universität
wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Structure and function of explosive fruits and seed dispersal in Acanthaceae"

verfasst von / submitted by

Marina Nigrowics

angestrebter akademischer Grad / in partial fulfillment of the requirements for the degree of

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2016 / Vienna, 2016

Studienkennzahl lt. Studienblatt / degree program code as it appears on the student record sheet:	A 190 445 344
Studienrichtung lt. Studienblatt / degree program as it appears on the student record sheet:	Lehramtsstudium UF Biologie & Umweltkunde, UF Englisch
Betreut von / Supervisor:	Univ.-Prof. Dr. Jürg Schönenberger

Table of Contents

Acknowledgements 1

Abstract 2

1. Introduction 3

2. Material and Methods 6

  2.1 Study species and sites 6

  2.2 Microtome sections, light microscopy and stereo microscopy 9

  2.3 Micro-Computer Tomography 10

  2.4 Scanning Electron Microscopy 13

  2.5 Experimental Methods – Explosive seed dispersal and High-speed video recording 13

3. Results 15

  3.1 General Findings 15

  3.2 Fruit Morphology and Anatomy (Stereo Microscopy, LM, SEM, MicroCT) 15

    3.2.1 Acanthoideae 15

    3.2.2 Nelsonioideae 21

    3.2.3 Thunbergioideae 25

    3.2.4 Comparative analysis of Acanthoideae, Nelsonioideae and Thunbergioideae 29

  3.3 Explosive seed dispersal and High-speed video recording 39

4. Discussion 39

  4.1 The explosive seed dispersal of all three subfamilies 39

  4.2 Morphological structures and tissues aiding seed expulsion 41

    4.2.1 The retinaculum 41

    4.2.2 The apical beak 44

    4.2.3 Specific tissues 46

  4.3 The opening mechanism – hygrochastic versus xerochastic triggering 48

  4.4 The explosive seed dispersal mechanisms and their evolutionary relationship 50

5. Conclusion 51

References 52

Supplementary Materials 53

Appendix 54

  Deutsche Zusammenfassung 54

Acknowledgements

I would like to thank Jürg Schönenberger, my supervisor, for all his help, motivation and patience. He offered me insights into the highly fascinating Acanthaceae and their explosion mechanisms and thoroughly supported me throughout this whole project. Special thanks go to Susanne Sonntag and Susanne Pamperl. I would not have been able to conduct this study without all their help. Additionally, I would like to extend my thanks to the Department of Botany and Biodiversity Research of the University of Vienna and also to the University of Vienna as such for their financial support by granting me the KWA scholarship. At this point, my gratitude is also due to Werner Huber who supported me at the Tropical Research Station La Gamba in Costa Rica and the staff of the Tropical Research Station who made my fieldwork in Costa Rica such a great experience.

I am also especially indebted to my family, particularly to my mother. Thank you so much for supporting me all my life and specifically during my studies at the University of Vienna! I know that my last year in particular was a tough time for all of you; I was not always the best company to have around.

Very sincere thanks go to Felix, my friend, best friend and companion for life who supported me more than I could have ever dreamed of. He truly contributed to the success I had within my studies and this work with his outstanding patience and knowledge about me and my weaknesses.

I am also very thankful for the enduring motivation and encouragement of my friends. In particular, I would like to mention Christian, the best company one can have during the years of university and my friends Nadine, Lisa, Patricia, Katharina and Anna Maria.

I thank all of you with all my heart! I highly appreciate everything you have done to support me during this chapter of my life.

Abstract

Acanthaceae, a mostly tropical and subtropical plant family of roughly 4000 species, is well known for its morphological diversity. This diversity confronted researchers of the last decades with several problems that partly remained unsolved up to the present. For instance, it is still not clear whether the three main subfamilies of Acanthaceae – namely Acanthoideae, Nelsonioideae and Thunbergioideae – all comprise species that feature fruits with explosive opening mechanisms to enable active seed dispersal. Already existing studies provide numerous contradictions concerning this particular issue. In this thesis, the existence of these extraordinary fruit explosion strategies within the three subfamilies at hand was investigated. Concerning the origin of these fruits in more detail, fruits of various Acanthoideae representatives, *Elytraria carolinensis* (as representative of Nelsonioideae) and *Thunbergia alata* (as representative of Thunbergioideae) were examined. In order to investigate the fruits, which were collected at the Botanical Garden of the University of Vienna and during my field studies at, or rather nearby, the Tropical Research Station La Gamba in Costa Rica, different approaches were implemented. On the one hand, fruit development and structure were investigated utilizing stereo microscopy, light microscopy, scanning electron microscopy and Micro CT-scans to provide new insights into these outstanding fruits. On the other hand, seed dispersal experiments were executed by using high-speed video recording of mature capsules on the verge of seed dispersion. The most significant findings of this study are, firstly, that despite all contradictions in the literature, species featuring explosively opening fruits are present in all three subfamilies of Acanthaceae. Secondly, as their morphological diversity might already reveal, the subfamilies possess similar tissue layers which are most likely causing the fruit explosion. However, the best compositions of these, in fact, three cell layers are located at different positions within the fruits of Acanthoideae, Nelsonioideae and Thunbergioideae, respectively. Thirdly, this study adds new results to the topic of hygrochastically or xerochastically opening capsules with the help of the seed dispersal experiments. In more detail, several species which were thought to explode only due to additional humidification, thus hygrochastically, were found to explode also during desiccation, therefore xerochastically. As a result, the investigations conducted within the context of this thesis offered new insights into the structure and function of the explosively opening fruits and might be of great significance for future studies on acanthaceous fruits.

Keywords: Acanthaceae, fruits, explosive seed dispersal, fruit morphology, Acanthoideae, Nelsonioideae, Thunbergioideae, Costa Rica.

1. Introduction

The family Acanthaceae comprises approximately 4000 mostly tropical and subtropical species and encompasses a wide range of morphological diversity. In addition, Acanthaceae species are adapted to different habitats and many subgroups exhibit complex biogeographic patterns (McDade et al. 2008). This great diversity has for a long time impeded the identification of phylogenetic relationships among major Acanthaceae lineages. Over the last century, a great collection of research on Acanthaceae was conducted and its findings have clarified numerous aspects. However, several issues are still remaining, as for example, the correct position of Acanthaceae within Lamiales, the relationships between the different subfamilies within Acanthaceae, or – at the structural-functional level – also the unique seed dispersal mechanisms investigated in the present study.

One of the first and most comprehensive classifications of Acanthaceae was produced by Lindau (1895) who appears to be the 'prime father' of the classification of Acanthaceae due to his rather early investigations into this plant family. Other scholars discussing such issues were, for instance, Schönenberger and Endress (1998) who focused on the systematic entanglement of two subfamilies (Mendoncioideae and Thunbergioideae), or also McDade et al. (2008) who investigated the overall phylogenetic composition of the Acanthaceae using molecular tools. Another study dealt with the phylogenetic relationships of *Avicennia* within the Acanthaceae and supported a close relationship to the subfamily Thunbergioideae (Borg & Schönenberger 2011). Furthermore, Wortley et al. (2005) discovered a more appropriate classification of *Thomandersia* (which earlier was generally referred to Acanthaceae) in the Lamiales. All these studies, again, illustrate that there are still various unsolved classification problems within and in the phylogenetic surroundings of the family Acanthaceae.

The subfamily Acanthoideae is probably the most thoroughly investigated clade within the family, and thus, some earlier research needs to be mentioned here. For instance, a great number of investigations were already performed enabling a better understanding of the tribe Ruellieae within the Acanthoideae (see Witztum & Schulgasser 1995; Tripp 2007; Tripp 2010; Tripp et al. 2013; Imam et al. 2013). Another tribe which already received significant attention is Justicieae. It was focused on in studies by Graham (1988) and by Wood (2015) who examined *Justicia* in more detail and described it "is the largest genus of Acanthaceae world-wide and is very diverse in the Neotropics" (2015). Acanthoideae have probably received particular attention due to the fact that this subfamily was often referred to as the 'real' Acanthaceae – also called "Acanthaceae sensu stricto" (McDade et al. 2000) or "'core'

Acanthaceae" (Borg 2012) – whereas subfamilies like Thunbergioideae and Nelsonioideae were just presented as the closest relatives together with the genus *Avicennia* (Borg 2012), which was already mentioned above. Borg et al. (2008) explain this discrepancy as follows:

> The delimitation of the family has been controversial due to morphological differences between the subfamily Acanthoideae (Acanthaceae s.str.), containing the vast majority of all species in the family, and the two other subfamilies, Nelsonioideae and Thunbergioideae sensu Scotland & Vollesen (2000).

Such investigations demonstrate that significant insights into some of the relationships within Acanthaceae were already gained by performing not only morphological but also molecular studies. Nevertheless, there are still numerous aspects of Acanthaceae and their representatives that remain unclear. This demonstrates the urgent need for further research.

Before describing areas that are in need of further investigation, another research area that already appears to be relatively well understood needs to be mentioned, namely the overall classification of the family. The general classification of Acanthaceae is important because it serves as the comprehensive phylogenetic framework needed for the present study. It is, for example, given by McDade (2008) and illustrates that there are at least three subfamilies of Acanthaceae: Acanthoideae, Thunbergioideae and Nelsonioideae (McDade 2008), which largely matches the classification by Scotland and Vollesen (2000). Furthermore, these two authors explain their system as an adaptation of Lindau's classification from 1895.

One possibility to find some more answers concerning the true relationships within the Acanthaceae – or simply to examine subfamilies and their distinctive structures and functions – is to investigate the fruits of different genera. Comparisons of the fruits of Acanthaceae genera were already conducted by some scholars but always with different foci. One of the most important features of Acanthaceae fruits – and this has usually been the main interest in studies investigating fruit morphology in this family – is their ability to explode in order to disperse their seeds. However, fruit morphology and fruit structure have up to now only been investigated in relatively few species. It is still controversial, which genera actually have such exploding capsules and which not. Borg and Schönenberger (2011), for instance, investigated some representatives of the three subfamilies mentioned above (Acanthoideae, Thunbergioideae, Nelsonioideae) and observed explosively dehiscent capsules in all three of them. McDade (2008) reached similar results showing that Nelsonioideae and *Thunbergia* (a genus of Thunbergioideae) feature explosive seed dispersal. On the other hand, there are studies (for example by Scotland & Vollesen 2000; Tripp 2007) assuming that specific species do not disperse their seeds explosively due to the absence of a retinaculum, which is a hook-like seed jaculator (also called a retinaculum) and will be discussed in the following paragraph.

The literature discussing explosive fruits in Acanthaceae mostly begins with examining whether the plants of interest have a retinaculum. This seed jaculator is supposed to be the most significant morphological structure of acanthaceous fruits supporting the explosive seed dispersal and it is even sometimes argued to have experienced scant attention (see Witztum & Schulgasser 1995). It appears to be obvious that the lever-like structure of the retinaculum is crucial for the act of dispersing the seeds away from its mother plant. Nonetheless, Scotland and Vollesen (2000), for instance, argue that "[t]he exploding capsules of Acanthaceae have an exactly congruent distribution to that of retinacula", and thus, emphasize that Acanthaceae genera without this retinaculum are not capable of exploding at all. Tripp (2007) seems to agree with Scotland and Vollesen (2000) because of her more definite argument that *Thunbergia* does not possess explosive fruits due to the lack of retinacula. Nevertheless, it seems unlikely that it is the retinacula themselves that cause the fruits to explode; these structures rather help to disperse the seeds more efficiently, just like some sort of catapult (Witztum & Schulgasser 1995). Apart from these studies, there are also some stating that *Thunbergia* does, in fact, have explosively dehiscent fruits, although the retinacula are missing (see McDade et al. 2008), which is, according to McDade et al. (2008), also true for some representatives of the Nelsonioideae. This controversy is important to this study due to the fact that it is a significant aspect that influences my second research question, which is stated below.

Apart from these basic aspects of fruit structure and function, there is a further issue that needs to be clarified. Irrespective of whether the fruits are explosive or not (and independent of the existence of a retinaculum), there is still the question of whether they open hygrochastically or xerochastically. The hygrochastic opening mechanism refers to additional humidification of the fruits – especially their distal beaks – that needs to occur in order to weaken "the pectic 'glue'" (Witztum & Schulgasser 1995) between the two fruit valves, which finally elicits the explosion. The xerochastic mechanism is also triggered by the availability of water but, as opposed to the hygrochastic mechanism, this type of seed expulsion "is due to the high stress in the bonding layer" of the two valves "at some degree of desiccation" (Witztum & Schulgasser 1995). These two types of seed expulsion mechanisms led to different conclusions in Acanthaceae research conducted in the last decades, or even centuries. Nevertheless, further details about investigations into this research area will be treated in the discussion (chapter 4).

Consequently, the goal of this study is to shed new light onto the enigmatic structure and function of the explosive, acanthaceous fruits and the significance of fruit morphology for our understanding of systematic relationships within Acanthaceae. In particular, my study focuses on the questions (1) whether there are representatives featuring explosively dehiscent fruits in all three subfamilies (Acanthoideae, Thunbergioideae and Nelsonioideae), (2) what the shared morphological features and differences of their fruits are, with special focus on the existence of a retinaculum and whether there are specific structures in the fruits (apart from the retinaculum), which elicit the explosion of the fruits, and finally (3) whether the explosive seed dispersal is a hygrochastic or a xerochastic process. Furthermore, the ontogenetic development of the retinaculum structure will be inspected in more detail in order to gain a better insight into the overall morphology of different Acanthaceae fruits.

2. Material and Methods

2.1 Study species and sites

Fruits in different developmental stages have been collected from a range of Acanthoideae, Thunbergioideae and Nelsonioideae. Part of the material (various species of Acanthoideae) was collected at the Tropical Research Station La Gamba, in the Province of Puntarenas, in the southwest of Costa Rica. My field research at this site – which encompasses the Piedras Blancas National Park, and thus, the Esquinas Rainforest, often better known as the "Rainforests of the Austrians" (see Sehnal & Zettel 1996; Weissenhofer & Huber 2001) – was conducted from February 8th to March 7th in 2015. The collection of acanthaceous fruits was executed at the Tropical Research Station La Gamba itself, along the roads connecting the villages La Gamba, Golfito and the Research Station and at some of the trails of the Esquinas Rainforest Lodge (mainly at Fila Trail, Ocelot Trail and Trail to Valle Bonito and back) given in Figure 1.

Material of additional taxa, especially of the subfamilies Thunbergioideae and Nelsonioideae, was collected at the Botanical Garden of the University of Vienna (HBV Wien). The extraordinary variety of species cultivated in the Botanical Garden enabled the selection of the best suitable species for this study. Species have proved to be appropriate for this research if the flower and fruit production occurred with some kind of regularity. In most of the cultivated species, fruit set through self-pollination was sufficient. However, the flowers of *Thunbergia alata* only produced fruits and seeds after cross-hand-pollination.

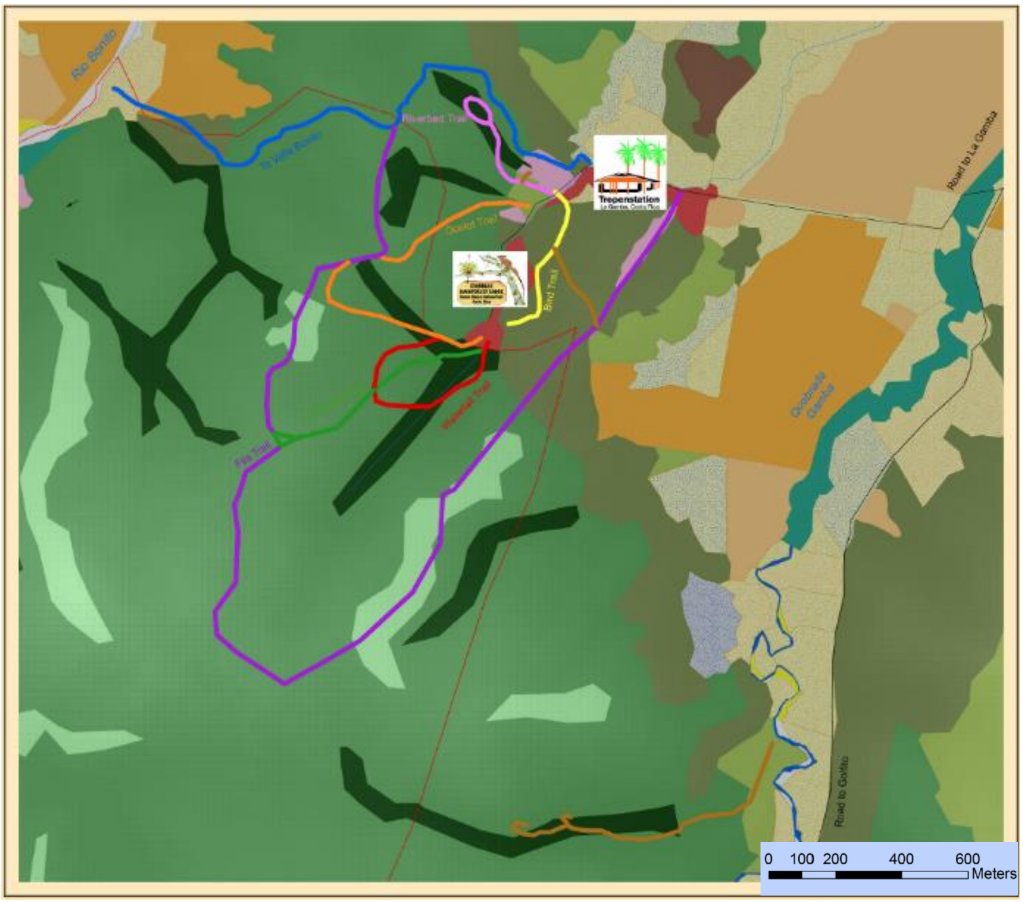

Figure 1. Trail map of the Esquinas Rainforest Lodge and Tropical Research Station La Gamba.

Samples collected in Costa Rica were fixed in 70% aqueous ethanol for storage and transportation to Austria. The common procedure of fixing the fresh plant material in formaldehyde - acetic acid - alcohol (FAA) before transferring it to 70% ethanol for storage and transportation was impossible to realize during the field study abroad, and thus, the plant material was immediately put into 70% ethanol. In comparison, most of the fruits collected at the Botanical garden of the University of Vienna were first fixed in FAA and after approximately seven days transferred into 70% ethanol. Further fruits collected at the HBV Wien, though, were stored as dried material for superficial morphological investigations using stereo microscopy (see 2.2), for the scanning electron microscopy (SEM) examinations specified in 2.4 or, if in a rather dry and mature stage of development, immediately used for the seed dispersal experiments using high-speed video recording (see 2.5).

In order to identify the species that were collected during the field study abroad, several books, articles and identifications keys, which were stored at the Tropical Research Station La Gamba, were used (to exemplify: Durkee 1986; Mabberley 1996; Gargiullo et al. 2008;

Utteridge & Bramley 2014). For the verification of the identification of the species cultivated at the Botanical Garden of the University of Vienna, Wasshausen (2013), theplantlist.org (last access 15th of January 2016) and tropicos.org (last access 15th of January 2016) were used and also some of the literature already utilized during the identification in Costa Rica were of great use.

Moreover, I collected two voucher specimens per species encountered abroad (sometimes only one voucher was possible); one is deposited in the herbarium CR of the National Museum of Costa Rica (Herbario Nacional de Costa Rica), which is in San José, the other one is deposited in the Herbarium WU, which is at the Department of Botany and Biodiversity Research of the University of Vienna. Further investigations on the acanthaceous fruits collected in and around the Tropical Research Station La Gamba were conducted at the Department of Botany and Biodiversity Research of the University of Vienna together with the studies of the fruits collected at the HBV Wien.

