micropyle more or less completely. In subsequent steps the micropylar thickening meristem of the outer integument enlarges, and the meristem step by step incorporates the entire funiculus until it finally encircles both the micropyle and the funiculus. The fleshy ring becomes thicker and thicker and develops a distinct rim towards the chalazal part of the ovule. In subsequent steps this ring forms a fleshy duplicature covering the outer integument (Fig. 2B–E). In contrast to a typical aril, this duplicature does not cover the micropyle, but leaves it free laterally to the funiculus. The duplicature leaves the chalazal region entirely free (Fig. 2F).

*Euonymus europaeus* L. has whorls of 4 perianth segments (Fig. 3A). The number of stamens equals the number of petals and carpels (Fig. 3A, B). The gynoecium is tetramerous (Fig. 3B) and starts its formation when the floral bud begins to close. The formation of the two ovules per locule starts prior to the complete closure of the carpel. The formation of the ovule starts with the differentiation of an obtuse narrow primordium (Fig. 3C). When the incurvation of the ovule starts (Fig. 3D), the inner integument is initiated. When the outer integument is formed, the nucellus is curved approximately 90 degrees and is turned towards the carpel margin (Fig. 3E). When the outer integument finally covers the inner integument and the nucellus, the ovule curves into a fully anatropous position (Figs. 3E, F; 4A). At this stage the micropyle points towards the proximal part of the gynoecium, the ovule is upright anatropous. When finally the ovule is completely anatropous, the ovule is slightly exostomatic (Fig. 4B). At this stage the exostome becomes thicker, and the thickening meristem at first encircles the micropyle more or less completely (Fig. 4C). In subsequent steps the micropylar thickening meristem of the outer integument enlarges, and the meristem step by step incorporates the entire funiculus

