

OBSERVATION OF REPRODUCTIVE ORGANS OF SARRACENIACEAE WITH  
SEM LV MODEL

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Abstract

The High Vacuum (HV) model of Scanning Electron Microscope (SEM) is commonly used to observe specimens coated with metal or carbon. To simplify the procedure and reduce the cost, we used the Low Vacuum (LV) model of Joel SEM to directly observe anthers, pollen, and seeds of *Sarracenia*, *Darlingtonia*, and *Heliamphora*. For the first time, these reproductive organs are recorded with SEM. We also compared their morphological similarities to test their inter-general affinities.

Introduction

Commonly, a HV model of SEM is used to observe morphology of small specimens that were previously coated with carbon or gold. However, recently some SEM (e.g., Joel) has been produced with a LV model allowing one to directly observe specimens, such as metal, china, plant leaves, pollen, seeds, feather, and insects (Symondson & Williams 1997), only if the sample surface is dense enough to reflect sufficient electron beams to be converted into images. Without coating specimens, the LV model is much more convenient and cost-saving than the HV model.

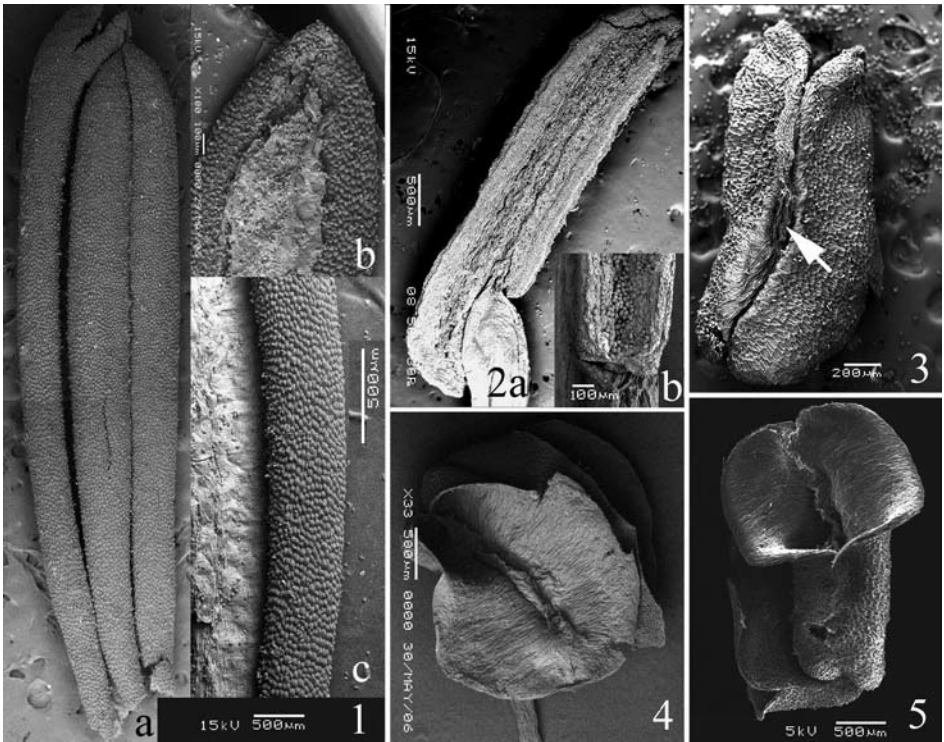
Sarraceniaceae include three genera, *Heliamphora* in northern South America, *Sarracenia* in southeastern North America, and *Darlingtonia* in California and Oregon. Recent molecular studies suggest that *Darlingtonia* should be sister to both *Heliamphora* and *Sarracenia* (Albert *et al.* 1992; Bayer *et al.* 1996; Neyland & Merchant 2006). Morphologically, their pollen have been studied with compound microscopes (Thanikaimoni & Vasanthy 1972) and some seeds have been briefly measured and described (McDaniel 1971; Maguire 1978; Godfrey & Wooten 1981; Ellison 2001; Schnell 2002), but they have not been well documented with SEM photos yet. As an attempt, we have used the LV model of Joel SEM to observe sarraceniacean anthers, pollen, and seeds. Rather than giving complete, detailed measurements, we present SEM photos of some sarraceniacean anthers, pollen, and seeds to demonstrate their similar and different characteristics, to be used to test the affinities among these geographically separated genera.