The main focus of my study is on several species of Acanthoideae and how their fruit morphology, which revealed to be rather homogenous within this subfamily, differs compared to one species of each of the other subfamilies: *Elytraria carolinensis*, as representative of the Nelsonioideae, and *Thunbergia alata*, as representative of the Thunbergioideae. I gathered fruits of the following Acanthaceae species at the Tropical Research Station La Gamba in February 2015 and of species provided by the Botanical garden of the University of Vienna from September 2014 to September 2015 and added where one can find a voucher of these findings (indicated by CR or WU):

Acanthoideae

Aphelandra cf. lingua-bovis Leonard, Nigrowics Marina, collection number (CN) 4, Costa Rica, WU

Aphelandra cf. tonduzii Leonard, Nigrowics Marina, CN 3, Costa Rica, CR and WU

Blechum cf. pyramidatum (Lam.) Urb., Nigrowics Marina, CN 7, Costa Rica, CR

Brillantaisia owariensis P.Beauv., Nigrowics Marina, not vouchered, cultivated at HBV Wien, 20358 Schönenberger Lehre

Crossandra cf. infundibuliformis (L.) Nees, Nigrowics Marina, CN 1, Costa Rica, CR and WU

Justicia cf. chamaephyton D. N. Gibson, Nigrowics Marina, CN 6, Costa Rica, WU

Justicia furcata Lam., Nigrowics Marina, not vouchered, cultivated at HBV Wien, ACA120104

Justicia cf. pectoralis Jacq., Nigrowics Marina, not vouchered, cultivated at HBV Wien, ACA120103

Razisea cf. spicata Oerst., Nigrowics Marina, CN 5, Costa Rica, WU

Ruellia cf. biolleyi Lindau, Nigrowics Marina, CN 15, Costa Rica, CR and WU

Ruellia blumei Steud., Nigrowics Marina, not vouchered, cultivated at HBV Wien, ACA120096

Ruellia cf. tonduzii Lindau, Nigrowics Marina, not vouchered, cultivated at HBV Wien, ACA000001

Ruellia cf. brevifolia (Pohl) C. Ezcurra, Nigrowics Marina, not vouchered, cultivated at HBV Wien (Jürg Schönenberger 21th of July 2014)

Schaueria calicotricha (Link & Otto) Nees, Nigrowics Marina, not vouchered, cultivated at HBV Wien, ACA110032

Nelsonioideae

Elytraria carolinensis (J.F.Gmel.) Pers., Nigrowics Marina, not vouchered, cultivated at HBV Wien, 32

Thunbergioideae

Thunbergia alata Bojer ex Sims, Nigrowics Marina, not vouchered, cultivated at HBV Wien, XX-O-DR-009745

2.2 Microtome sections, light microscopy and stereo microscopy

For microtome sectioning, the fruits of three species representing the three different subfamilies were investigated with particular attention; these are *Ruellia cf. brevifolia* (Acanthoideae), *Thunbergia alata* (Thunbergioideae) and *Elytraria carolinensis* (Nelsonioideae). The following steps were taken into action for the sectioning of these fruits. Firstly, fruits stored in 70% ethanol have been dehydrated during an ascending ethanol series up to 100% ethanol over a period of several days. Secondly, the fruits were transferred into an infiltration liquid consisting of 100ml Technovit 7100 and 1g hardener I. In order to remove excess air, samples were treated in a vacuum desiccator. Subsequently, the objects were stored in a refrigerator for at least two more days for further infiltration. The next step was the embedding of the fruits in an infiltrate composition (for approximately 10 objects: 8ml of the infiltration liquid the fruits were fixed in and 0,712ml hardener II). After some hours in a heating cabinet at roughly 40°C, the objects were hardened, and thus, finally prepared for the sectioning with the rotary microtome Microm HM 355S (for more information on microtome sectioning preparation, consult Gerlach 1984). Longitudinal and cross serial sections were produced with a slice thickness of 6-7µm, placed on microscope slides in a mixture of H_2O and 96% ethanol and then dried on a heating plate at approximately 75°C. Concerning the microtome sectioning in general, it should be mentioned shortly that it involved some difficulties to yield complete sections of the fruits' tissues due to the lignification, which already took place to some extent. Thus, the pictures, for example illustrated in Figure 8, should be considered with this information in mind. In order to obtain objects featuring enough contrast to see differences in tissue structure etc., the slices were stained by the use of a multiple general stain. In more detail, Ruthenium red (0.2%) and Toluidine blue (0.5%) were utilized for staining. Before examining the objects with light microscopy, they were dried in a heating cabinet once more and afterwards, mounted with Entellane or Roti-Mount.

The observation of the longitudinal and cross sections of the fruits was realized on an Olympus BX 50 microscope. For capturing the best representative sections, photographs were taken with a Nikon DS-Fi1. Afterwards, the photographs were processed with the Adobe program Photoshop CS6 Extended.

The same camera (Nikon DS-Fi1) was also made use of for photographing fresh as well as dried acanthaceous fruits using an Olympus SZH 10 (stereo microscope). The fresh plant material for this part of the research represented fruits from young to rather mature, but not dried and lignified material and was also cut open to enable rough analyses of the fruits' morphology. Dried, and thus, lignified material was photographed in the conditions I originally stored them. Therefore, I took photographs of mature capsules which were still closed, but also photographs of already dehisced ones. In the latter case, the two halves – whether there were still some seeds to be found in them or not – were captured, and sometimes also the dispersed seeds were photographed.

2.3 Micro-Computer Tomography

For sample preparation for Micro CT-scans, samples were transferred from 70% ethanol to a mixture of 85% ethanol and 0.5% PTA (phosphotungstic acid, as a contrast agent) for two to four days (depending on sample size and stage of maturity), and then transferred to 96% ethanol and 1% PTA, in which the samples remained for further seven days, to foster slow dehydration. Relatively mature fruits, especially the already dried capsules of *Thunbergia alata*, were scanned directly in this infiltration liquid. Scans were conducted on a Zeiss Xradia MicroXCT-200 system. Younger fruit stages with less differentiated tissues were critical-point-dried in an Autosamdri-815 before scanning. This procedure allowed for better resolution of the scans due to the fact that only air surrounded the objects instead of liquid solvents (for further details concerning the protocol, see Staedler et al. 2013). I also tried to critical-point dry some rather mature fruits of *Thunbergia alata* for higher resolution of the scans but they exploded during this procedure, or at least opened their beaks, which was also not desired in this part of the research.

Reconstruction of the tomographic raw data was conducted in the program XM Reconstructor in which one receives a volume viewer file as output. The program XM Controller was the tool used in this research to transform this file into a two-dimensional stack of images. Amira is the 3D software, which finally implemented the conversion of these stacks into three-dimensional objects which were then adapted for investigations within Amira. This program allows – amongst other things – to view the reconstructed 3D-images from any perspective. Scan settings and additional parameters for each scanned sample are given in Table 1.

Table 1. Scan settings including all additional parameters for the Micro CT-scans of the fruits investigated within this study.

Description	sample condition	part	pixelsize µm	source	detector	voltage	power	µA	exposure	bin	magn.	pictures
Elytraria carolinensis	CP-dried	mature fruit	5,0580	-18,0	6,0	20	3,0	150	2,5	2	4x	728
Thunbergia alata	CP-dried	fruit valve with seeds	19,7020	-24,0	9,0	20	3,0	150	7,0	2	1x	1000
Ruellia cf. brevifolia	CP-dried	mature fruit, partly dehisced	5,7400	-40	7,0	22	4,0	181	6	2	4x	728
Elytraria carolinensis	CP-dried	mature fruit	4,7100	-22,0	9,5	22	4,0	181	5,0	2	4x	1000
Justicia furcata	CP-dried	mature fruit	18,0608	-22,0	11,0	22	4,0	181	6,0	2	1x	1000
Thunbergia alata	CP-dried	mature fruit	6,3140	-110,0	7,5	22	4,0	181	30,0	2	4x	1600
Brillantaisia owariensis	CP-dried	detailed section of mature fruit	4,8510	-20,5	8,0	22	4,0	181	3,0	2	4x	1600
Elytraria carolinensis	CP-dried	mature fruit, partly dehisced	4,7260	-20,0	8,5	22	4,0	181	6,0	2	4x	1000
Thunbergia alata	CP-dried	immature fruit	4,7010	-23,0	10,0	28	5,5	196	2,0	2	4x	1200
Thunbergia alata	CP-dried	immature fruit	4,9400	-26,0	9,5	24	4,5	187	3,0	2	4x	1200
Elytraria carolinensis	CP-dried	immature fruit	4,8870	-25,0	9,5	22	4,0	181	4,0	2	4x	1200
Elytraria carolinensis	CP-dried	immature fruit	4,8870	-25,0	9,5	22	4,0	181	4,0	2	4x	1200
Thunbergia alata	CP-dried	mature fruit	12,6810	-22	25,0	22	4,0	181	10,0	2	1x	1600
Schaueria calicotricha	in alcohol atmosphere	mature fruit	12,4860	-24,5	28,5	50	8,0	160	6,0	2	1x	1600
Ruellia cf. brevifolia	in alcohol atmosphere	mature fruit	12,4860	-24,5	28,5	37	7,0	200	6,0	2	1x	1600
Thunbergia alata	CP-dried	mature fruit	16,7710	-26	16,0	27	5,0	184	11,0	2	1x	1600
Ruellia cf. brevifolia	CP-dried	immature fruit	4,8319	-24,0	9,5	22	4,0	181	4,0	2	4x	1200
Ruellia cf. brevifolia	CP-dried	immature fruit	4,7543	-21,5	9,0	22	4,0	181	3,0	2	4x	1200
Ruellia cf. brevifolia	CP-dried	immature fruit	4,6438	-21,0	9,5	22	4,0	181	3,0	2	4x	1200
Thunbergia alata	CP-dried	immature fruit	4,6777	-21,5	9,5	24	4,5	187	4,0	2	4x	1200
Thunbergia alata	in alcohol atmosphere	detailed section beak, mature fruit	4,4370	-25,0	13,0	25	5,0	200	10,0	2	4x	1200
Ruellia cf. brevifolia	CP-dried	Bottom of mature fruit Middle of mature fruit Top/Beak of mature fruit	2,172 3,007 1,981	-28 -18,5 -21	9,5 23 7,5	23	4,5	195	10 5 5	2	10x 4x 10x	1000 1200 1000
Thunbergia alata	in alcohol atmosphere	beak, almost mature fruit	4,6690	-22,0	12,0	35	7,0	200	4,0	2	4x	1200

2.4 Scanning Electron Microscopy

For scanning electron microscopy (SEM) investigations, samples previously fixed and stored in ethanol were dehydrated in an ascending ethanol series (70% - 85% - 100%), which was then followed by critical-point drying (CPD) in CO_2 with an Autosamdri-815. Furthermore, fully mature and already dry fruits were collected and stored in dried condition; no further preparation was required for the next phases. All dried samples were then mounted onto SEM stubs and subsequently sputter-coated with gold using a Sputter-Coater SCD 050. Finally, the fruits were studied by means of a JEOL JSM-6390 Scanning Electron Microscope.

2.5 Experimental Methods – Explosive seed dispersal and High-speed video recording

In order to better understand the explosive mechanism, high-speed video recording of the explosive dehiscence of some acanthaceous fruits was also applied. These fruits were all collected at the Botanical Garden of Vienna due to the fact that it was necessary to have mature but fresh fruits, which were not separated from the mother plants for a long period before the start of these experiments. Unfortunately, it was not easy to gather the fruits at the absolutely right time – namely on the verge of their natural dehiscence – and thus, several samples were lost shortly before the start of the high-speed video recording (these fruits exploded in the transport boxes to the experimental setting) or they were still too far away from exploding due to immaturity.

I collected mature fruits with, if possible, part of the pedicel still attached to them in order to be able to mount the fruits in front of the camera (Casio Exilim EX-ZR 100). Furthermore, holes in different sizes were drilled into small metal pieces in which the fruit pedicels were inserted. Metal was used due to the fact that it is a heat conducting material. This leads to the description of the surroundings the fruits were put in during the video recordings. One significant aspect was heat. Some of the species included in this experiment were said to be xerochastic, and thus, should be exploding due to desiccation (see Putzer 2015 who studied a species of *Justicia*).

Concerning the other species investigated here, no such information of their opening mechanisms was available. Therefore, I used the same mounting technique for all fruits – trying to elicit, or at least quicken, xerochastic dispersal processes through adding heat. Greuter and Rankin Rodríguez (2010) noted that:

> [T]he Acanthaceae appear to be playing a dual game, as exemplified by *Crossandra nilotica* Oliv., in which half of the capsules open xerochastically and the other half hygrochastically, depending on whether the initial tension that builds up in drying suffices to break the apical clamp or whether it must be weakened by moisture (Sell 1969: 434-435). If, as in this case, there is a subtle balance between both strategies, that is tilted by slight quantitative, non-structural changes, it is safe to assume that the evolutionary step from xerochasy to hygrochasy and back is an easy one that may frequently happen. (Greuter & Rankin Rodríguez 2010)

According to these researchers, it could be asserted that – at least some – acanthaceous fruits are capable of opening xerochastically as well as hygrochastically upon the same mother plant. Thus, testing only the reaction of fruits on desiccation should simply be seen as an attempt to examine this idea.

Heat was provided by a heating lamp, which was situated next to the camera, also in order to illuminate the fruits from an appropriate angle and light quality for video recording. High-speed video recording requires external lighting as relatively high light intensity is needed to produce enough photons that can reach the recording device during the frames (Cross 2013), which, in the end, leads to better resolution. However, there was even a further source of light needed in my experiments – the sun. After trying several different settings indoor, I came to the conclusion that the sun would improve my video recordings significantly. I suppose that the sun as additional light source is not always required, but the camera utilized here (Casio Exilim EX-ZR 100) yielded better resolutions if the sun was present at the setting. This was, of course, reducing the options for optimal video recordings according to the dependence of good weather; however, the roof terrace of the Department of Botany and Biodiversity Research of the University of Vienna proved to be a suitable place for including sunlight in my video recording equipment (most of the recordings took place in May to August 2015). Moreover, to achieve more contrast between the fruits and their surroundings, black background was arranged (for further useful tips concerning the set-up of appropriate parameters or also more theoretical background knowledge of physics, one might consult the thesis by Cross, 2013). In addition, the set-up resembled some kind of cave due to the four walls which were built around the fruits and the camera to reduce the impact of wind on the objects of interest. Obviously, wind was another problem which arose the moment the setting required relocation from indoor to outdoor.

For the video recording, I intended to use the maximum of possible frames with the camera at hand (1000fps with a pixel size of 224x64). This mode would have enabled slow and detailed recordings of the exploding fruits. Unfortunately, recording the fruit explosions – which took an unpredictably long time to take place – was not practicable with this camera setting due to the fact that the rechargeable battery did not last longer than 10 minutes when recording in this mode and also the memory capacity set its limits to that. Therefore, the recordings were mostly realized with the mode of “HS480” which means that 480 frames per second were recorded with a resolution of 224x160.

3. Results

3.1 General Findings

The gynoecium of Acanthaceae always comprises two fused carpels – it is syncarpous – each of which encloses a locule (chamber) and the placentation is axile (e.g. Figures 6 H; 10 G). The fruits are predominantly loculicidal capsules, which disperse their seeds explosively. The distal part of the ovary is usually drawn out into a more or less long beak-like structure (Figures 2, 7, 11 A). Considering Acanthoideae in more detail, the capsules are mostly stipitate and ovoid to oblong (Figure 2), their two to numerous, distinctly flattened seeds are borne on retinacula (Figures 4 A, B, D – L; 5 B, D, E, H; 16; 17), which derive from the funicles and remain in their position within the capsule after seed expulsion. *Elytraria carolinensis*, the representative of Nelsonioideae investigated in this study, possesses similar capsules to those of the Acanthoideae (Figure 7): they are explosive, but are sessile, mostly ovoid to subconic, their axile placentae are rather minute, retinacula are absent and the seeds are shaped irregularly. The representative of Thunbergioideae, *Thunbergia alata* (Figure 11), produces two fertile, collaterally arranged, semiglobose ovules with two seeds on axile placentae per carpel and its fruits also lack retinacula (as in Nelsonioideae). In addition, the capsules of *Thunbergia alata* possess extraordinary beaks (Figure 11 A) that derive from sterile apical prolongations of the ovary (and not the style).

3.2 Fruit Morphology and Anatomy (Stereo Microscopy, LM, SEM, MicroCT)

3.2.1 Acanthoideae

The fruits of the Acanthoideae species studied here, share several features in their morphology and anatomy (see Figure 2). Each species has its seeds, which are arranged in two alternate rows per carpel, mounted on retinacula (Figure 2 B, C, E, F). Additional details of the retinacula will be presented in chapter 3.2.4. In most Acanthoideae species investigated here, the part of the gynoecium where the seeds are located, is distinctly thickened (Figure 2 A – H). Moreover, these species also proved to be similar in their external structure due to the existence of a relatively short beak-like structure at the apex of their fruits (Figure 2 A – H). The fruits of *Ruellia blumei* and *Brillantaisia owariensis*, on the other hand, are linear in shape, and thus, do not possess a conspicuously thickened ovary (Figure 2 I – L). Due to the fact that the acanthoid species share many similarities among each other, the following results will mainly focus on one species, namely *Ruellia cf. brevifolia*, and only some extraordinary deviations in the other species will be mentioned in addition.

The investigations into the anatomy of *Ruellia cf. brevifolia* revealed that already young and immature fruits feature a beak-like structure at their apex (Figure 3 A – C). Furthermore, the cells in the beak of this species display different degrees of lignification in their cell walls and compactness in arrangement, especially in rather mature fruits which are generally close to lignification (Figure 3 F). The overall morphological structure of the beaks of the different Acanthoideae representatives appears to be similar in the acanthoid species presented here. However, the length of the apical beaks varies significantly (compare for example Figure 4 C and H).

The retinacula of *Ruellia cf. brevifolia* – and probably also of the other – acanthoid species is most likely already developing in an early state of fruit maturity. However, this funicular structure was only clearly detected in more mature fruits (Figure 3 D, E, retinacula indicated with arrows). Concerning the retinacula of *Ruellia cf. brevifolia* compared to the ones of other acanthoid species, this hook-shaped structures exposed to be shaped differently depending on the species they belong to (Figure 4 A, B, D – L). Some species possess retinacula which appear to perfectly surround the proximal side of their respective seeds (Figure 4 D), whereas others display a looser connection to their seeds (Figure 4 H – J). A further interesting result shows where exactly the seed is attached to the retinaculum, or *was* attached if the seed is already mature enough to be separated from the retinaculum's tissue and is in waiting position for the explosive dispersal. Seeds are generally attached close to the proximal end of the retinaculum (Figure 4 G, indicated with an arrow).

Figure 2. Acanthoideae. Fruits at different maturity stages. Stereo Microscopy. (A) *Justicia cf. pectoralis*, early lignification. (B) *Ruellia cf. tonduzii*, capsule valves after explosion. (C) *Ruellia cf. tonduzii*, immature fruit cut open; on the left, a seed on its retinaculum removed from the fruit. (D), (E) *Schaueria calicotricha*, mature fruit before and after manual opening of the valves. (F) *Justicia furcata*, capsule valves after explosion. (G), (H) *Ruellia cf. brevifolia*, immature fruit before and after manual opening of the valves. (I), (J) *Ruellia blumei*, immature fruit before and after manual cutting into ovary locule. (K), (L) *Brillantaisia owariensis*, mature fruit before and after manual cutting along the septum.

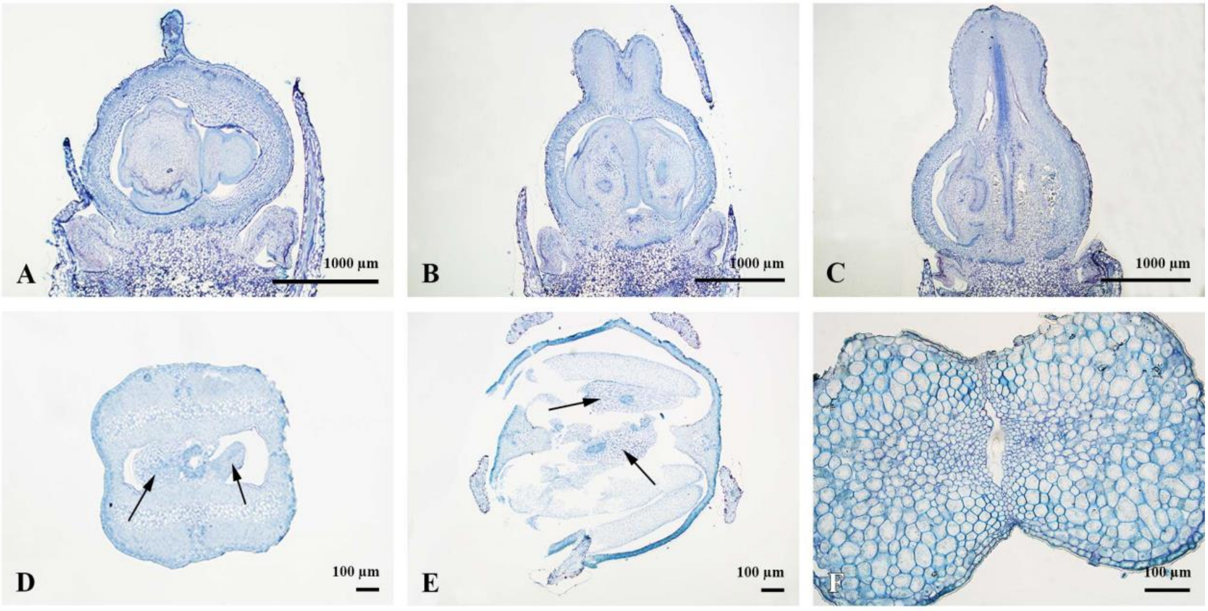

Figure 3. Acanthoideae. *Ruellia cf. brevifolia*. Light Microscopy. RT-stain. (A) – (C) Longitudinal sections of a young fruit. (D) – (F) Cross sections of a more mature fruit. (D) Lowest two retinacula within the fruit, arrows indicate retinacula. (E) The middle of the ovary locule, arrows indicate retinacula. (F) The beak of the fruit.