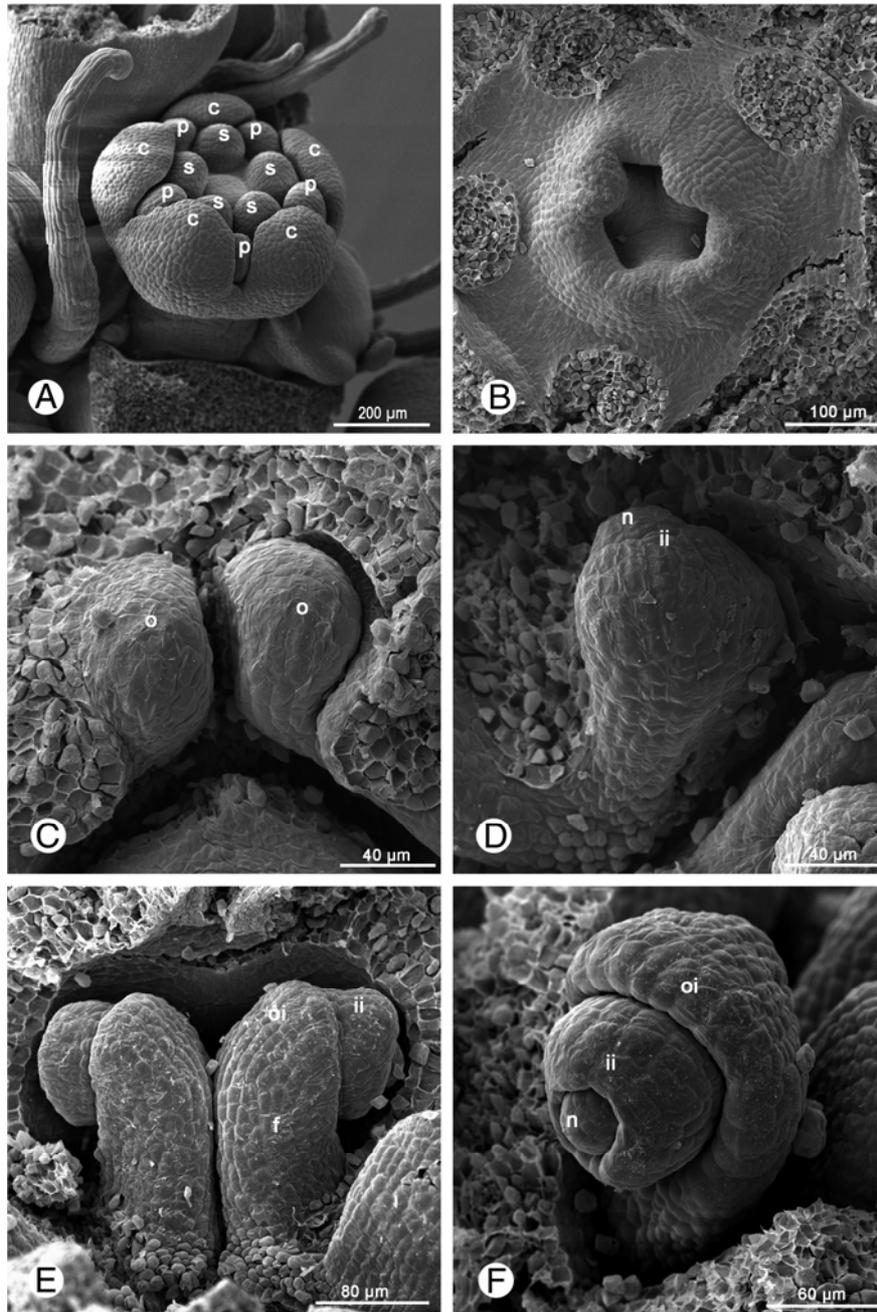


Fig. 1

*Celastrus orbiculatus* I: Ovule, successive developmental stages. A — young flower with perianth and stamen primordia; B — trimerous gynoecium initiating; C — initiation of 2 ovules per locule; D — beginning development of the inner integument; E, F — formation of the outer integument and beginning ovule incurvation. c = calyx, f = funiculus, ii = inner integument, n = nucellus, o = ovule, oi = outer integument, p = petal, s = stamen