Material and Method

Reproductive organs of *Darlingtonia californica* were provided by Barry Rice (Davis, California, USA), while those of *Heliamphora* sp. were given by Andreas Wistuba (Mannheim, Germany). *Sarracenia purpurea* specimens were collected from Mount Davis Bog (Somerset, Pennsylvania, USA), while other *Sarracenia* species samples were from Meadowview Biological Research Station (Woodford, Virginia, USA). Specimens of seven species, *Sarracenia alabamensis*, *S. minor*, *S. oreophila*, *S. psittacina*, *S. purpurea*, *Darlingtonia californica*, and *Heliamphora* sp., have been collected and observed, although not all of their results are presented in this report. Samples were not treated but directly laid on double-side adhesive aluminum tape on SEM stubs. The JSM-6060LV was set with 15 kv, pressure 30 pa, spot size 50, and shadow 1.

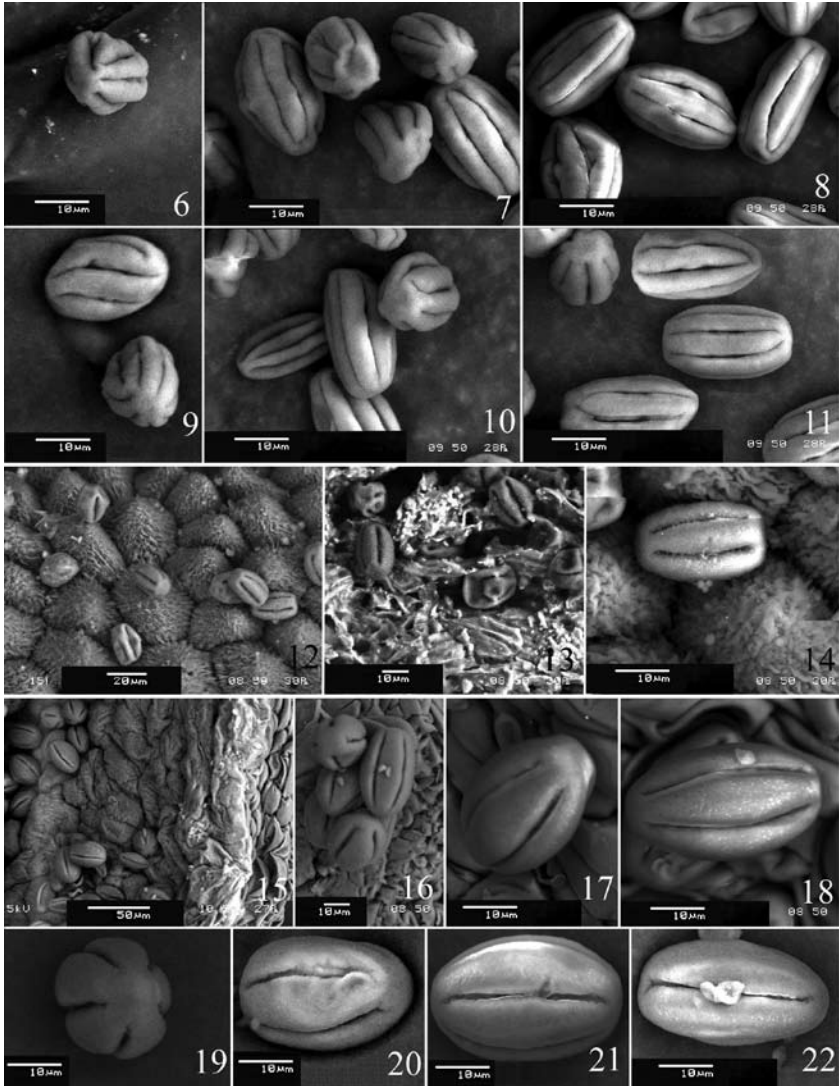
## Results

Anthers, pollen, and seeds of the three sarraceniacean genera were photographed with SEM. Anthers (see Figures 1-5) are morphologically very different from each other. All anthers have two pollen sacs and two longitudinal dehiscent slits. Anthers of *Heliampora* sp. are the longest among all three genera, up to 7.5 mm long, and each pollen chamber is about 1 mm in diameter (see Figure 1). The samples were received without the bottom part where should connect the filament. The anther dehiscent slit runs from the tip (see Figure 1b) through the bottom. The pollen sac is very thick, and the outside is covered with small round bumps (see Figure 1c), but some of them are so densely packed that they deformed from round to polygonal (see Figure 12). Each is about 30~40  $\mu\text{m}$  in diameter and covered with erect flake-like structures (see Figures 12, 14). The inside of pollen sac has pollen and degenerated cells (see Figure 13). The anther of *Darlingtonia californica* has a tetra-angular cross view. It is about 4.0~4.5 mm long, about 0.5 mm wide (see Figure 2b), and the lateral side is about 0.8 mm wide (see Figure 2a). The filament bears the anther at about 1/5 anther length to the bottom (see Figure 2a). The outer surface is covered with crowded bumps that are relatively smooth (see Figure 15 left side), but the inside of pollen sac is filled with pollen and flattened-ball-like degenerated cells (see Figures 16-18). Anthers of *Sarracenia* are the shortest among all three genera. Among five observed species, anthers range from 1.36 to 3.89 mm long and the outer diameter of pollen sacs are 0.3 to 1.3 mm (Table 1). Filament is attached to the center on the backside of the anther (see Figure 3 arrow). Dehiscent slits initially open from the top to certain length downwards (see Figure 5), but eventually open fully (see Figure 4), flat or flipping outwards (see Figures 4-5, Table 1). Outer surface has reticulated structure and inner surface has horizontal striations (see Figures 4-5).