Young and immature fruits of *Ruellia cf. brevifolia* revealed that their seeds are still connected to the placenta through the funicles, which did not develop their retinacula yet (Figure 6 A). This is most likely also the case in the other acanthoid species, but only mature, or nearly mature, fruits of these species were investigated (see Figure 5). In more mature stages of *Ruellia cf. brevifolia* fruits, it is visible that the retinacula are at least partially lignified (visible as brighter areas in the Micro CT-scans due to their higher density; especially in Figure 6 B, E, F, H; indicated with arrows). These nearly hook-shaped structures are, furthermore, obviously positioned underneath each single seed and even surround them to some extent (Figure 6 D – G, I, in D & I: indicated with stars). The position of the retinacula in the fruits of other Acanthoideae species is very similar to that in *Ruellia cf. brevifolia* (Figure 5, 6).

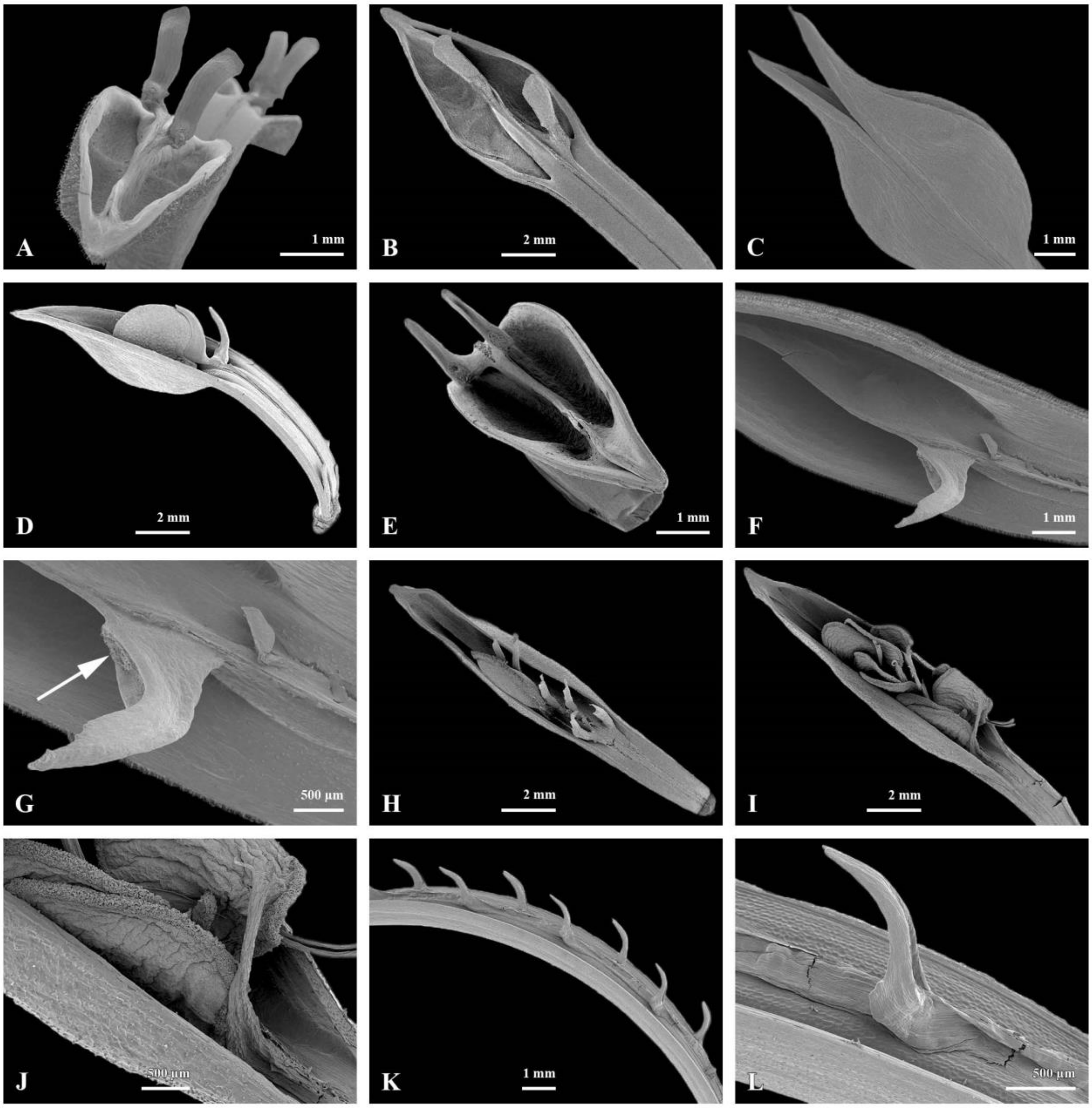

Figure 4. Acanthoideae. Scanning Electron Microscopy. (A) *Ruellia cf. tonduzii*, both capsule valves after explosion, observing them from the top-end of one valve. (B) *Ruellia cf. tonduzii*, one valve, view of ovary locule. (C) – (E) *Schaueria calicotricha*. (C) Mature capsule, beak already opening. (D) One valve with a seed remaining on its retinaculum. (E) Empty valve, view from above / from beak downwards. (F), (G) *Justicia furcata*, ovary locule and retinaculum in detail, arrow indicates seed’s point of attachment. (H) – (J) *Ruellia cf. brevifolia*, valves with one to many seeds and a detailed inspection of seeds on retinacula. (K), (L) *Brillantaisia owariensis*, several retinacula on one valve and one retinaculum in detail.

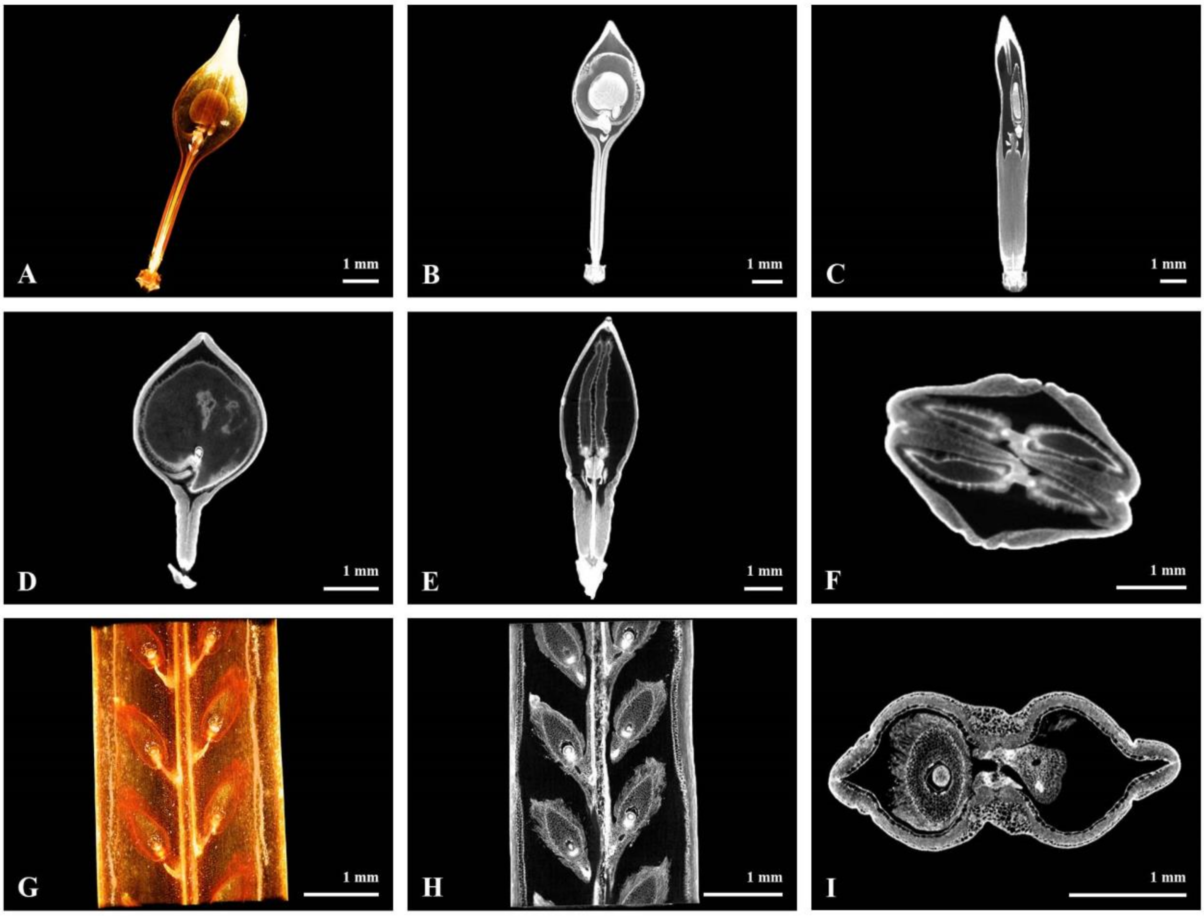

Figure 5. Acanthoideae. MicroCT. (A) – (C) *Schaueria calicotricha*, nearly mature fruit, no lignification of capsule yet. (A) Semitransparent view of whole capsule. (B) Longitudinal section along septum, inspection of one seed. (C) Longitudinal section perpendicular to septum. (D) – (F) *Justicia furcata*, nearly mature fruit, no lignification of capsule yet. (D) Longitudinal section along septum, close-up of one seed. (E) Longitudinal section perpendicular to septum. (F) Cross section through ovary locules. (G) – (I) *Brillantaisia owariensis*, nearly mature fruit, no lignification of capsule yet. (G) Semitransparent view of ovary locule section. (H) Longitudinal section perpendicular to septum. (I) Cross section of ovary locule, one seed (left side) and one retinaculum (right side).

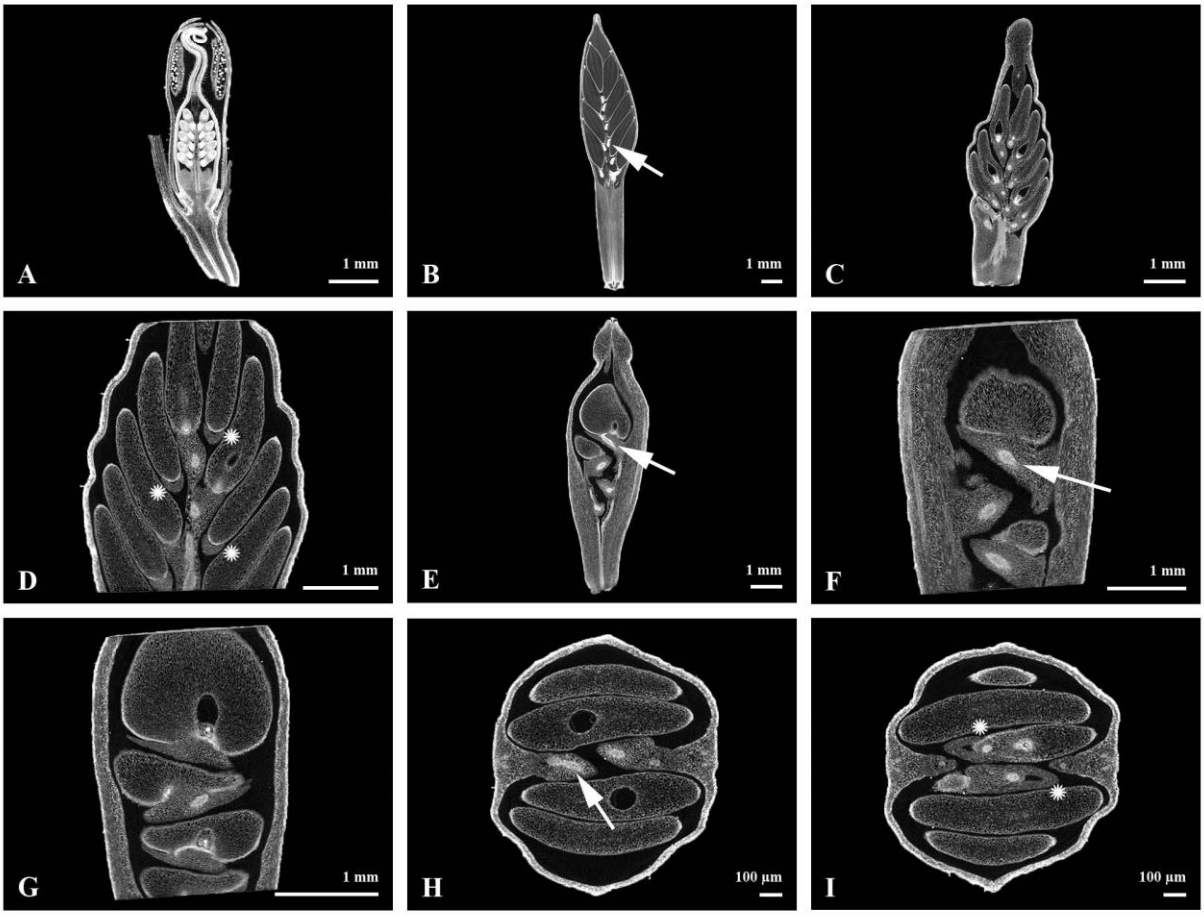

Figure 6. Acanthoideae. *Ruellia cf. brevifolia*. MicroCT. All arrows indicate beginning lignification. All stars indicate retinacula surrounding seeds. (A) Young/immature fruit. (B) Semimature fruit, longitudinal section perpendicular to septum. (C), (D) Almost mature fruit, longitudinal sections perpendicular to septum. (E) – (G) Almost mature fruit, longitudinal sections along septum. (H), (I) Cross sections of ovary locule.

3.2.2 Nelsonioideae

Investigations of the fruits of *Elytraria carolinensis* exhibited that this species possesses no retinacula (see Figure 7 B; 8; 9 A, B; 10). The funicles connecting the seeds to the placentae are short and inconspicuous without any outgrowths (Figures 9 E, F; 10 G). Each locule contains 8 – 10 irregularly shaped seeds, which are arranged in two alternating rows on the placentae (Figures 8 A; 10 C). Taking a closer look at the inner surface of dehisced fruit valves, it was revealed that there are remnants of placental connections – funicular regions – to the already missing seeds visible as slightly darker spots in the capsule valves (Figure 7 B, indicated with arrows). However, these structures (Figure 8 D, I, J; 9 E, F; 10 G) do clearly not correspond to the conspicuous, hook-like retinacula of acanthoid species. These funicular regions simply connect the seeds to the placenta during seed development.

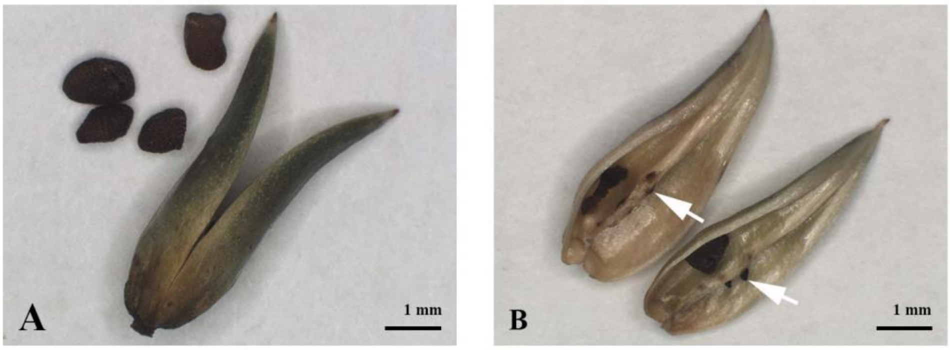

Figure 7. *Elytraria carolinensis*, Nelsonioideae. Nearly mature fruit with part of the ovules/seeds not fully developed. Stereo Microscopy. (A) Nearly mature fruit, manually opened, with seeds next to it. (B) Valves after manual opening, one seed remaining, placenta / funicular region indicated with arrows.

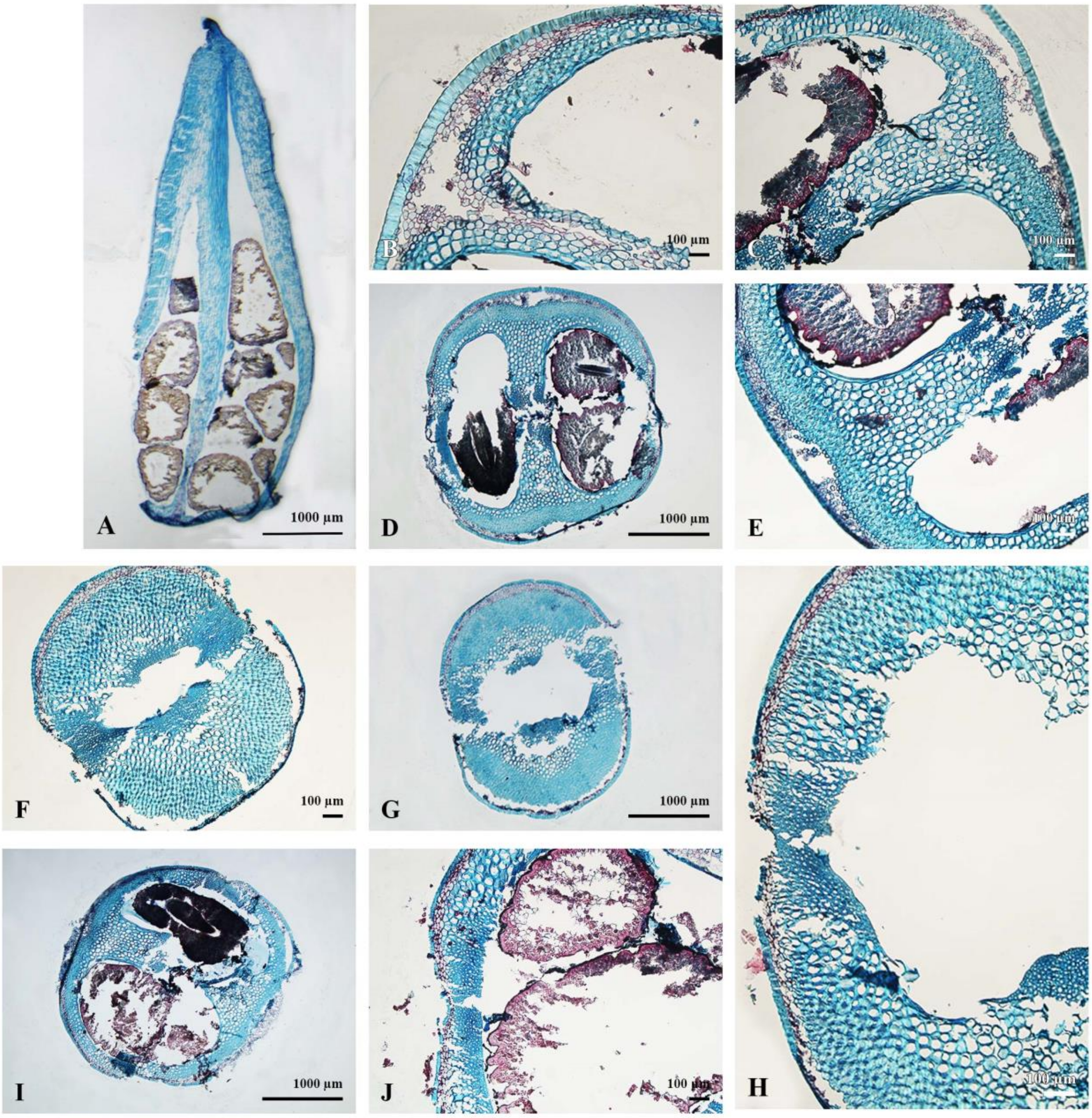

Figure 8. *Elytraria carolinensis*, Nelsonioideae. Almost mature fruit, no lignification of capsule yet. Light Microscopy. RT-stain. (A) Longitudinal section. (B) – (E) Cross sections, whole ovary locule and details of valve structure. (F) – (H) Cross sections of beak. (F) Approximately 2 mm above top-end of ovary locule. (G) Approximately 1 mm above top-end of ovary locule. (H) Transition zone between beak and locule, shortly above the two uppermost seeds. (I), (J) Almost mature fruit, cross sections of ovary locule including seeds.