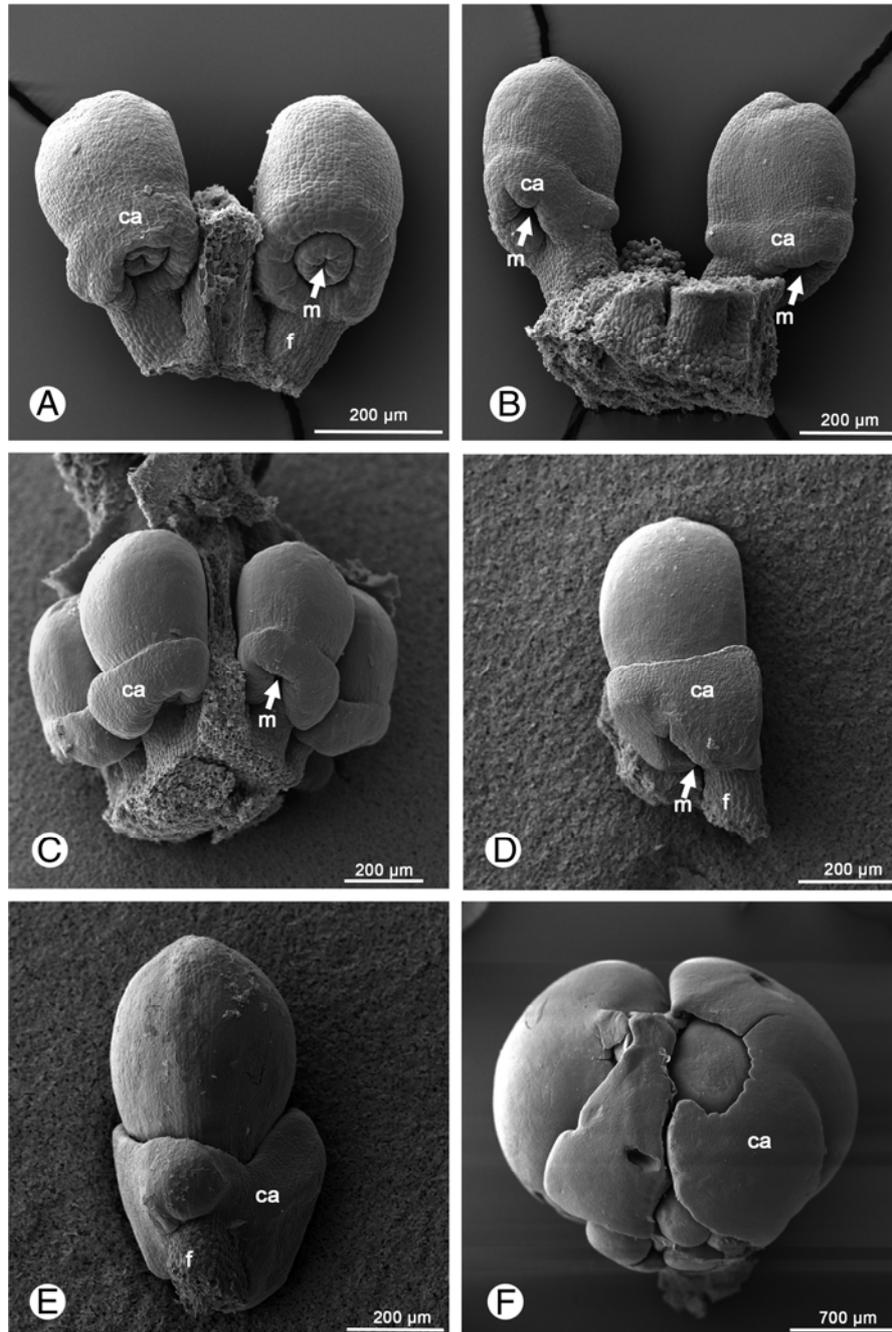


Fig. 2  
*Celastrus orbiculatus* II: Caruncula, successive developmental stages. A — caruncula originating from the margin of the outer integument; B–D — enlargement of the caruncula and incorporation of the funiculus; E — beginning formation of the fleshy seed duplicature; F — mature seed. ca = caruncula, f = funiculus, ii = inner integument, m = micropyle, oi = outer integument