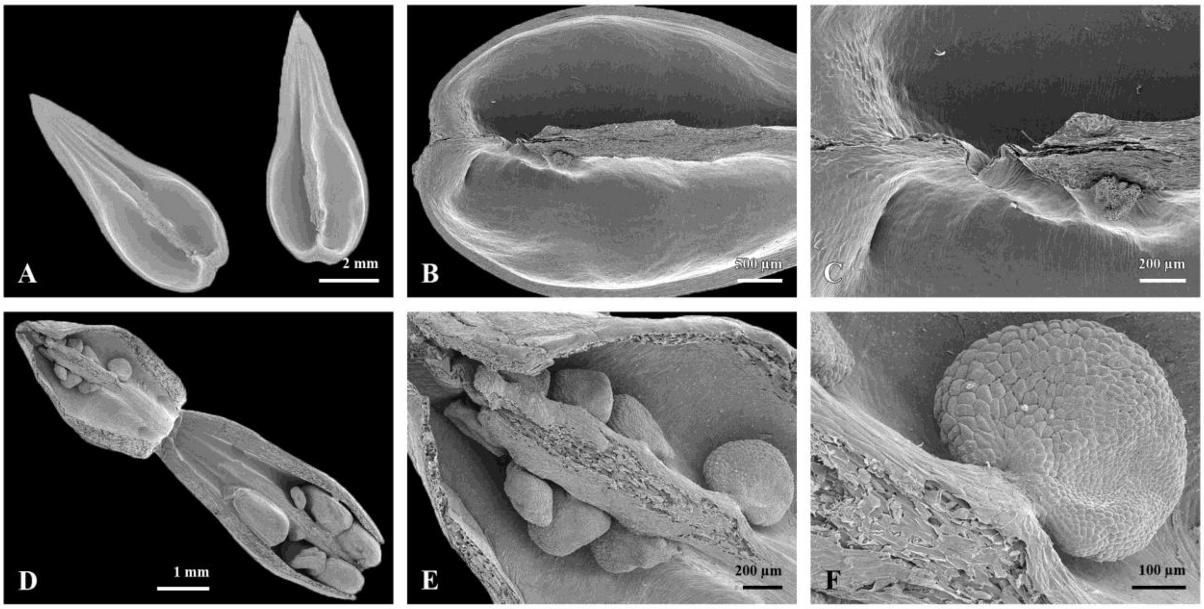

Figure 9. *Elytraria carolinensis*, Nelsonioideae. Scanning Electron Microscopy. (A) – (C) Valves after fruit explosion. (D) – (F) Nearly mature fruit with some seeds not fully developed. (F) Seed connected to placenta.

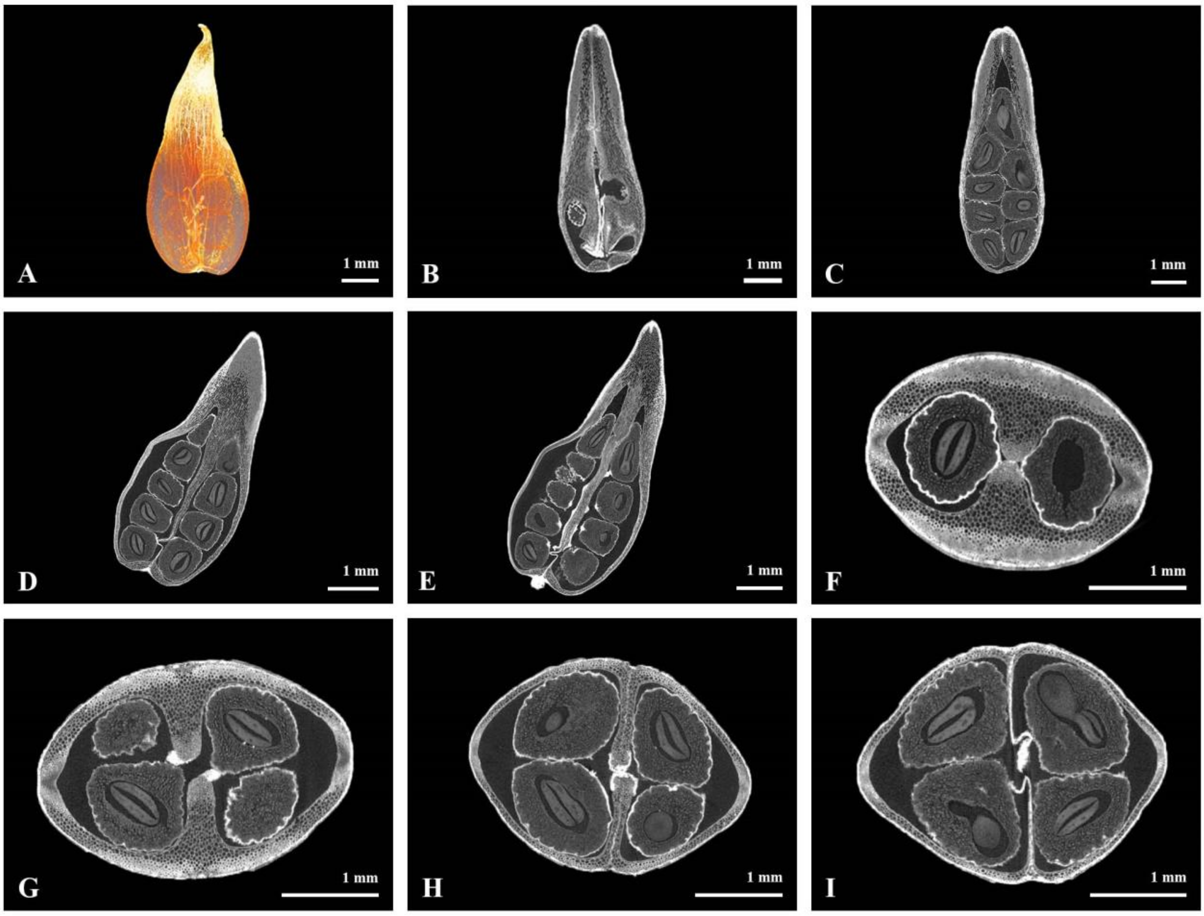

Figure 10. *Elytraria carolinensis*, Nelsonioideae. Nearly mature fruits, no lignification of capsule yet. MicroCT. (A) Semitransparent view of whole capsule. (B), (C) Longitudinal sections along septum.

(B) Inspection of septum. (C) Inspection of seeds in one valve. (D), (E) Longitudinal sections perpendicular to septum. (F) – (I) Cross sections, from two uppermost seeds to lowest seeds within ovary locule.

3.2.3 Thunbergioideae

The fruits of *Thunbergia alata* normally contain four seeds enclosed in a conspicuously globose ovary (Figure 11). Two semiglobose seeds are arranged collaterally in each locule and the ovary possesses a remarkably long, sterile and flattened apical beak (Figure 11 A). At the transition zone itself, the convex upper parts of the locules (usually two per carpel) are visible as empty areas (Figure 12 B, indicated with stars) – according to the number of seeds which will come into sight some sections further down. Apart from the beak itself, this transition zone between the beak and the ovary was also discovered to be significant for the fruit explosion due to its specific arrangements of cell layers (see Figures 12 B; 13 C; 23 K) which will be considered more closely in the next chapter (3.2.4). Furthermore, the center of the proximal end of the ovary locules (Figure 13 D) might also be of significance for the seed expulsion due to its compactness and thicker appearance compared to the rest of the valve areas surrounding the ovary locules. In immature and not yet lignified fruits, the seeds are still connected to the placenta (Figures 12 C; 14 B, D, E) but, as it was the case with *Elytraria carolinensis*, no retinacula exist in these fruits (Figures 11 A; 13 A, E).

The septum between the two carpels is present in young fruits (Figure 15 C) as well as in more mature fruits (Figure 15 H – L). In even later stages of maturity (Figure 13), it largely degenerates (see Figure 13 A, E, F, in F: indicated with arrows). The seeds, if still present, are connected to the placenta with a simple funicle (Figure 15 I, L) which, again, illustrates that the fruits of this species do not develop retinacula in any form.

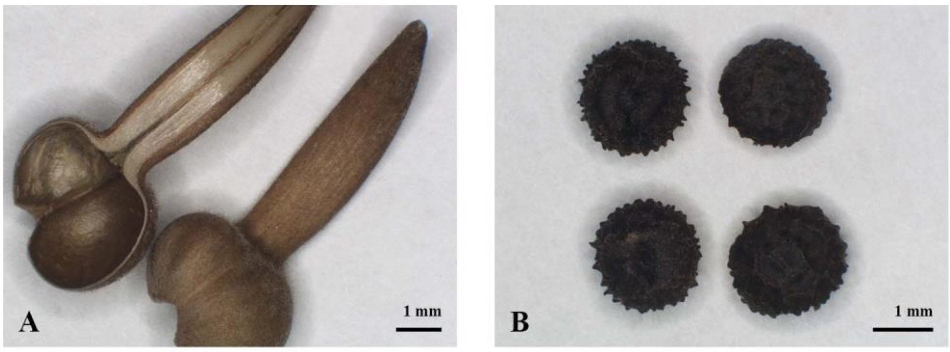

Figure 11. *Thunbergia alata*, Thunbergioideae. Stereo Microscopy. (A) Capsule valves after fruit explosion. (B) The four dispersed seeds after fruit explosion.

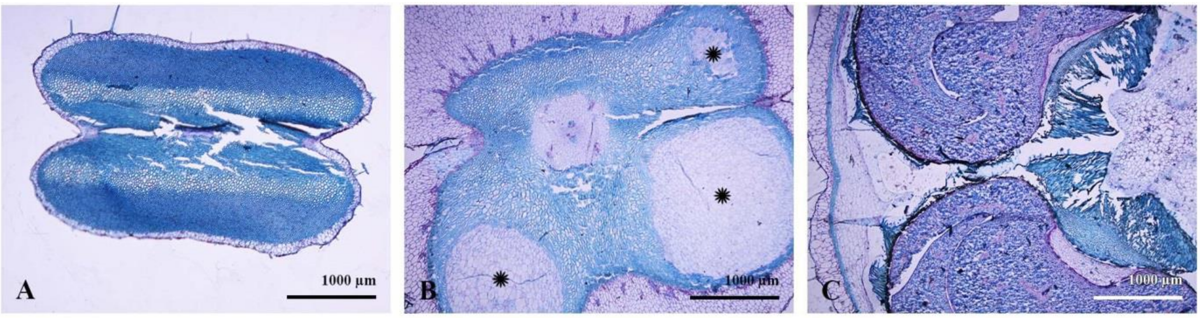

Figure 12. *Thunbergia alata*, Thunbergioideae. Almost mature fruit, no lignification of capsule yet. Light Microscopy. RT-stain. Cross sections. (A) Middle of the beak. (B) Transition zone between beak and ovary, convex upper parts of locule indicated with stars. (C) One half of the ovary locule, including two seeds.

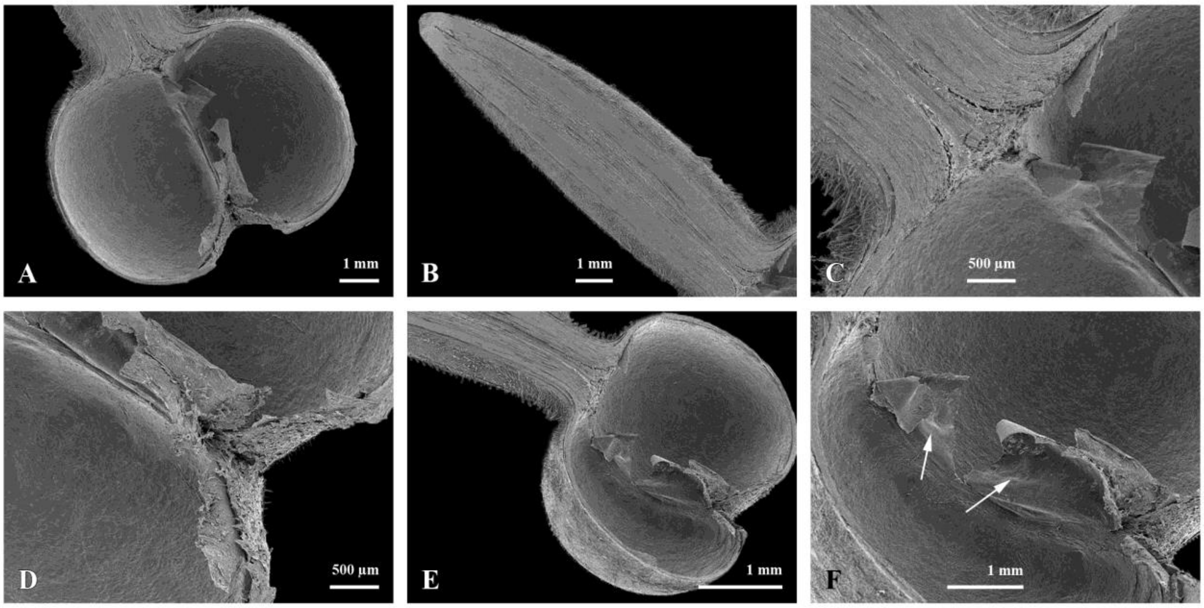

Figure 13. *Thunbergia alata*, Thunbergioideae. Capsule valve after fruit explosion. Scanning Electron Microscopy. (A) Ovary locule for two seeds. (B) Inner surface of beak structure of one valve. (C) Transition zone between beak and ovary locule. (D) Lowest central point of ovary locule. (E) Ovary locule and parts of beak from oblique view. (F) Septum, indicated with arrows.

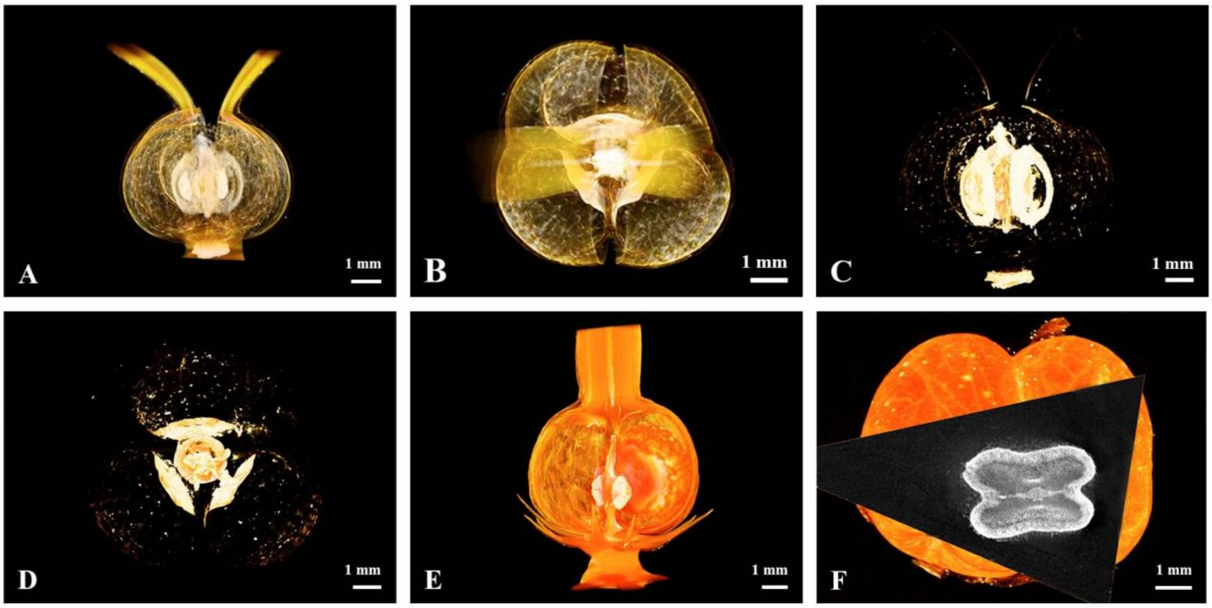

Figure 14. *Thunbergia alata*, Thunbergioideae. Almost mature fruits, already parts of capsules lignified. MicroCT. (A), (B) Semitransparent views of capsule with slight crack between valves, beak halves totally separated, lateral view and view from top (beak) downwards. (C), (D) Placenta structures in the middle highlighted, seeds and capsule surrounding almost transparent. (E) Lateral view of one valve with one seed and placenta. (F) Cross section of beak, ovary locule in background.

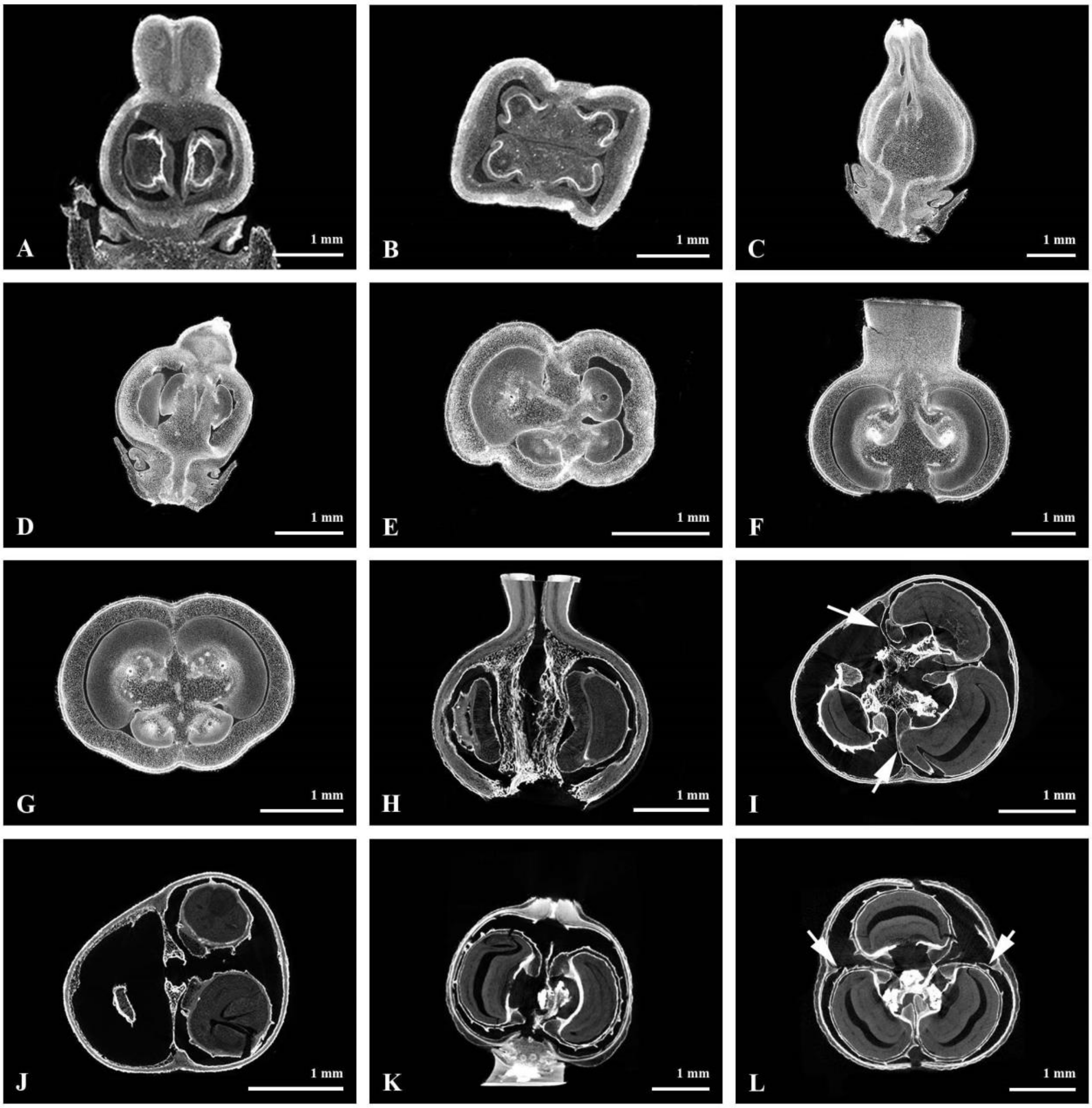

Figure 15. *Thunbergia alata*, Thunbergioideae. MicroCT. (A), (B) Young, immature fruit, longitudinal and cross section, focus on early seed development. (C) Immature fruit, longitudinal section along septum. (D) Immature fruit, longitudinal section. (E) Immature fruit, cross section, small abnormality: one seed more developed than the others. (F) More mature fruit, longitudinal section, detail of two seeds connected to placenta. (G) More mature fruit, cross section, small abnormality: two seeds more developed. (H) – (L) Almost mature fruits, already parts of capsule lignified. (H) Longitudinal section, crack of valves visible. (I), (J) Cross section of ovary locule, only three seeds developed. (I) Septum still present and indicated with arrows. (J) Septum still present. (K) Longitudinal section of ovary locule and transition zone between beak and ovary locule. (L) Cross section of ovary locule, only 3 seeds developed, only remnants of septum visible and indicated with arrows.

3.2.4 Comparative analysis of Acanthoideae, Nelsonioideae and Thunbergioideae

As mentioned above, a retinaculum is only present in the fruits of the subfamily Acanthoideae. Among representatives of this subfamily, shape and size of retinacula differ conspicuously in spite of their overall identical basic structure (Figures 16 A, B, D – I; 18). In the supplementary materials of this study, one can find an animated display of a seed resting on its retinaculum (given in Figure 18) in order to visualize these unique fruit structures in another, even more detailed illustration (https://phaidra.univie.ac.at/detail_object/o:424298). Nonetheless, differences in size and shape of the retinacula are correlated with the differences in seed size between the individual species. The smallest (0.8 mm in diameter) are found in *Ruellia blumei* (Figure 2 J); here the retinacula are 0.5 mm long and slender and acute in shape. At the other end of the range is species *Justicia furcata* with a seed diameter of up to 3 mm; here there are also distinct differences in the retinacula’s size and shape. They can be 2.5 mm long, are vertically thicker in shape and surround their corresponding seeds to a greater extent (Figures 2 F; 4 F; 16 G). At the same time, they still have fundamental similarities such as the rudimental seed-embracing shape, the indentation in which the seeds reside (Figure 16 F, G) and, finally, the fact that they remain on the capsule valves after the explosive seed dispersal has taken place (see for example Figure 16 B, D, J, L). Moreover, the attachment point where the seeds were connected to the retinacula before reaching absolute maturity is always at the proximal-most point on the within the inner (upper) surface of the retinaculum (Figure 4 G, indicated with an arrow). Capsule valves of *Schaueria calicotricha* proved to be useful samples for this investigation due to their rather big retinacula (Figure 16 F, indicated with arrows). At the anatomical level, the retinacula consist of mainly two different tissue types – a mostly already lignified area with tightly packed cells close to the attachment point of the seed (Figures 16 C, indicated with a star; 17 A, E – H, some indicated with arrows) and more loosely arranged cells in the remaining parts of this structure (Figure 17 E – H).

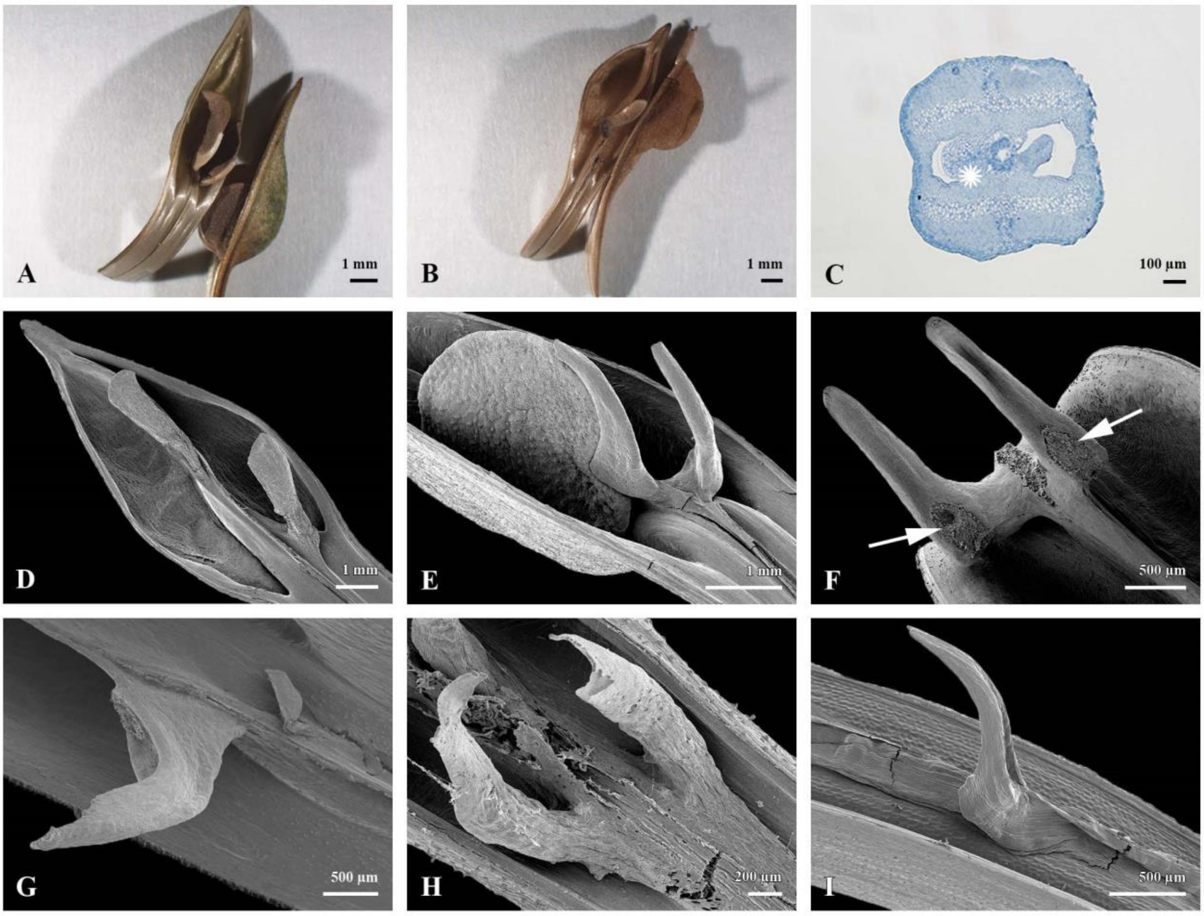

Figure 16. Acanthoideae. Retinacula. (A), (B) Stereo Microscopy. (C) Light Microscopy. (D) – (I) Scanning Electron Microscopy. (A) *Schaueria calicotricha*, mature fruit after manual opening of valves. (B) *Justicia furcata*, valves after explosion. (C) *Ruellia cf. brevifolia*, cross section of almost mature fruit, lowest two retinacula within ovary locule, lignifications indicated with a star. (D) *Ruellia cf. tonduzii*, one valve, view of ovary locule. (E) *Schaueria calicotricha*, valve with a seed remaining on its retinaculum. (F) *Schaueria calicotricha*, two retinacula, view from above / beak downwards, seed attachments indicated with arrows. (G) *Justicia furcata*, retinaculum in detail. (H) *Ruellia cf. brevifolia*, two retinacula in detail, view from below. (I) *Brillantaisia owariensis*, retinaculum in detail.

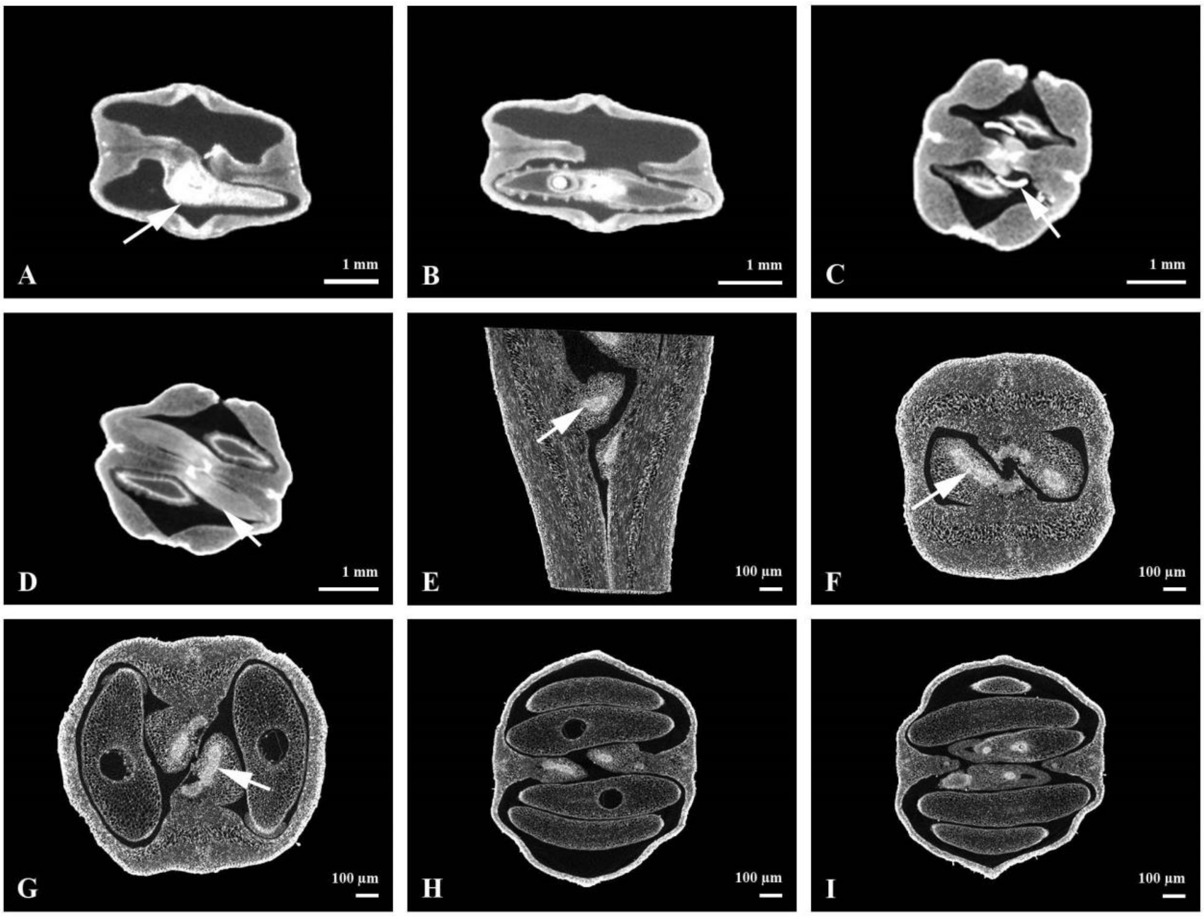

Figure 17. Acanthoideae. Retinacula. MicroCT. (A), (B) *Schaueria calicotricha*, cross sections of retinacula and seed embraced by retinacula (on the right). (C), (D) *Justicia furcata*, cross sections of retinacula and seeds. (E) – (I) *Ruellia cf. brevifolia*. (E) Longitudinal section of lowest part of ovary locule, lowest three retinacula. (F) – (I) Cross sections from lowest two retinacula two middle of ovary locule. All arrows indicate lignifications in retinacula structure.

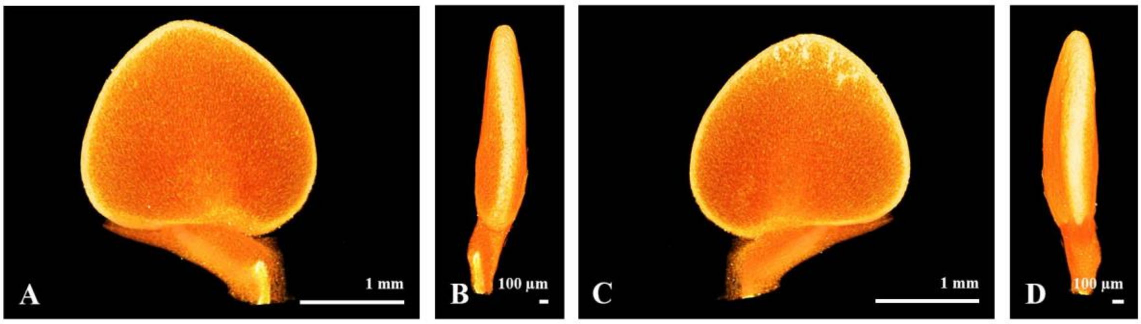

Figure 18. *Ruellia cf. brevifolia*, Acanthoideae. Retinacula. MicroCT. (A) – (D) Segmentation of one seed resting on its retinaculum, four different perspectives (always turning 90° clockwise).

As already mentioned above, *Elytraria carolinensis* and *Thunbergia alata* do not feature retinacula, which is illustrated in Figure 19, in which no such structures can be found.

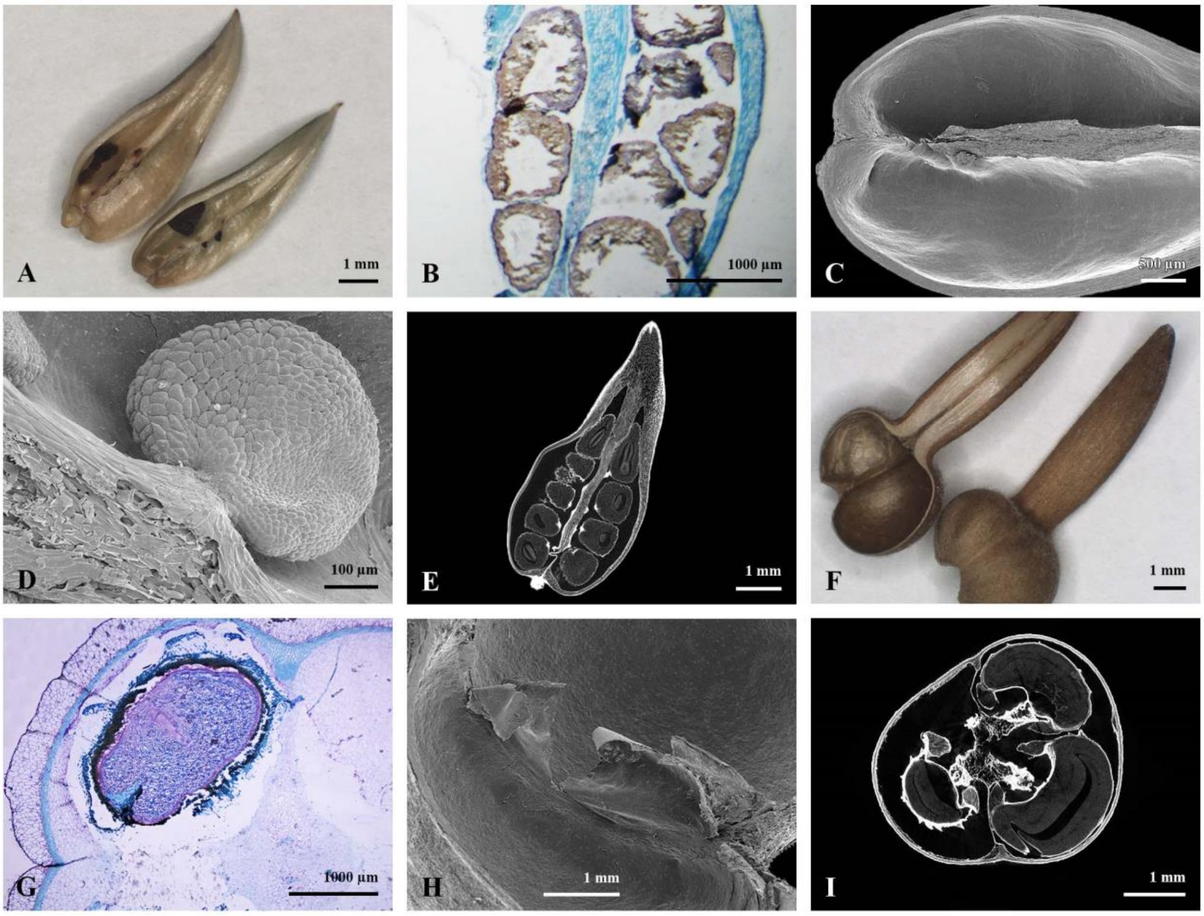

Figure 19. Taxa without retinacula. (A) – (E) *Elytraria carolinensis*, Nelsonioideae. (F) – (I) *Thunbergia alata*, Thunbergioideae. (A) Stereo Microscopy, inner surfaces of valves. (B) Light Microscopy, longitudinal section of ovary locule. (C) Scanning Electron Microscopy, smooth inner surface of ovary locule. (D) Seed connected to placenta. (E) MicroCT, longitudinal section. (F) Stereo Microscopy, valves after explosion. (G) Light Microscopy, cross section of ovary locule, abnormality: only one seed developed. (H) Scanning Electron Microscopy, smooth inner surface of ovary locule. (I) MicroCT, cross section of ovary locule, abnormality: only three seeds developed.

Another significant structure within acanthaceous fruits appears to be the beak, which is present in most of the species studied here, although its shape and length varies greatly from one species to another. In all cases, the beak corresponds to the distal-most part of the ovary and not to the style (Figure 20). In *Ruellia cf. brevifolia*, the beak is relatively short (ca. 1.5 mm; Figure 20 C) and at fruit maturity it is composed of relatively tightly packed cells that are smaller in the center of the beak than in its periphery (20 D – F). Already in immature fruits, the line of dehiscence between the two fruit valves is clearly visible (20 E, F; 21 A –

F). The ovary locules extend into the proximal part of the beak (20 C, D; 21 D). The beak structure of the other acanthoid species studied here is basically similar. Differences worth mentioning are found in *Justicia cf. pectoralis* (Figure 2 A) and *Justicia furcata* (Figure 2 F). In these two species, the beaks appear to be of approximately the same length compared to *Ruellia cf. brevifolia* but the transition zones between the ovary locules and the beaks appear to be more abrupt – as if the beaks were truly distinct parts of the capsules. *Schaueria calicotricha* (Figures 2 D; 4 C; 20 A) seems to present a mixture of these two extremes, especially when comparing *Ruellia cf. brevifolia* (Figure 2 G) to *Justicia furcata* (Figure 2 F). In this comparison, *Schaueria calicotricha* appears to display some kind of transition form of these two contrasting capsule shapes.

The beak of *Elytraria carolinensis* presents a similar picture because it is also simply an apical continuation of the locules and there is also a hollow space between the capsule valves of these fruits (Figure 21, G – J). Nonetheless, at the anatomical level, the beaks' tissues appear to be more differentiated in the capsules of *Elytraria carolinensis* (Figures 20 G – I; 23 H) when comparing them to the tissues of acanthoid beaks (Figures 20 B, C; 23 D).

Whereas the acanthoid beaks appear at the first sight to be organized in rather two cell tissues – large cells at the periphery and smaller and more compact cells towards the center of the beak (Figure 20 E, F; 21 B; 23 D) –, the cell tissues of the beaks of *Thunbergia alata* revealed to be organized in three distinguishable areas (Figure 20 M, N; 22 A – C; 23 L). The outermost tissue consists of small and tightly packed cells (Figure 22 A, indicated with stars), the next tissue displays an arrangement of bigger cells (Figure 20 M, N; 22 A, darkest areas), and the innermost tissue is an area of small and compact cells which are arranged more loosely than the ones of the outermost layer (Figure 20 M, N). Moreover, it was revealed how the distribution of the different tissue types in *Thunbergia alata*'s beaks changes from the top-end towards the locules (Figure 22 A – E). The distal-most part of the beak seems to portray a rather equal distribution of all three layers, only with the tissue in the middle being slightly more narrow (Figures 20 M, N; 23 L). This distribution changes towards the mid-section were the middle tissue with its larger cells takes over more space resulting also in a narrowing mainly of the innermost layer (Figure 22 A, D, indicated with arrows). Close to the ovary, this change in the distribution is continued while, furthermore, the more compact and lignified outer cell tissue appears to be marginalized due to the rather unconstrained and less lignified cells of the middle tissue taking over more space around the center of the fruits (Figure 23 K). In more detail, the outermost layer alters from being just along two lengths of the pericarp to surrounding the whole ovary locule along the entire inside of the pericarp in a narrow strip of cell tissue (strongly stained areas in Figure 20 O; bright areas in Figure 22 A, E, indicated with stars; Figure 23 K). Another perspective to the cell structure of the beak exhibited that the hollow space in the middle of the beak is present until it meets the apical end of the ovary locule, as it is an extension of the locule (Figure 22 F – I). Further investigations uncovered that the density of the cells changes from the periphery to the center of the beak (Figure 22 J – L) – in other words, the density decreases while the number of intercellular spaces increases.