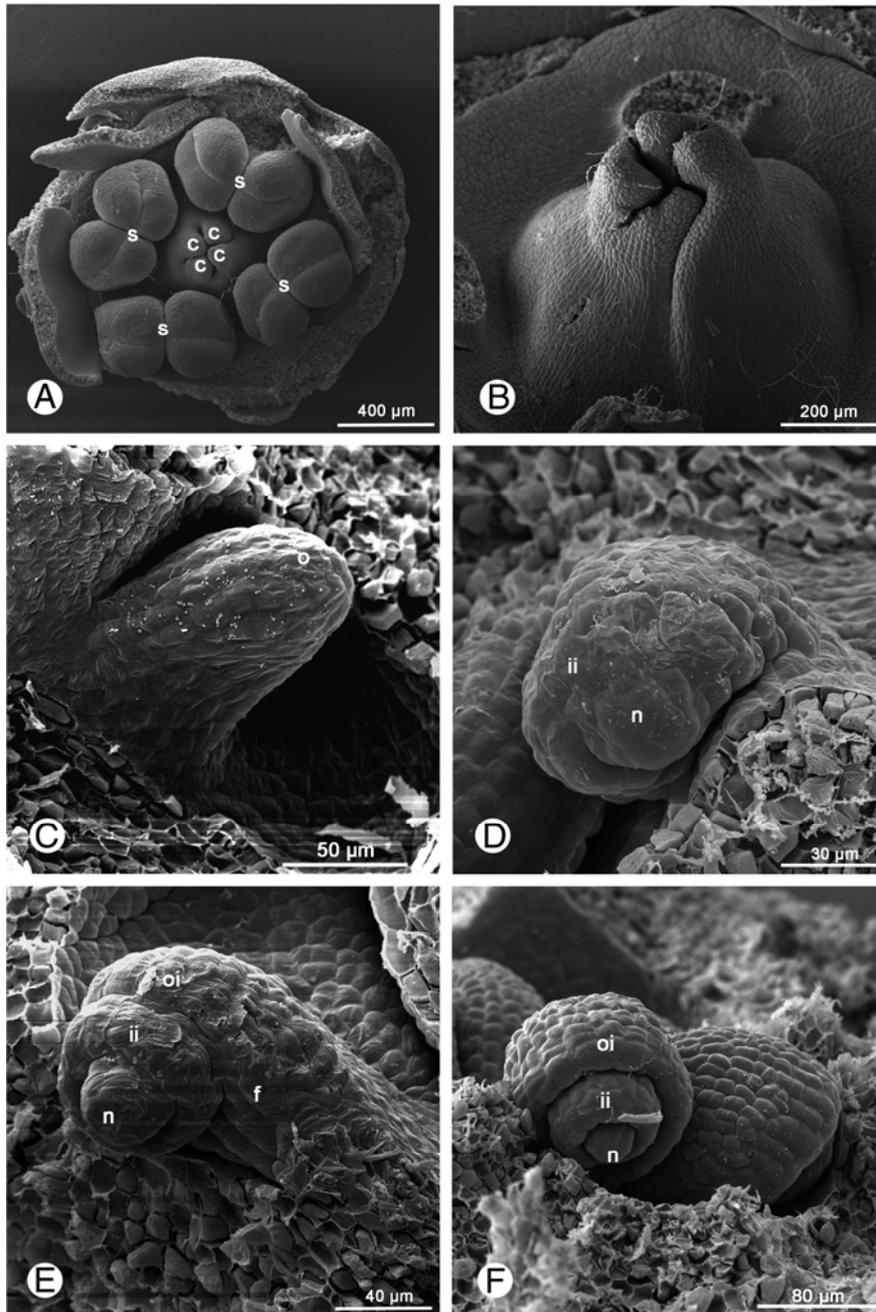


Fig. 3

*Euonymus europaeus* I: Ovule, successive developmental stages. A — preanthetic flower bud showing stamens and carpel primordia; B — young tetramerous gynoecium; C — ovule initiation; D — beginning development of the inner integument; E, F — formation of the outer integument and beginning ovule incurvation. c = carpel, f = funiculus, ii = inner integument, n = nucellus, o = ovule, oi = outer integument, s = stamen

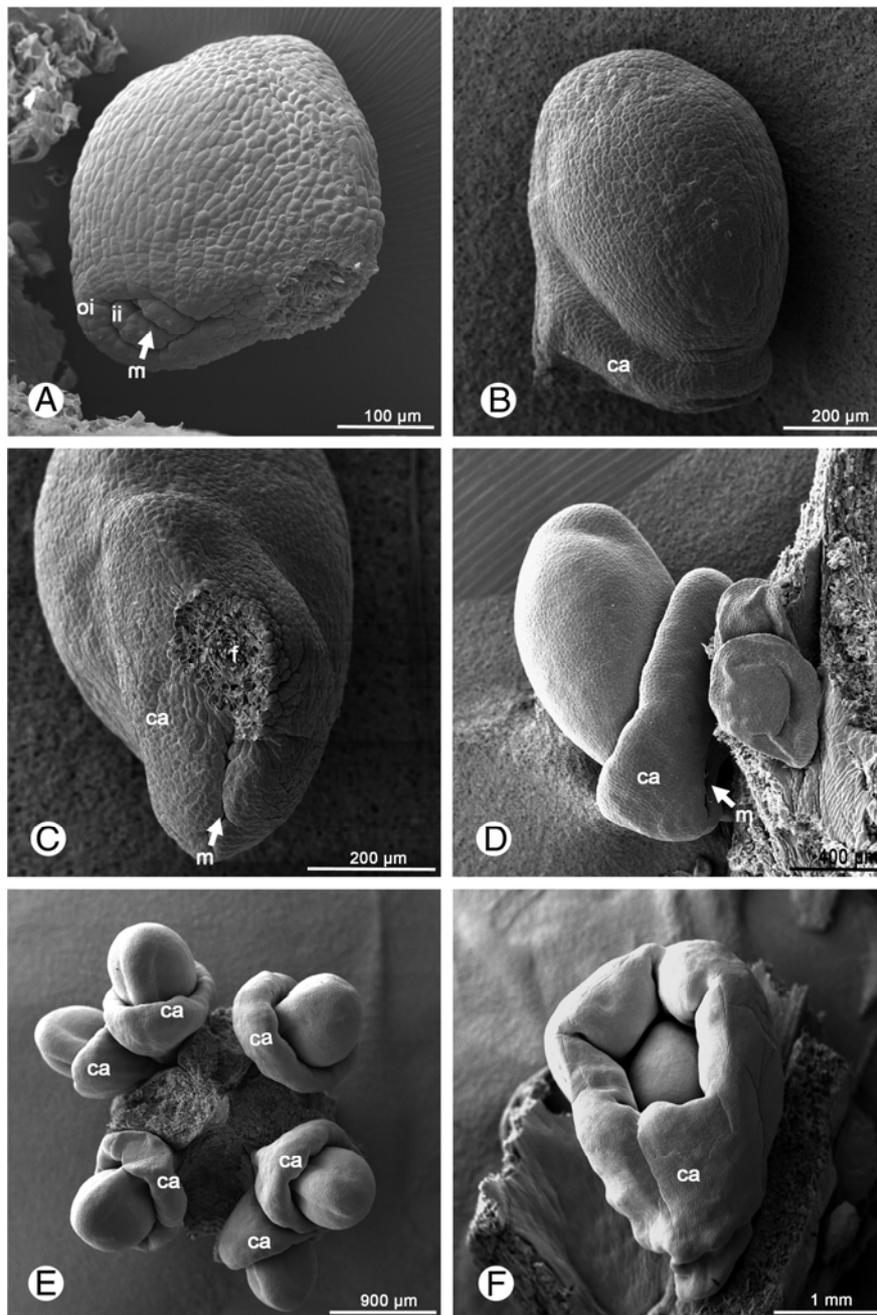


Fig. 4

*Euonymus europaeus* II: Caruncula, successive developmental stages. A — micropyle formed by 2 integuments prior to caruncula formation; B — caruncula originating from the margin of the outer integument; C — incorporation of the funiculus in the formation of the caruncula; D — beginning formation of the fleshy duplicature; E — slightly older stage in seed development than in D (6 of 8, 2 seeds removed); F — mature seed. ca = caruncula, f = funiculus, ii = inner integument, m = micropyle, oi = outer integument

until it finally encircles both the micropyle and the funiculus (Fig. 4B–D). The fleshy ring becomes thicker and thicker and develops a distinct rim towards the chalazal part of the ovule. In subsequent steps this ring forms a fleshy duplicature covering the outer integument (Fig. 4E, F).

*Euonymus planipes* (KOEHNE) KOEHNE has a pentamerous imbricate perianth (Fig. 5A). The number of stamens equals the number of petals (Fig. 5A, B). The gynoecium may be pentamerous or sometimes tetramerous, and starts its formation when the floral bud begins to close (Fig. 5B). The formation of the two ovules per locule starts prior to the complete closure of the carpel. Unlike in *Euonymus europaeus* L. and *Celastrus orbiculatus* THUNB., the anatropous ovules are pendulous from the top of the locule to the cup-shaped base of the gynoecium. When the incurvation of the ovule starts the inner integument is initiated. When the outer integument is formed the nucellus is positioned at right angle to the funiculus (Fig. 5C, D). During further development, the ovule gradually turns into its final anatropous position (Fig. 5E). When finally the ovule is completely anatropous, the ovule is slightly exostomatic, the micropyle nearly touches the funiculus (Fig. 5E). At this stage the exostome becomes thicker, and the thickening meristem at first encircles the micropyle more or less completely (Fig. 5F). In subsequent steps the micropylar thickening meristem of the outer integument enlarges, and the meristem step by step incorporates the entire funiculus until it finally encircles both the micropyle and the funiculus (Fig. 6A, B). The fleshy ring becomes thicker and thicker and first develops a distinct circular rim towards the chalazal part of the ovule (Fig. 6C). Further growth of the duplicature is asymmetric and leads to an oblique shape of the duplicature (Fig. 6D). In subsequent steps this ring forms a fleshy duplicature covering the outer integument (Fig. 6E, F).

## Discussion

The term aril is historically used in a rather broad sense. This may lead to an ambiguous understanding of structures described as arils. In diagnostics this might be acceptable as this

character is generally used together with others. However, if the focus is on character evolution, processes starting in a different way and leading to different final stages should be distinguished carefully. GAERTNER (1788) was the first to describe the aril as an accessory integument. Botanists commonly use the term aril for fleshy structures arising from the funiculus that enclose the ovule more or less totally. Nevertheless, different terminologies were used by different workers in different research contexts (PLANCHON 1845; BAILLON 1876; CORNER 1949, 1953, 1976; VAN DER PIJL 1972; KAPIL et al. 1980). One of the defining properties of an aril is that it is generally fleshy, but the most important property of an aril is how it is initiated and how it develops. A true aril originates from the funiculus, and it can be described as a the third seed envelope of the ovule (ENDRESS 2011). This organ can be fleshy or hairy or can form wings, it may have its own vasculature, and sometimes produces a mucilaginous pulp filling the locule.

PLANCHON (1845) described the putative arils of *Celastrus scandens* and *Euonymus latifolius* as arillodes – false arils – and cited these false arils as derived from the exostome of the outer integument rather than from the funiculus. This is very similar to our results, but not according to PLANCHON's drawing. Here the arillode originates from two distinct primordia, one coming from the funiculus and the other from the exostome. Despite this unusual development PLANCHON regarded it as an aril. In contrast to that, MIERS (1856) described the same structure as originating exclusively from the funiculus and treats it as a normal aril.