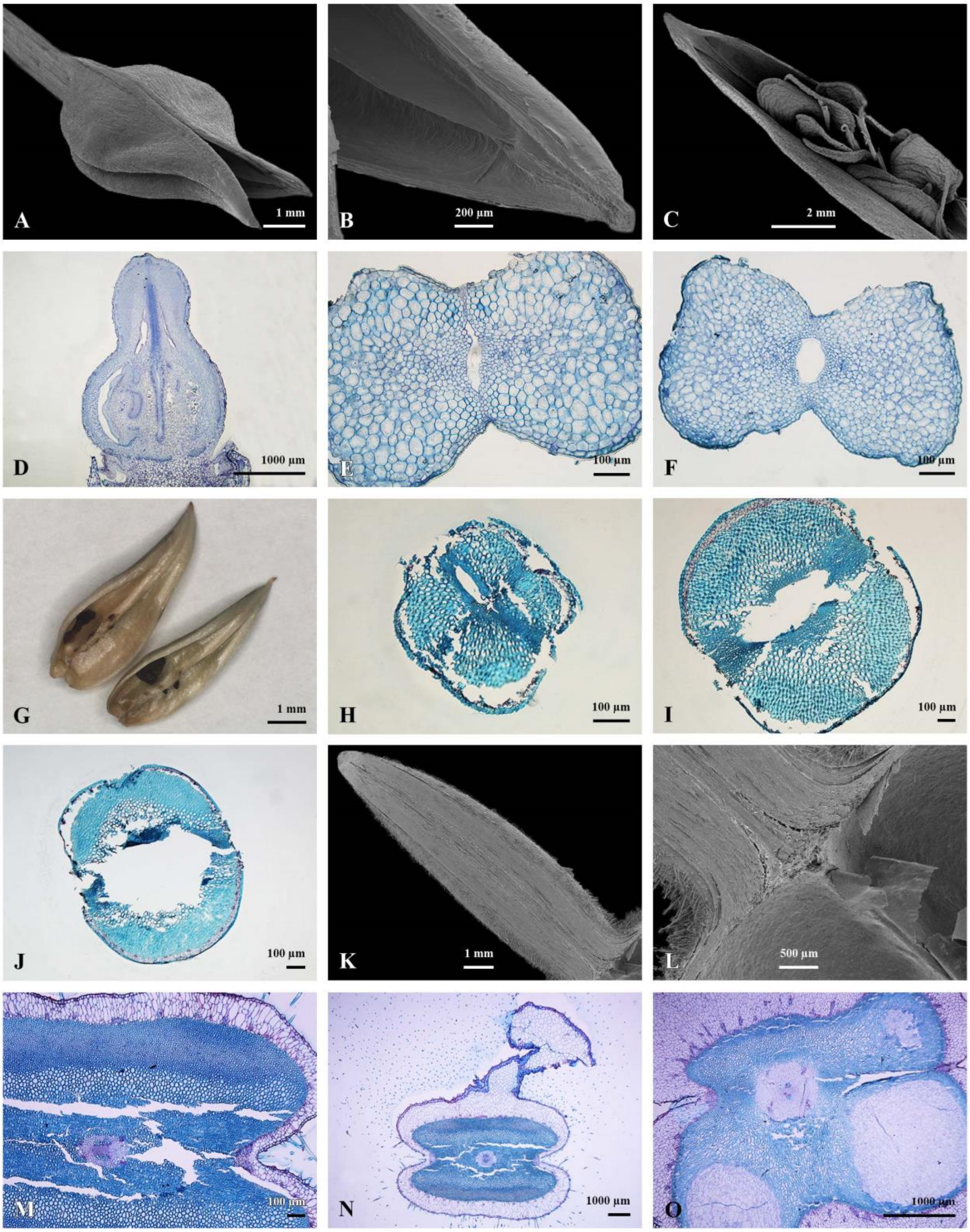

Figure 20. Apical beaks, at different stages of maturity. (A) – (F) Acanthoideae. (G) – (J) *Elytraria carolinensis*, Nelsonioideae. (K) – (O) *Thunbergia alata*, Thunbergioideae. (A), (B) *Schaueria calicotricha*, Scanning Electron Microscopy, mature fruit, view of ovary locule with beak and inner surface of one beak half in detail. (C) – (F) *Ruellia cf. brevifolia*. (C) Scanning Electron Microscopy, mature fruit, view of seed chamber with inner surface of one beak half. (D) – (F) Light Microscopy. (D) Longitudinal section of immature fruit with beak at top-end. (E), (F) Cross sections of beak of nearly mature fruit, approaching seed chamber. (G) Stereo Microscopy, view of inner surfaces of mature capsule valves with beak. (H) – (J) Light Microscopy, cross sections of beak of nearly mature fruit, approaching ovary locule. (K), (L) Scanning Electron Microscopy, mature fruit. (K) Inner surface of one beak half. (L) Transition zone between beak and ovary locule in one valve. (M) – (O) Light Microscopy, nearly mature fruit, cross sections of beak, approaching ovary locule.

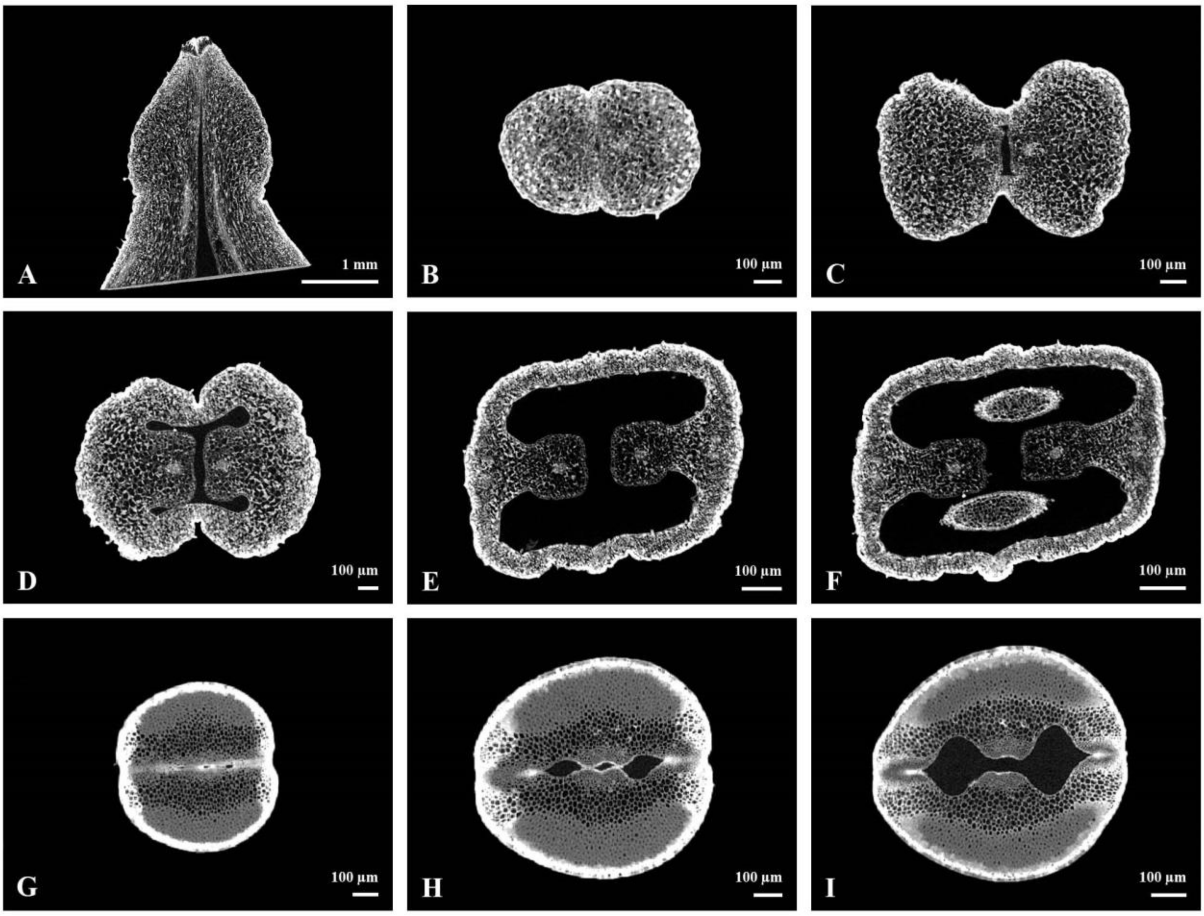

Figure 21. Apical beaks of nearly mature fruits. MicroCT. (A) – (F) *Ruellia cf. brevifolia*, Acanthoideae. (G) – (I) *Elytraria carolinensis*, Nelsonioideae. (A) Longitudinal section, hollow space between valves. (B) – (F) Cross sections, approaching ovary locule with two uppermost seeds, hollow space develops into ovary locule. (G) – (I) Cross sections, approaching ovary locule, hollow space develops into ovary locule.

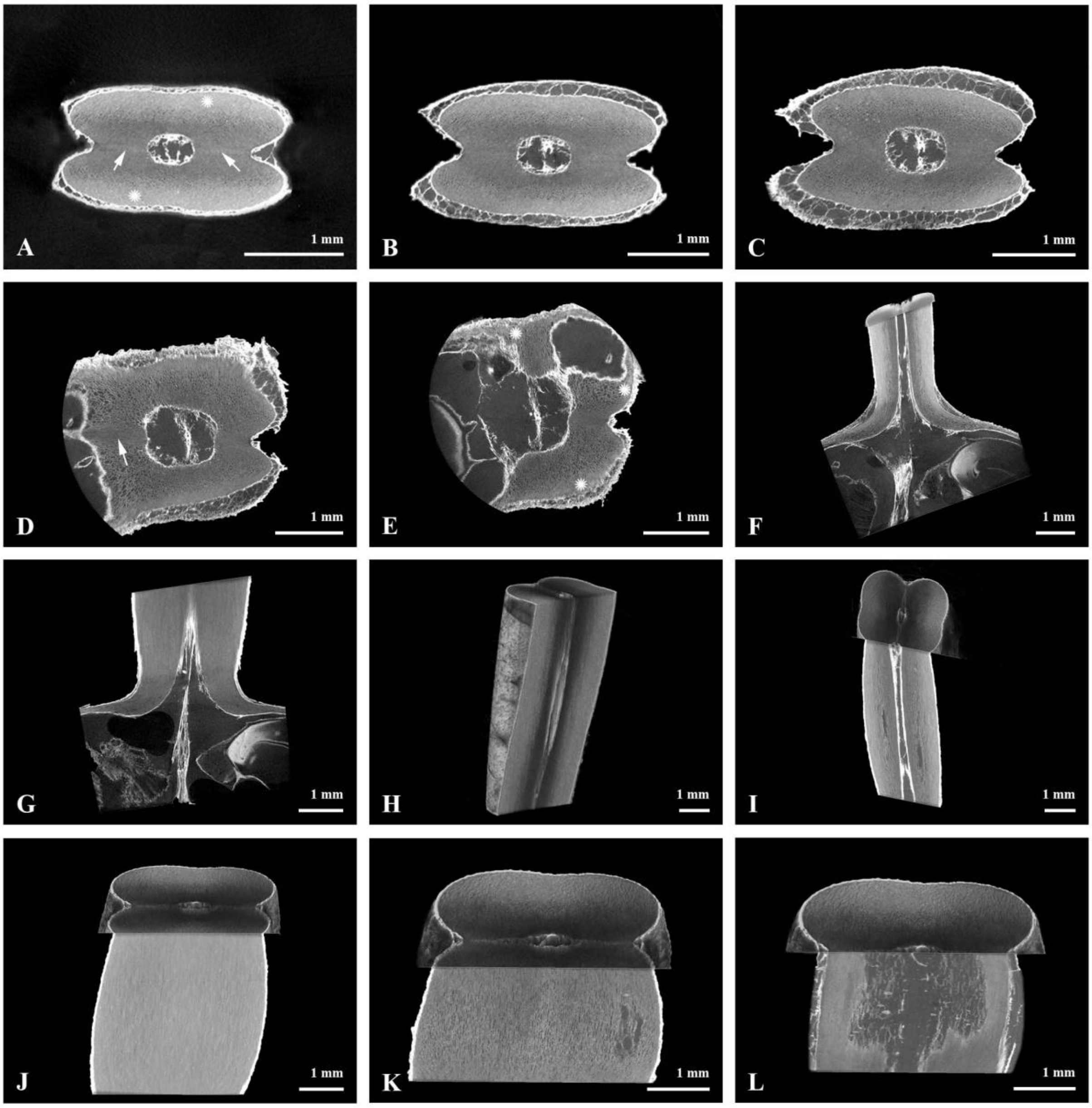

Figure 22. Apical beaks of a nearly mature fruit. MicroCT. *Thunbergia alata*, Thunbergioideae. (A) – (E) Cross sections, approaching ovary locule. (F) – (L) Longitudinal sections. (F), (G) Transition zone between beak and ovary locule, perpendicular to septum. (H), (I) Beak itself, perpendicular to septum. (J) – (L) Beak itself, along septum, approaching meeting point of beak halves.

Finally, there are specific tissue layers present in all three subfamilies. However, their most prominent displays are located at different areas within the fruits. In Acanthoideae, they are best visible in the lower parts of the locules. More specifically, one can see them most clearly in cross sections, which are exactly located at the two lowest retinacula, as the schematic drawing of an Acanthoideae cross section in Figure 23 B shows. The outermost layer (Figure 23, depicted in medium gray) can also be called the ‘active layer’ (according to Witztum & Schulgasser 1995) and consists of rather small, compact and closely arranged cells. The next layer, which is mostly a rather narrow strip of tissue (Figure 23, depicted in bright gray), is generally referred to as the ‘intermediate layer’. It consists of larger and more loosely arranged cells and is always located between the active layer and the ‘resistance layer’. This third and final layer of importance is located even closer to the center of the fruit (Figure 23, depicted in dark gray) and appears to smoothly fade into the retinacula due to the fact that the cells do not change their close and narrow composition at the retinacula attachment points. Figure 23 C and D show that this specific cell structure can be detected in other areas of the fruits as well but they are not traceable in such a clear manner due to the fact that the overall shape of the fruit changes from its base to its tip, and thus, also its cell composition changes.

These three types of cell layers were also detected in *Elytraria carolinensis*, but instead of showing their biggest extensions in the basal part of the ovary locule as in the fruits of Acanthoideae, it is rather located at the apical end of the ovary locules (Figure 23 G). In this species, one can discover the same three layers but they differ in their extension and distribution (Figure 23 G). The intermediate layer is broader and bigger in the fruits of *Elytraria carolinensis*, whereas the other two layers appear to be slightly narrower in shape. One can also find all three layers in cross sections of the middle of the ovary locule (Figure 23 F) and the beak of the fruits (Figure 23 H), but not as clearly identifiable as at the two highest seeds, and thus, close to the top-end of the ovary locule (as in Figure 23 G).

In *Thunbergia alata*, the active, the intermediate and the resistance layer were most distinctly found in the beaks of the fruits (Figure 23 L). Detection of these three layers in other areas of the fruits was difficult but the results can be seen in Figure 23 J and K. In the middle of the ovary locule (Figure 23 J), the active layer surrounds all four seeds in a nearly perfect circle, while the intermediate and the resistance layer are only located at the two connation points of the two carpels. Considering the layers’ positions in the transition zone between the ovary locule and the beak (Figure 23 K), the active layer appears to constitute rather two tissue circles. Furthermore, the intermediate layer occupies more space at this part of the fruit, while the resistance layer is still rather limited to a narrow space in the middle of the cross section.

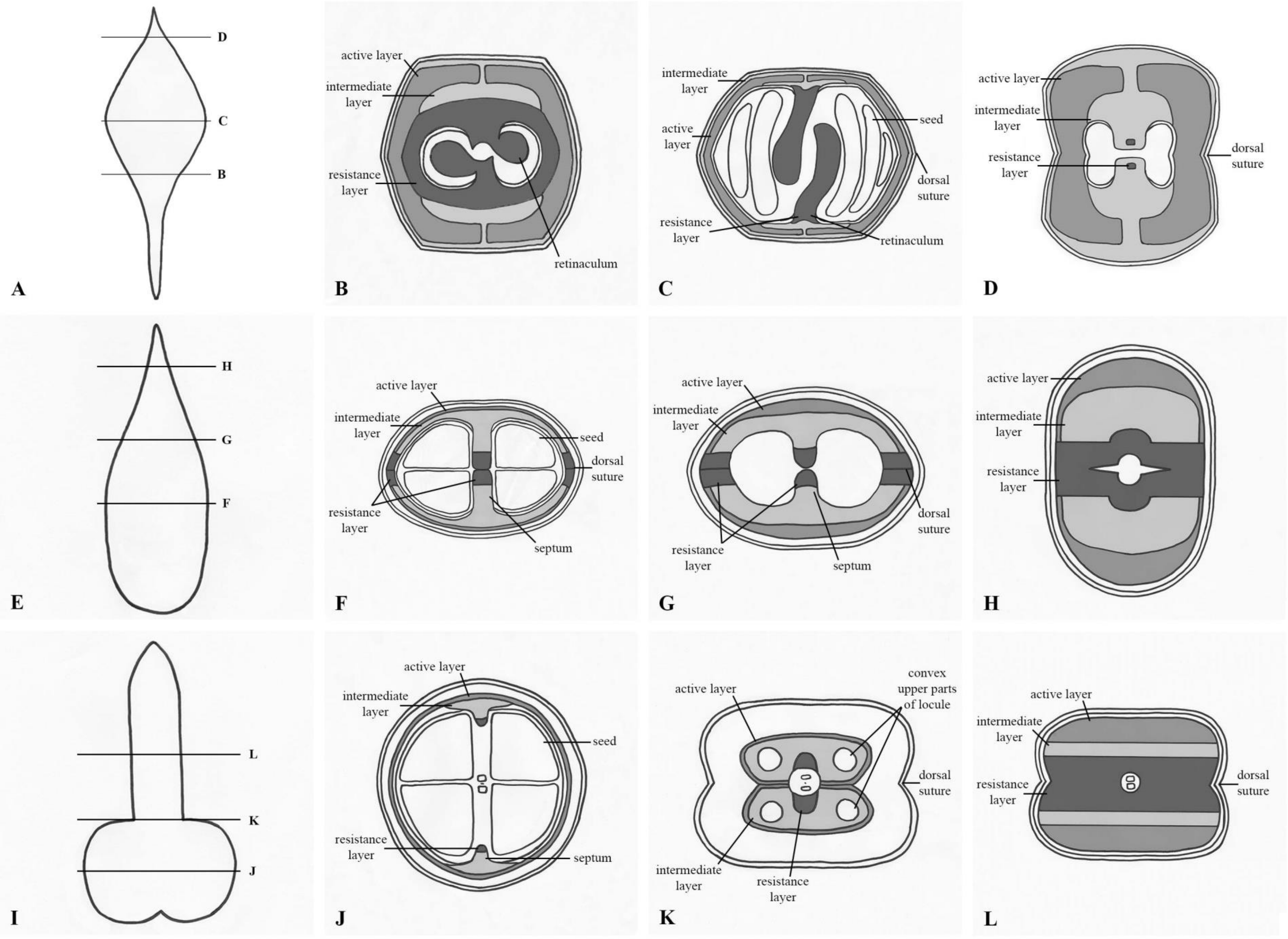
A
D
C
B
B
active layer
intermediate layer
resistance layer
retinaculum
C
intermediate layer
active layer
seed
dorsal suture
resistance layer
retinaculum
D
active layer
intermediate layer
resistance layer
dorsal suture
E
H
G
F
F
active layer
intermediate layer
seed
dorsal suture
resistance layer
septum
G
active layer
intermediate layer
resistance layer
septum
dorsal suture
H
active layer
intermediate layer
resistance layer
I
L
K
J
J
active layer
intermediate layer
seed
resistance layer
septum
K
active layer
convex upper parts of locule
dorsal suture
intermediate layer
resistance layer
L
active layer
intermediate layer
resistance layer
dorsal suture

Figure 23. Schematic drawings. Cross sections. (A) – (D) Acanthoideae. (E) – (H) Nelsonioideae. (I) – (L) Thunbergioideae. (A) Whole capsule, the approximate levels of the cross sections given in (B) – (D) are indicated with lines. (B) Lowest point of ovary locule, first two retinacula visible. (C) Middle of ovary locule, fully developed retinacula and seeds. (D) Beak. (E) Whole capsule, the approximate levels of the cross sections given in (F) – (H) are indicated with lines. (F) Middle of ovary locule, four seeds visible. (G) Highest point of ovary locule (shortly above highest seeds), beginning of beak structure. (H) Beak. (I) Whole capsule, the approximate levels of the cross sections given in (J) – (L) are indicated with lines. (J) Middle of ovary locule, all four seeds visible. (K) Transition zone between ovary locule and beak. (L) Beak.