Our results on *Euonymus europaeus*, however, are completely different from those of MIERS, and from PLANCHON as well. One reason is that MIERS did no complete developmental study, but analyzed only seeds and rather late stages of seed development in *Euonymus europaeus* (MIERS 1856). PFEIFFER (1891) described the arils of *Celastrus*, *Euonymus*, and *Gymnosporia cassinoides* as derived from the exostome and the hilum. CORNER (1976) described the aril of *Euonymus glandulosus* as entirely derived from the funiculus, and the aril of other species (*Catha edulis*, *Celastrus paniculatus*, *Sarawakodendron filamentosum*) as derived from the exostome and the funiculus.

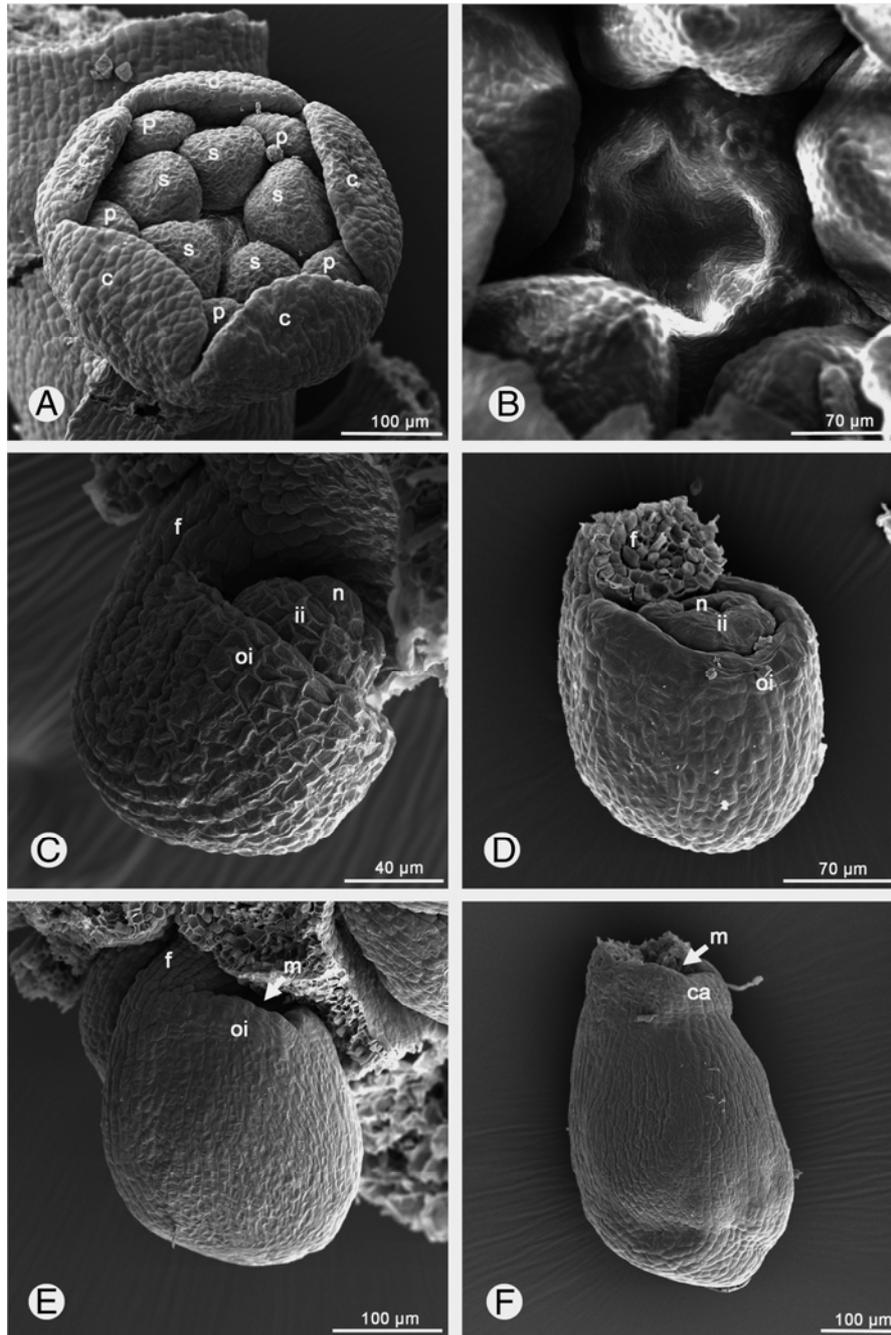


Fig. 5  
*Euonymus planipes* I: Ovule, successive developmental stages. A — young flower with perianth and stamen primordia; B — pentamerous gynoecium initiating; C–E — formation of the outer integument and ovule incurvation; F — caruncula initiating from the margin of the outer integument. c = calyx, ca = caruncula, f = funiculus, ii = inner integument, n = nucellus, oi = outer integument, p = petal, s = stamen

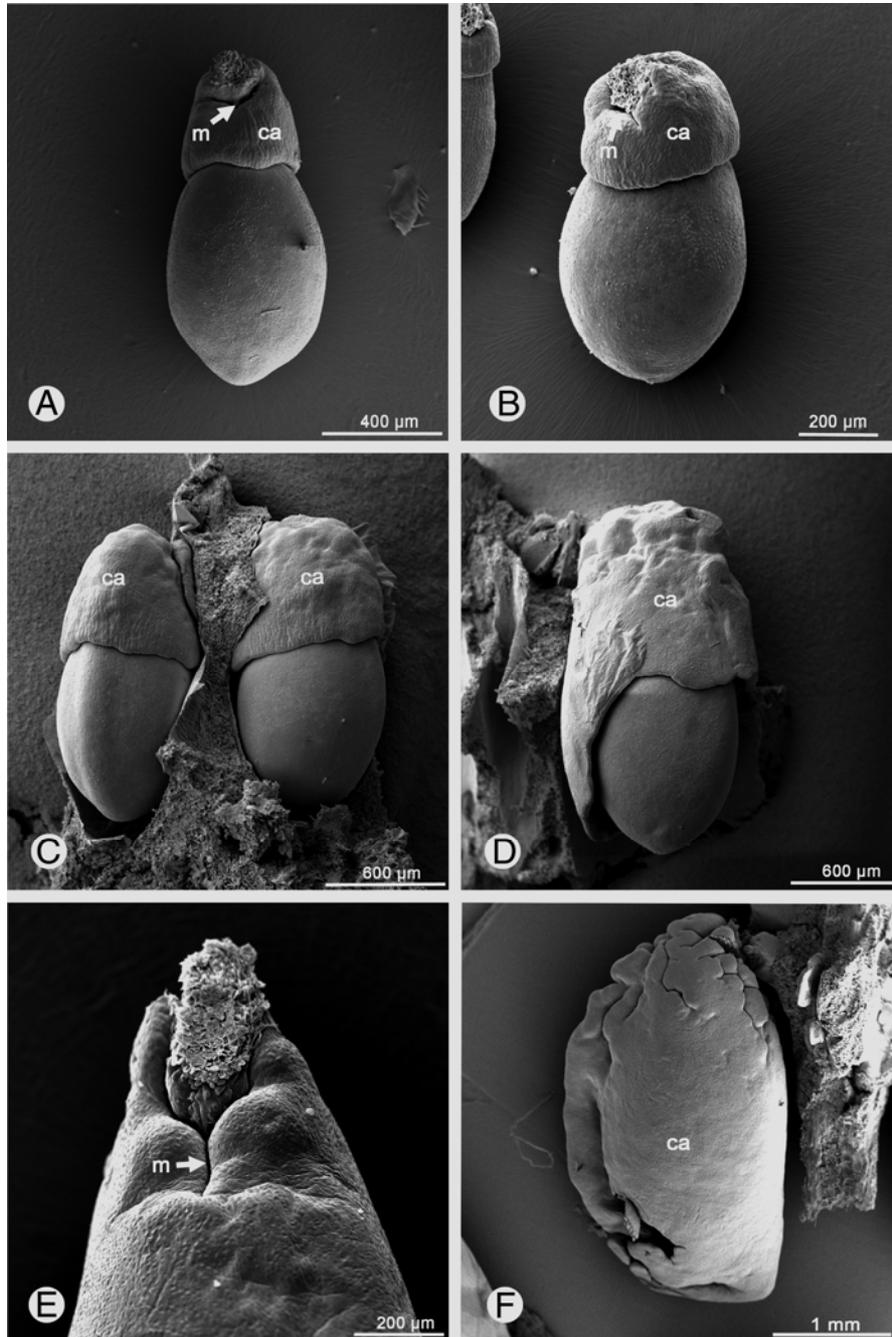


Fig. 6

*Euonymus planipes* II: Caruncula, successive developmental stages. A, B — caruncula originating from the margin of the outer integument; C — older circular stage of the caruncula; D — asymmetric growth of the caruncula; E — mature stage of the seed, view from the hilum; F — entire mature seed, lateral view. ca = caruncula, f = funiculus; m = micropyle