3.3 Explosive seed dispersal and High-speed video recording

Although approximately 50 fruits were collected for high-speed video recording of their explosive dehiscence, only 13 explosions were in the end videotaped. Eleven of these explosions occurred in the species *Ruellia cf. tonduzii*, one in *Brillantaisia owariensis* and one in *Ruellia cf. brevifolia*. In addition, I was able to observe some fruits of *Elytraria carolinensis* explode by eye, as well as several fruits of *Thunbergia alata*. Unfortunately, none of these explosions could be filmed. *Brillantaisia owariensis* (Acanthoideae) proved to be another interesting representative of Acanthaceae during this experiment due to its fast capsule explosions; these capsules always exploded on the way to the experimental setting. The seed expulsions observed during this experiment, whether in front of the camera or not, resulted in seed dispersal up to five meters away from the mother plant. In more detail, only the seeds of Acanthoideae were ejected for approximately five meters; the seeds of *Thunbergia alata* reached a distance of roughly three meters, whereas *Elytraria carolinensis*' seeds flung away only about one meter.

4. Discussion

This chapter is structured according to the three research questions determined at the beginning of this thesis and will finish with a short depiction of which subfamilies' explosive opening mechanism evolved earlier when considering their phylogenetic relationships.

4.1 The explosive seed dispersal of all three subfamilies

First of all, the first research question specified in the introduction, namely, whether explosive seed dispersal can be found in all three subfamilies of Acanthaceae, should be answered here. After previous researchers clarified the phylogenetic relationships between these three subfamilies, which had been highly controversial for a long time beforehand (Borg 2012), the explosive seed dispersal in these related subfamilies was another fascinating aspect of Acanthaceae which still remained largely unexplored. It was often argued that only species of Acanthoideae are capable of featuring explosive fruit opening mechanisms (Scotland & Vollesen 2000; McDade et al. 2000) due to their exceptional fruit type within which the seeds are positioned on retinacula. According to Scotland and Vollesen (2000), the lack of these retinacula in Nelsonioideae and Thunbergioideae is the ultimate reason for these subfamilies to be deficient in explosively opening capsules. However, this argument was already refuted by other researchers stating that probably all subfamilies may have species with explosive seed dispersal (McDade et al. 2008, Borg & Schönenberger 2011). In other words, also Nelsonioideae may include at least some species which disperse their seeds explosively, although they lack retinaculate fruits (Daniel & McDade 2014). Furthermore, it had already earlier been mentioned that at least some species of Thunbergioideae display explosive dehiscence in their fruits without the hook-like retinacula (Schönenberger 1998, Borg et al. 2008). Therefore, the retinacula are most likely not causing the capsules to explode but rather enable the Acanthoideae to achieve greater dispersal distances. More details concerning the retinacula will be discussed in the next chapter (4.2.1). Subsequently, these still rather vague findings can now be supported by the study at hand due to the fact that capsule explosions were observed in species of all three subfamilies. As described in the results section on the experiment utilizing high-speed video recording, the observation of explosions of several Acanthoideae species, as well as explosions of *Elytraria carolinensis* (Nelsonioideae) and *Thunbergia alata* (Thunbergioideae) were realized within this experiment and some of these explosions were also captured with the high-speed video camera. Therefore, not only the subfamily Acanthoideae features the explosive mechanism for effective and far-reaching seed dispersal in its fruits, which was already known and described in the earlier literature, but also the other two subfamilies, Nelsonioideae and Thunbergioideae, are capable of this form of seed dispersal. Obviously, these three subfamilies display different strategies and mechanisms within its fruit structures to enable an explosion, and these differing tissue structures, which in the end showed to have more similarities than expected, are also discussed in this study (see 4.2.3). Unfortunately, the videos recorded with this camera (Nikon DS-Fi1) do not feature a high resolution; however, a video showing the explosions of four acanthoid capsules (one of *Brillantaisia owariensis*, three of *Ruellia cf. tonduzii*) can be found in the supplementary materials of this thesis (https://phaidra.univie.ac.at/view/o:426865). For future research, it should be mentioned that other cameras will most likely feature different – maybe more suitable – settings for high-speed video recordings and should therefore be taken into consideration. For example, Cross (2013) conducted her high-speed recordings of exploding Acanthaceae with a Redlake HG-XR high-speed video camera which enabled her movies to feature up to 9860fps. This camera is even capable of capturing up to 100000fps, and thus, appears to be a highly useful device for shooting high-speed movies. Probably another suitable camera is the X-PRI high-speed camera by AOS Technologies, which is used in capsule explosion experiments conducted by Hayashi et al. (2010). In general, the studies by Cross (2013) and Hayashi et al. (2010) were of particular relevance to this study because they both provide detailed descriptions of their video-recording settings, and these were of great help. Moreover, Cross (2013) or also Witztum and Schulgasser (1995) illustrate enlightening picture sequences taken from high-speed video recordings, which should be taken into account if similar experiments were to be reproduced or also if one simply desires to see an illustration of an actually occurring capsule explosion summarized in some pictures.

Subsequently, this experiment showed, although its effective outcome in form of videos is incomplete, that representatives of all three subfamilies of Acanthaceae are capable of explosive seed dispersal. Nonetheless, what should also be shortly mentioned at this point is the fact that not all acanthaceous fruits are woody and loculicidal capsules that are capable of exploding. The genus *Avicennia*, for instance, possesses asymmetric capsules (Borg & Schönenberger 2011). In addition, there is even another fruit type present in species of Acanthaceae, namely, fleshy drupes as in the Thunbergioideae genus *Mendoncia* (Borg & Schönenberger 2011; Borg 2012).

4.2 Morphological structures and tissues aiding seed expulsion

4.2.1 The retinaculum

The retinaculum is the first morphological structure which should be discussed here due to its prominence in earlier studies and the controversies it elicited. First of all, this hook-like structure is only present in fruits of Acanthoideae (see for example Witztum & Schulgasser 1995; or see Figures 16 – 18). Nevertheless, this structure is neither triggering nor affecting the explosion of these acanthoid capsules but rather extending the ejection of the acanthoid seeds away from their mother plants to several meters due to its lever-like shape (Borg et al. 2008), as well as simply influencing the trajectory (Ulbrich 1928). This is important to mention because it refutes the argument saying that Nelsonioideae and Thunbergioideae are not exploding only because of their lack of retinacula, as stated by Scotland and Vollesen

(2000), for two reasons. Firstly, this is in fact disputable because they do explode – as described in the discussion above; and secondly, as shown here, they do have explosive capsules although they possess no retinaculate fruits.

Turning back to the retinaculum itself, its origin and development should be depicted shortly. Analyses showed that retinacula derive from lignified, elongate funicles (Corner 1976; McDade et al. 2000; Scotland & Vollesen 2000; McDade & Tripp 2007; McDade et a. 2008). On account of these structures, retinacula are connected to the placentae in the center of the capsule until and beyond the fruit explosion occurs (Putzer 2015). This origin can be supported by the fact that retinacula are only fully developed if their corresponding seeds reach maturity (Wortley et al. 2005). Therefore, the retinacula are most likely derivatives of funicles because these outgrowths are associated with the actual seeds and not the placenta, which would still display normal development although the seeds might have been aborted (Wortley et al. 2005). The actual attachment points of the seeds on the retinacula furthermore reinforce the hypothesis that a retinaculum actually corresponds to a funicle (see Figures 4 G; 16 F).

Comparing retinacula to the structures connecting the seeds of *Elytraria carolinensis* to their placentae, a significant difference can be easily detected. The only connections visible between the seeds and the placenta of this Nelsonioideae species are remnants of funicular regions (Figures 7 B; 19 A). This can be asserted due to their different shape and the fact that they are still present in order to sustain the developing seeds and not to form some kind of catapult for them as the acanthoid retinacula do (see Figures 9 F; 10 G; 19 D). Also *Thunbergia alata* as representative of Thunbergioideae, (Figures 11 A; 19 F) only has connections between the seeds and the placentae during their maturation, just as the seeds of *Elytraria carolinensis* (Figures 15 E, G; 19 I).

Another aspect one might think of when comparing Acanthoideae and their retinaculate fruits with *Elytraria carolinensis* and *Thunbergia alata*, which lack retinacula, is the distance the seeds are dispersed during fruit explosion. Do the retinacula significantly affect the dispersal distance? The seed expulsion experiment conducted within this study showed that Acanthoideae can fling their seeds away up to five meters. This corresponds to the observations made by Cross (2013) who found out that some *Ruellia* and *Yeatesia* seeds also reach a flight distance of approximately five meters, as well as Witztum and Schulgasser (1995) who observed *Ruellia* seeds flying up to three meters, and finally Ulbrich (1928) who even argued that seeds of *Acanthus* are flung away for about nine meters. This far-reaching launch distance might be positively affected by the retinacula which act as jaculator during the ejection of the seeds. However, the seed distribution of *Thunbergia alata* was also reaching roughly three meters. Therefore, one should be cautious hypothesizing that only Acanthoideae seeds can fly long distances within this plant family. In other words, although the acanthoid seeds' range was obviously greater, *Thunbergia alata* seeds probably have evolved another effective strategy for ejecting their seeds several meters away. It could be assumed that the beak is of great importance in this species; thus, this fruit structure will be discussed in the next chapter (4.2.2). The seeds of *Elytraria carolinensis* did also fly away for a significant distance, but they did not exceed a range of approximately one meter. These nelsonioid fruits might have developed another structure that enables far-reaching dispersal distances of their seeds. Therefore, the comparatively long flight distance of the acanthoid seeds might be caused by the existence of retinacula. However, this is just an assumption which was not clarified within this study.

Regarding the retinacula of the studied Acanthoideae species in more detail now, their different shapes should be analyzed shortly. Apart from the fact that these funicle structures are present in all species of this subfamily, they display variations when considering them in detail. For example, whereas *Justicia furcata*, *Schaueria calicotricha* and *Brillantaisia owariensis* possess rather similar looking retinacula (Figures 4 D – F; 16 F, G, I), the ones of *Ruellia cf. brevifolia* have a different appearance (Figures 4 H – J; 16 H) due to their somehow ragged top-ends. Also the retinacula of *Ruellia cf. tonduzii* exhibit a slightly different shape because their apical ends are broader and more curved (Figures 4 A; 16 D). Interestingly, the retinacula which expose similar appearances – thus, the ones of *Justicia furcata*, *Schaueria calicotricha* and *Brillantaisia owariensis* – are present in fruits with conspicuously different shapes. *Brillantaisia owariensis*' fruits are long and linear in shape (Figures 2 K, L; 4 K), and therefore, do not look like the fruits of the other two species, which in fact share some overall similarities in fruit shape (Figures 2 D – F; 16 A, B). This leads to the assumption that the fruit shape might not directly be correlated with the retinacula shapes. What might be of more importance is the shape of the seeds the retinacula are surrounding. In fact, it would make sense if the form of the retinacula is adapted to the form of the corresponding seeds. This can also be seen in the investigations conducted within this study (Figures 2 C, E; 4 D; 5 A, B, D; 16 A, E). Furthermore, the curved-out seed borne on its retinaculum given in Figure 18 (A – D) is supposed to illustrate exactly this perfect compatibility between the seed and its retinaculum, which is mostly the case in all acanthoid seeds. One might also presume this when looking at the pictures of Figure 6 (especially D, F, G, I), which therefore could be considered as further support of this hypothesis.

Finally, it should be mentioned that the explosive seed dispersal aid of the retinaculum can also be found in other plant families in, obviously, different shapes, sizes and implementations. For instance, *Cardamine parviflora* (Brassicaceae) also disperses its seeds with catapult-like structures (Hayashi et al. 2010). Additionally, some tropical orchid species are said to possess some kind of ‘slinging hairs’ which react hygroscopically and fling the seeds away when wetted (Ulbrich 1928), which might be argued as comparable structure too due to its same goal – ejecting seeds away from the mother plant.

4.2.2 The apical beak

One taxon should get special attention in this part of the paper, and this is *Thunbergia alata* and the genus *Thunbergia* in general, because this lineage has an extraordinarily long apical beak which is probably of significance for the explosive opening of these fruits. The tissues within this fruit prolongation appear to have an impact on the explosion mechanism; therefore, the different layers one can find within these beaks (see for example Figures 12 A; 14 F; 20 M, N; 22 A – C, J – L) will be topic of the following chapter (4.2.3.; Figure 23 J – L).

Firstly, the hollow space between the beak halves is most likely a continuation of the locules that extend up into the beak (Figure 22, especially A – E). This might aid the valves’ separation during the fruit explosion because of a lack of cohesion between the capsule valves in this area. Moreover, comparable hollow spaces in the middle of the beak can be found in representatives of Acanthoideae and in one of Nelsonioideae (Figure 21 A – C, G – I). This presence across the subfamilies might support that such a hollow space within the beak, whether it is a long one as in *Thunbergia alata* or not as in the other species at hand, can aid the fruit explosion in all three subfamilies. However, apart from the length of the beaks, there is another significant difference between the capsules’ apical ends of *Thunbergia alata* and the other ones, namely the transition zones between the beak and the ovary locule. The transition zone in *Thunbergia alata* fruits is more abrupt than in the other species (Figures 11 A; 13 C; 20 L; 22 D – G; 23 I). Thus, the beaks of *Ruellia cf. brevifolia* and *Elytraria carolinensis* fruits are simply more like a prolongation of the ovary locule and do not appear to be detachable of the ovary locule or profoundly different in their visual appearance to the rest of the fruit as in *Thunbergia alata* (for *Ruellia cf. brevifolia* and *Elytraria carolinensis* see Figures 2 G; 7 A; 20 C, G).

Secondly, there are conspicuous intercellular spaces within the beak halves of *Thunbergia alata* (Figures 12 A; 20 M, N; 22 I, K, L). These spaces might also aid during the separation of the capsule valves because they represent a further lack of cohesion between the tissues of the beak. According to the cell layers that will be analyzed in the next chapter, these intercellular spaces are located in the intermediate layer (Figure 22 K), but paradoxically they are most present in the ‘resistance’ layer (Figure 22 L). Nonetheless, according to this layer’s position being in the middle of the beak halves (see also Figure 23 L), it might be clear again that this tissue possesses rather large lumina between its cells, as these hollow spaces appear to aid the separation of the beak halves, and thus, of the capsule valves.

Regarding the cell compositions in the acanthoid beaks, it could be argued that rather solid parts, thus sclerenchymatous cells (Figure 20 E, F), act as some kind of connecting tissue between the two capsule valves. Therefore, the whole process of fruit explosion could depend on these cells to disintegrate, for example, by additional humidification or by desiccation (Bremekamp 1926; Witztum & Schulgasser 1995). It could be argued that this concept is adaptable to the fruits of *Thunbergia alata* as well as *Elytraria carolinensis*, due to their still rather similar beak structure and the three layers which are present in all three species at hand. The next chapter will present more details concerning these cell layers (see also Figure 23).

Although this study argues that the beak is probably of significance for the capsule explosion, it is, according to the observations made within this study, possible that the beak halves already open partly (starting at the apex) without the lower rest of the capsule valves to immediately separate as well (see Figures 4 C; 14 A, B; 15 H; 20 A), which is especially the case in *Thunbergia alata* (Figures 14 A, B; 15 H). For this reason, another assumption which can be made is that the transition zone between the beak and the ovary is important for the fruit explosion (Figure 12 B; 13 C; 22 D, E). This area might store potential energy for the explosive fruit opening. This assumption could be compared to Witztum and Schulgasser (1995) stating that there is an “elastic potential energy stored in the valves”. Additionally, the middle of the bottom-end of the ovary locule (Figure 13 D) might also store such energy in its thickened and strongly lignified cell composition, and therefore, these areas might be important tissue areas for the explosive seed dispersal.

4.2.3 Specific tissues

Investigations of the different tissue types in the fruits in the studied species uncovered several similarities between the acanthoid species, as well as between the species of all three subfamilies. Overall, three different cell layers were found to occur in these subfamilies; however, their most prominent development is located in different areas within the three different subfamilies' fruits (see Figure 23). These layers are argued to be important for the capsule explosion, and thus, they probably play significant roles in the mechanics of the seed expulsion (Witztum & Schulgasser 1995). Before referring to these specific layers and their positions in more detail, the overall concept of Witztum and Schulgasser (1995) should be summarized. First of all, one should consider the capsules – in previous research only acanthoid capsules were described and are therefore best known – as "bimetallic strips" (Witztum & Schulgasser 1995; Cross 2013). In this concept, the active layers, which are located towards the outside of the fruits (Figure 23, colored medium gray), show more intense reactions to desiccation than the resistance layers (Figure 23, colored dark gray). Thus, the active layers are more likely to bend, or shrink, which would simply result in the capsule valves to separate. However, the capsule valves primarily build up some tension because they do not immediately conform to the outward movement of the active layers. This tension is probably resisted by a type of 'internal glue' which holds the antagonist capsule valves together (Cross 2013). The capsule valves being torn between the pressure to separate due to the shrinking of the active layer and the glue holding them together results in a stress on the whole fruit. Referring back to Witztum and Schulgasser (1995), this specific anatomy of the fruits concerning the storage of elastic potential energy which is then released in form of kinetic energy aids the maximization of the flight distance the seeds can reach when the valves finally separate. The investigations of the study at hand agree with this concept; however, this study here can also add some new insights to this idea. On the one hand, fruits of *Elytraria carolinensis*, the nelsonioid representative, were observed to explode too and such layers as described in Witztum and Schulgasser's study (1995), as well as in Cross' thesis (2013), were also discovered in this species (Figure 23 F – H). On the other hand, the fruits of the Thunbergioideae species studied here, *Thunbergia alata*, was also found to explode and to possess such specific cell layers (Figure 23 J – L). Therefore, one of the main conclusions of this study is that not only acanthoid fruits feature such layers which, together with some kind of internal glue as antagonist, create a tension for the final fruit explosions, but also species belonging to the other two subfamilies exhibit to have three different types of cell layers and are, thus, capable of this extraordinary mechanism.

Interestingly, referring to the bimetallic strip once more, a truly resembling outwards movement can be observed in fruits of *Thunbergia alata*. As described in the previous section concerning the apical beak (4.2.2), this extraordinarily long beak can bend by splitting its two halves without already eliciting the entire capsule explosion (see Figures 14 A, B; 15 H). This preceding opening of the beak might even increase the tension between the capsule valves, and thus, it might be another significant factor for these specific seeds to reach such long flight distances (approximately 3 meters).

One might argue now that only two differing layers are necessary for resembling a bimetallic strip that builds up tension, however, one layer in the middle of them – the intermediate layer (Figure 23, colored bright gray) – is also highly important. According to earlier investigations, this type of layer is most significant for ensuring the seeds the best elevation angle possible (Witztum & Schulgasser 1995). Nevertheless, this function is also said to be mastered by the retinacula of the Acanthoideae capsules (see chapter on retinacula; Witztum & Schulgasser 1995). Therefore, this intermediate layer might be of more importance to the other two species (representatives of Nelsonioideae and Thunbergioideae) due to their lack of retinacula.

Concerning the organization and texture of these three layers, it can be claimed that they share some similarities although their obvious differences should not be disregarded. The active layer consists of rather small and compact cells with lignified cell walls (see for example Figure 20 M). The resistance layer exhibits a mostly similar cell composition due to its small and narrowly arranged cells which also show some lignification (Figure 20 I, M). Nevertheless, intercellular spaces can be found in this tissue which is rather unique for the resistance layer compared to the other two types (see also Figure 20 I, M). The final tissue type – the intermediate layer – is mostly composed of larger praenchymatous cells which are more loosely arranged (Figure 20 H, I, M, N; Witztum & Schulgasser 1995). These cells have, moreover, also partly lignified cell walls, which displays a further similarity between these three tissue types. The varying degrees of lignification in the different layers should not be neglected though.