This can be interpreted as diversity within the Celastraceae which also gives us a way to understand the phylogeny of Celastraceae and the order of Celastrales. VAN DER PIJL (1972) concluded that *Euonymus* has an arillode, not an aril. VAN DER PIJL used the term arillode rather as an ecological term than as a morphological one. In his interpretation, the fleshy structures which cover the seed and do not fulfil his definition of an aril are all summarized as arillodes. This, however, is not really a solution as it shifts ambiguities to another term instead of solving them in a meaningful way. Together with the aril there are four different types of fleshy seed appendages including the sclerotesta in *Magnolia* and the strophiole and the caruncula in Euphorbiaceae.

It is of some interest that all these secondary fleshy appendages are either associated with the micropyle or with the funiculus or hilum. Both the micropyle and the hilum are weak points in the protective structure covering endosperm and embryo. They can get closed by mere shrinking of the surrounding tissues, but they can also close by active growth of adjacent cells. This could be a preadaptation leading to the fleshy appendages by stepwise enlargement. In orthotropous seeds, fleshy structures originating from micropyle or funiculus are clearly separate from each other, and usually only one option is present. In anatropous seeds, the two zones are close together. Only minor meristem incorporation is necessary to build up mixed forms in which micropylar appendages and funicular appendages merge. In the same way the fleshy parts originating only from the micropyle or only from the funiculus may incorporate the complementary structure easily. It can be thus expected that structures that are very similar at maturity display rather different developmental patterns. For diagnostic purpose, it is not meaningful to distinguish these structures in detail. Used in an evolutionary context, fleshy seed appendages might supply even more information than actually used.

*Lophopyxis* was regarded as a genus with an arillate structure belonging to Celastraceae, but according to SIMMONS (1999) *Lophopyxis* is better placed in Euphorbiaceae. This conclusion is also supported by our results saying that the aril in Celastraceae should be called carun-

cula. In the system of APG III (2009), Celastrales is only sister group of the orders of Malpighiales and Oxalidales. So the relationship between the Celastraceae and Euphorbiaceae is closer than generally suggested. In the papers by SIMMONS & HEDIN (1999), SIMMONS (2004), and SIMMONS et al. (2008, 2011) the character aril is used without any clear description, they regard this structure in Celastraceae as a true aril. In 2012, SIMMONS et al. concluded that “current intrageneric classifications of *Euonymus* are not completely natural and require revision”. But prior to all tests, the development of the morphological characters of this family should be clearly understood.

## Conclusion

*Celastrus orbiculatus* has a pentamerous perianth and five anthers, but a trimerous gynoecium. *Euonymus europaeus* is tetramerous throughout the genus. However, the flower of *Euonymus planipes* has five sepals, five petals, and five carpels (seldom four carpels). The developmental process in *Celastrus orbiculatus*, *Euonymus europaeus*, and *Euonymus planipes* is very similar. The visible difference is the position of the ovule primordium. The ovule primordium of *Euonymus planipes* is on the top of the placenta, so the micropyle is directed towards the style.

The structure in Celastraceae called aril originates from the margin of the micropyle which is here formed by the outer integument. The funiculus is incorporated in the formation of the fleshy part of the seed rather late in the primary morphogenesis. The relevant steps are so early that the development differs markedly from what has to be expected for a true aril. The pattern of development described here was hitherto unknown. According to this pattern, the micropyle is not inside the aril, but at the base of the fleshy structure which is thus better referred to as a caruncula.

## Acknowledgements

The Botanical Garden of the Ruhr-Universität Bochum has generously allowed us to collect the material for the present and several other studies from its collections. The first author is especially grateful to Sabine Adler for her efforts in improving the manu-

script linguistically and structurally. He also gratefully acknowledges the financial support by the Chinese Scholarship Council.

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