Regarding the strongest development of these layers within the species of the three subfamilies, truly contrasting positions can be observed. In Acanthoideae fruits, the tissues are most prominently developed close to the base of the ovary locules, more precisely, where the first, and thus, lowest two retinacula are located (Figure 23 B). In comparison to that, *Elytraria carolinensis* fruits revealed to display such a clear representation of all three layers shortly above the ovary locules, i.e., between the two uppermost seeds and the beak (Figure 23 G). Finally, in the fruits of *Thunbergia alata,* the three layers are most prominent in the beaks (Figure 23 L), which, again, stresses the importance of the beak for this species. Moreover, it is located further away from the ovary locule when comparing this finding to the other two once more. However, as mentioned in the results of this study, all three layers were discovered throughout the fruits' anatomy, as one can also see in Figure 23. These descriptions should merely emphasize that there were certain areas which illustrated these layers most strikingly. They also indicate which specific areas of the fruits are most important for the fruit explosions in the different subfamilies.

Consequently, the three specific tissue layers discussed here are present in all three subfamilies. Furthermore, they are most likely responsible for the explosion of all acanthaceous fruits, although their most distinctive representations are located at different areas within the fruits. Before turning to the penultimate aspect of this discussion, the elastic energy which is stored in the dried capsules should be mentioned once again. As described in other studies, additional humidification could reduce the strengths of the internal glue, and thus, elicit the fruit explosion (Witztum & Schulgasser 1995). Nevertheless, also additional desiccation could lead to capsule explosion due to the seam between the valves failing to deal with the stress between the layers (Witztum & Schulgasser 1995). This means that the different layers do not really contribute to the understanding of hygrochastic and xerochastic opening processes. Other investigations would be needed to clarify which opening type is elicited within which fruit and at what time. The following chapter is an attempt to describe how these two types occur and what their differences as well as similarities are.

4.3 The opening mechanism – hygrochastic versus xerochastic triggering

Hildebrand (1873) – one of the first researchers who recognized that Acanthaceae have exploding capsules – described the seed expulsion of two Acanthoideae species as xerochastic, and thus, as exploding due to desiccation. This scholar goes on with assuming that all Acanthaceae (at that time only Acanthoideae were investigated as representatives of Acanthaceae) could disperse their seeds explosively through this xerochastic mechanism, which was also supported by the more specific argument stating that the genus *Justicia* opens after desiccation (Putzer 2015). However, some decades later, it was argued that all acanthaceous fruits (probably also referring to Acanthaceae sensu stricto) are actually hygrochastic because they appear to open when moistened (Bremekamp 1926). This was, at least partly, supported by some scholars rather recently. For instance, the seed dispersal of the genus *Ruellia* was described to be hygrochastic (Witztum & Schulgasser 1995; Imam et al. 2013; Wasshausen 2013). From an ecological point of view, it should be added here that seed dispersal during wet conditions does, in fact, make sense due to the beneficial conditions for germination, and thus, for the establishment of the seedlings. Nevertheless, the study by Greuter and Rankin Rodríguez (2010) portrayed a highly interesting summary of the most probable reasons for all these confusing findings as follows:

> One tends to assume that the capsules tear open when drying (xerochasy), but Bremekamp (1926) came to the contrary conclusion, that acanthoid capsules open hygrochastically. He discovered that hygrochasy is so to say a phenomenon superimposed on xerochasy, insofar as first a tension must build up in the fruit as a result of desiccation, only then can wetting of the capsule beak, which holds the capsule halves together, trigger the release. (Greuter & Rankin Rodríguez 2010)

This finding is especially the case in *Ruellia*, as this was the genus investigated within the study by Greuter and Rankin Rodríguez (2010). However, this might be an explanation for how this general controversy about hygrochastic or xerochastic acanthaceous fruits primarily came to existence. This controversy appears to be important to consider when investigating the explosive seed dispersal in Acanthaceae and, for this reason, it led to the third and final research question of this paper and should be treated here.

In general, this thesis agrees with the concept claiming that the explosive opening process of acanthaceous fruits can occur under wet conditions – hygrochastically – as well as under dry conditions – xerochastically (Greuter & Rankin Rodríguez 2010). However, it should be mentioned here that this study did not test the hygrochastic opening mechanism itself (see chapter 2.5 for more information), but, as mentioned above, several researchers already examined this type and discovered it to occur in several species. Thus, the seed dispersal experiment conducted within this study was supposed to demonstrate that fruits of all three subfamilies are, in addition to the hygrochastic opening process, capable of opening xerochastically, and this was finally the case. For this reason, the outcomes of this study conform to Greuter and Rankin Rodríguez (2010) – acanthaceous fruits can, at least in some cases, open hygrochastically (according to previous literature) as well as xerochastically (according to previous studies and this study at hand).

Additionally, the explosive seed dispersal of fruits of *Crossandra cf. infundibuliformis* would have also been interesting to watch but samples of this Acanthoideae representative were only collected in Costa Rica; after having fixed them in ethanol for their transport to Vienna, no such experiments were possible anymore. Thus, one can only refer to the results of Greuter and Rankin Rodríguez (2010) who, as already mentioned in chapter 2.5, state that the fruits of this species are likewise using fruit explosions for effective seed dispersals and, interestingly, explosions under, once again, dry as well as wet conditions were observed.

4.4 The explosive seed dispersal mechanisms and their evolutionary relationship

A final question should also be discussed shortly, namely, which type of explosive seed dispersal, most likely developed first – the mechanism found in Nelsonioideae and Thunbergioideae which takes place without retinacula, or the more complex mechanism present in Acanthoideae which feature lever-like structures, i.e. retinacula.

Before discussing the two most likely hypotheses concerning the evolutionary order of these two explosive dispersal mechanisms, the recent state of knowledge regarding the phylogenetic relationships among the Acanthaceae subfamilies should be described. Phylogenetic studies showed that Thunbergioideae are probably sister to Acanthoideae (Borg et al. 2008; McDade et al. 2000), followed by Nelsonioideae, which is sister to the clade with the aforementioned subfamilies. Comprehensive phylogenetic trees are for example shown in McDade et al. (2008: Fig. 2) or also in Tripp et al. (2013: Fig. 1).

The first possible evolutionary sequence of changes would involve that already the most recent common ancestor of all three subfamilies had explosive capsules with retinacula. In this scenario, we would have to assume that retinacula were lost twice, once along the branch leading to the Nelsonioideae and once along the branch leading to the Thunbergioideae. The second plausible sequence of steps in the Acanthaceae evolution would start with the ancestors of all three subfamilies being in the possession of explosive fruits but without retinacula. Thus, this would already represent the seed dispersal mechanism as it is displayed by Nelsonioideae and Thunbergioideae. The only change which would have been needed to occur in this hypothesis is the gain of retinacula along the branch leading to the Acanthoideae.

It can be argued now that the second, more parsimonious (only one change instead of two as in the first hypothesis) of these hypotheses is the more likely one to have occurred in the phylogenetic development of the Acanthaceae subfamilies. Therefore, this hypothesis suggests that the rather simple explosive seed dispersal mechanism of Nelsonioideae and Thunbergioideae evolved earlier than the more complex retinaculate explosion mechanism of Acanthoideae.

5. Conclusion

In conclusion, this paper reveals some fascinating insights into the fruit morphology of Acanthoideae, Nelsonioideae and Thunbergioideae and the mechanisms of the capsule explosion within these three subfamilies of Acanthaceae. This study clearly shows that capsule explosions do, in fact, occur in all three subfamilies, which is the first and probably most crucial finding of this study. Further insights include the main similarities and most significant differences between several Acanthoideae species and *Elytraria carolinensis,* a Nelsonioideae species, as well as *Thunbergia alata*, a representative of Thunbergioideae, in terms of their morphological structures, which are most likely aiding the remarkably effective seed expulsion. These structures investigated in more detail were first of all the retinacula, which are hook-like derivatives of funicles and only exist in Acanthoideae. Secondly, the apical beaks, which are most striking in *Thunbergia alata*, and are thus maybe also most significant for the capsule explosions within this species. Thirdly, the three specific tissue layers that were found to be existent in all these acanthaceous fruits investigated within this study are probably the main elicitors for the capsule explosions across all three subfamilies due to the tension they create between the capsule valves. Finally, the xerochastic dehiscence of all these acanthaceous fruits was investigated and it can be claimed now that not only hygrochastic opening processes are present in the fruits of Acanthaceae.

References

Borg, Agneta Julia. 2012. *Evolutionary relationships in Thunbergioideae and other early branching lineages of Acanthaceae*. Stockholm: Universitetsservice US-AB.

Borg, Agneta Julia; McDade, Lucinda A.; Schönenberger, Jürg. 2008. “Molecular phylogenetics and morphological evolution of Thunbergioideae (Acanthaceae)”. *Taxon* 57(3), 811-822.

Borg, Agneta Julia; Schönenberger, Jürg. 2011. “Comparative floral development and structure of the black mangrove genus Avicennia L. and related taxa in the Acanthaceae”. *International Journal of Plant Sciences* 172(3), 330-344.

Bremekamp, C. E. B. 1926. “On the opening mechanism of the acanthaceous fruit”. *South African Journal of Science* 13, 488-491.

Bremekamp, S. 1965. “Delimitation and subdivision of the Acanthaceae”. *The Bulletin of the Botanical Survey of India* 7, 21-30.

Corner, Edred John Henry. 1976. *The Seeds of Dicotyledons*. Cambridge: University Press.

Cross, Carolyn. 2013. “Slinging Spinning Seed Substitutes: An investigation into the explosive seed dispersal of the Acanthaceae”. Bachelor thesis, Pomona College, Claremont.

Daniel, Thomas F.; McDade, Lucinda A. 2014. “Nelsonioideae (Lamiales: Acanthaceae): Revision of Genera and Catalog of Species”. *Aliso* 32(1), 1-45.

Durkee, L. H. 1986. Family #200 Acanthaceae. In Burger, William (ed), *Fieldiana: Botany – Flora Costaricensis*, 1-87. Chicago: Filed Museum of Natural History.

Gargiullo, Margaret B.; Magnuson, Barbara; Kimball, Larry. 2008. *A Field Guide to Plants of Costa Rica*. Oxford: University Press.

Gerlach, Dieter. 1984. *Botanische Mikrotechnik*. Stuttgart: Georg Thieme Verlag.

Graham, Victoria A.W. 1988. “Delimitation and infra-generic classification of *Justicia* (*Acanthaceae*)”. *Kew Bulletin* 43(4), 551-624.

Greuter, Werner; Rankin Rodríguez, Rosa. 2010. “Notes on some endemic Cuban species of *Ruelliinae* (Acanthaceae), on their seeds, pollen morphology and hygroscopic features”. Willdenowia 40(2), 285-304.

Gutterman, Y.; Witztum, A.; Evenari, M. 1967. „Seed dispersal and germination in *Blepharis Persica* (Burm.) Kuntze”. *Israel Journal of Botany* 16, 213-234.

Hayashi, Marika; Gerry, Shannon P.; Ellerby, David J. 2010. “The seed dispersal catapult of *Cardamine parviflora* (Brassicaceae) is efficient but unreliable”. *American Journal of Botany* 97(10), 1595-1601.

Hildebrand, F. 1873. “Die Schleuderfrüchte und ihr im anatomischer Bau begründeter Mechanismus“. *Jahrbücher für Wissenschaftliche Botanik* 9, 235-276.

Imam, Farid; Haryadi, Bagus; Sumarmo, Pamungkas B.; Miftakudin; Chandra, Zulga M.; Soetedjo, Hariyadi. 2013. “Explosion Phenomenon Observed from Seed Capsules of *Pletekan* (*Ruellia Tuberosa* L.)”. *Journal of Natural Sciences* 3(9), 96-101.

Lindau, G. 1895. “Acanthaceae”. In Engler, A.; Prantl, K. (eds.). *Die natürlichen Pflanzenfamilien*. Leipzig, Germany, 4(3b), 274-353.

Mabberley, David J. 1997. *The Plant-Book: A portable dictionary of the vascular plants*. Cambridge: University Press.

McDade, Lucinda A.; Daniel, Thomas F.; Kiel, Carrie A. 2008. “Toward a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae S.L. (Lamiales)”. *American Journal of Botany* 95(9), 1136–1152.

McDade, Lucinda A.; Masta, Susan E.; Moody, Michael L.; Waters, Elizabeth. 2000. “Phylogenetic Relationships among Acanthaceae: Evidence from Two Genomes”. *Systematic Botany* 25(1), 106-121.

McDade, Lucinda A.; Tripp, Erin A. 2007. “A synopsis of Costa Rican Ruellia

(Acanthaceae), with descriptions of four new species”. *Brittonia* 59(3), 199-216.

Putzer, Irene Eleonore. 2015. „Morphologische und anatomische Untersuchungen an Blütenelementen von *Justicia secunda* VAHL (Acanthaceae)“. Diploma thesis, Vienna, Austria.

Schönenberger, Jürg. 1999. “Floral structure, development and diversity in *Thunbergia* (Acanthaceae)”. *Botanical Journal of the Linnean Society* 130, 1-36.

Schönenberger, Jürg; Endress, Peter K. 1998. “Structure and development of the flowers in *Mendoncia*, *Pseudocalyx*, and *Thunbergia* (Acanthaceae) and their systematic implications”. *International Journal of Plant Sciences*, 159(3), 446-465.

Scotland R.W.; Vollesen, K. 2000. “Classification of Acanthaceae”. *Kew Bulletin* 55(3), 513-589.

Sehnal, Peter; Zettel, Herbert. 1996. *Esquinas-Nationalpark – Der Regenwald der Österreicher in Costa Rica*. Wolkersdorf: Druckerei Paul Gerin.

Staedler, Yannick, M.; Masson, David; Schönenberger, Jürg. 2013. “Plant tissues in 3D via X-ray tomography: simple contrasting methods allow high resolution imaging”. *PLoS One* 8(9): e75295.

Tripp, Erin A. 2007. “Evolutionary Relationships within the species-rich genus *Ruellia* (Acanthaceae)”. *Systematic Botany* 32(3), 628-649.

Tripp, Erin A. 2010. “Taxonomic Revision of *Ruellia* Section *Chiropterophila* (Acanthaceae): a Lineage of Rare and Endemic Species from Mexico”. *Systematic Botany* 35(3), 629-661.

Tripp, Erin A.; Daniel, Thomas F.; Fatimah, Siti; McDade, Lucinda A. 2013. “Phylogenetic Relationships within Ruellieae (Acanthaceae) and a revised classification”. *International Journal of Plant Sciences* 174(1), 97-137.

Ulbrich, E. 1928. *Biologie der Früchte und Samen (Karpobiologie)*. Berlin: Julius Springer.

Utteridge, Timothy; Bramley, Gemma. 2014. *The Kew tropical plant families identification handbook*. United Kingdom: Royal Botanic Gardens.

Wasshausen, Dieter C. 2013. Acanthaceae. In Persson, Claes; Ståhl, Bertil (eds), *Flora of Ecuador*, 1-328. Gothenburg: Department of Biological and Environmental Sciences.

Weissenhofer, Anton; Huber, Werner. 2001. “Der ‘Regenwald der Österreicher’: Biodiversität, Vegetation und Besonderheiten ‚unseres‘ Regenwaldes in Costa Rica“. *ÖKO L* 23(3), 3-18.

Witztum, A.; Schulgasser, K.1995. “The Mechanics of Seed Expulsion in Acanthaceae”. *Journal of theoretical Biology* 176, 531-542.

Wood, John R. I. 2015. “New species of Acanthaceae from Bolivia”. *Kew Bulletin* 70(34).

Wortley, Alexandra H.; Scotland, Robert W.; Rudall, Paula J. 2005. “Floral anatomy of Thomandersia (Lamiales), with particular reference to the nature of the retinaculum and extranuptial nectarines”. *Botanical Journal of the Linnean Society* 149, 469-482.

Supplementary Materials

https://phaidra.univie.ac.at/detail_object/o:424298 – Movie of a seed on its retinaculum

https://phaidra.univie.ac.at/view/o:426865 – Movie of 4 explosions of acanthoid capsules

Appendix

Deutsche Zusammenfassung

Die Pflanzenfamilie Acanthaceae, auch bekannt unter dem Namen ‚Akanthusgewächse', umfasst schätzungsweise 4000 Arten, die hauptsächlich in den Tropen und Subtropen heimisch sind und eine hohe morphologische Diversität aufweisen. Es wurden in den letzten Jahrzehnten bereits verschiedene Studien zu dieser Pflanzenfamilie durchgeführt; besonders die phylogenetischen Beziehungen zwischen den Unterfamilien waren oft im Fokus der Wissenschaft. Ein Forschungsbereich der jedoch bisher oftmals für Verwirrung sorgte konzentriert sich auf die Frage welche Vertreter der Acanthaceen explosive Früchte bilden und welche dies nicht tun. Zu diesem Thema findet man zahlreiche Widersprüche verteilt über das letzte Jahrhundert. Diese Studie versucht etwas Licht ins Dunkel zu bringen und aufzuklären ob es nun tatsächlich explosive Samenausbreitung in den drei wichtigsten Unterfamilien – Acanthoideae, Nelsonioideae und Thunbergioideae – der Acanthaceen gibt. Dafür wurden verschiedenste Arten der Acanthoideae und jeweils eine Art der beiden anderen Unterfamilien (*Elytraria carolinensis* von den Nelsonioideae und *Thunbergia alata* von den Thunbergioideae) untersucht. Von der erstgenannten Unterfamilie wurden nicht nur Früchte aus dem Botanischen Garten der Universität Wien gesammelt, sondern auch Früchte von Vertretern, die während der Feldstudie im Februar 2015 in Costa Rica in der Nähe der Tropenstation La Gamba gesucht wurden. Mit Hilfe von Laborexperimenten, wurde festgestellt, dass tatsächlich Vertreter aus allen drei Unterfamilien zur explosiven Samenausbreitung befähigt sind. Zusätzlich wurden die Gemeinsamkeiten und Unterschiede in der Fruchtmorphologie und Anatomie der drei Unterfamilien mit Hilfe von Lichtmikroskopie, Rasterelektronenmikroskopie und Computertomographie im Detail herausgearbeitet. Diese Untersuchungen zeigten, dass Acanthoideae Retinacula – eine Haken-Struktur, die die Auswurfweite der Samen beträchtlich erhöhen kann – besitzen. Manche Wissenschaftler der vergangenen Jahre vermuteten, dass diese Struktur die Explosion der Fruchtkapseln gar erst ermöglicht. Dies wurde mit der vorliegenden Studie widerlegt, da, wie schon erwähnt, auch *Elytraria carolinensis* und *Thunbergia alata* explodierende Kapseln hervorbrachten obwohl diese beiden Vertreter der zwei anderen Unterfamilien keine Retinacula in ihren Früchten besitzen. Im Weiteren zeigen die morphologischen Studien im Rahmen dieser Arbeit, dass andere Strukturen in den Kapseln bedeutend für die tatsächliche explosive Öffnung der Kapseln sind. Solche Strukturen wurden in allen drei Unterfamilien in Form von drei unterschiedlichen Gewebsschichten gefunden, jedoch sind diese an

unterschiedlichen Positionen innerhalb der verschiedenen Kapseln unterschiedlich stark entwickelt. Diese Schichten, bestehend aus der ‚aktiven Schicht‘, einer ‚Zwischenschicht‘ und der ‚Resistenzschicht‘ (in den Kapseln von außen nach innen aufzufinden), bewerkstelligen höchstwahrscheinlich die Fruchtexplosionen. In den Acanthoideae erkennt man die eindeutigste Darlegung dieser Schichten im Querschnitt des unteren Endes der Samenkammer an welchem die beiden untersten Retinacula zu sehen sind. Beim Nelsonioideae-Vertreter *Elytraria carolinensis* sind diese Zellschichten am apikalen Ende des Ovars am stärksten ausgeprägt. Bei *Thunbergia alata* (Thunbergioideae) schließlich, sind die drei Schichten im Schnabel der Früchte am klarsten zu erkennen. Weiters wurde versucht die Problematik der hygrochastischen, beziehungsweise der xerochastischen Dehiszenz aufzuklären. So wurde innerhalb dieser Studie aufgedeckt, dass die untersuchten Spezies, trotz allen Widersprüchen in vorangegangenen Studien, zur xerochastischen Dehiszenz befähigt sind und somit nicht nur das Zuführen von Wasser, wie in der hygrochastischen Dehiszenz erforderlich, zur Auslösung der Fruchtexplosion führt. Schlussendlich hat diese Arbeit interessante Ergebnisse hervorgebracht, die einen wichtigen Beitrag zum derzeitigen Kenntnisstand der Acanthaceae und ihrer außergewöhnlichen explosiven Früchte inklusive deren Morphologie liefern.