Figures 1-5: Sarraceniacean anthers. 1, *Heliampora* sp.: a, dorsal view; b, ventral view with dehiscent part; c, inner side (left) and inward-curving pollen chamber edge. 2, *Darlingtonia californica*: a, side view; b, ventral view. 3, *Sarracenia alabamensis*: the arrow pointing at the remnant of filament in the middle of dorsal side. 4-5, *Sarracenia purpurea*: ventral views.

All observed pollen grains are in the same colporate type, but in different sizes, and with different numbers of colpi. Both *Heliamphora* sp. (see Figures 12-14) and *Darlingtonia californica* (see Figures 15-22) have pollen grains with 4-6 colpi, but most pollen of the former have 4 colpi and the latter are mostly pentacolpate. Pollen of *Sarracenia purpurea* have 6-8 colpi (see Figures 6-11), mostly have 7 colpi. All taxa have the pollen in the similar shape, ranging from isopolar, prolate, oblate, to spheroidal, depending on their maturity. Young pollen grains are elongated or oblate, while fully developed pollen grains are in isopolar oval to spheroidal shapes. Colpi are mostly straight, but some of them are twisted (see Figure 9). Pollen grains of *Darlingtonia* and *S. purpurea* have smooth surface, but those of *Heliamphora* sp. have tiny bumps, especially along the apertures (see Figure 14).



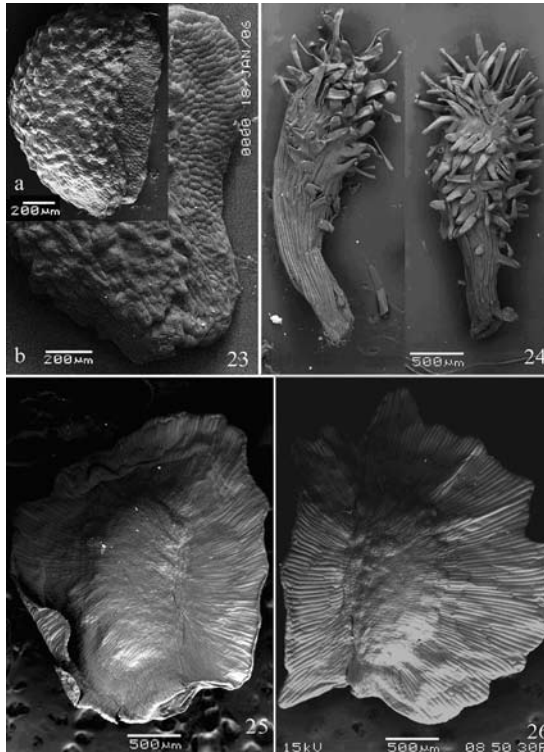
Figures 6-22: Sarraceniacean pollen. 6-11, pollen of *Sarracenia purpurea* in the same magnification. 12-14, *Heliamphora* sp.: 12 and 14, pollen grains on the outer surface of a pollen sac; 13, pollen grains and degenerated cells inside of a pollen sac. 15-22, *Darlingtonia californica*: 15, pollen grain on the outer bumpy surface (left side); 16-18, pollen on nutritious tissue or immature pollen inside of a pollen sac. 17-22, pollen in the same magnification.

Table 1. Some anthers of five species of *Sarracenia*.

Species	Length (mm)	Width (mm)	Dehiscence	
<i>Sarracenia alabamensis</i>	1.36~1.56	0.3	Entire length	Flat slit
<i>Sarracenia psittacina</i>	2.4	1.0	1.80 mm	Flat slit
<i>Sarracenia minor</i>	2.39	0.935	1.96 mm	Flip outwards
<i>Sarracenia purpurea</i>	2.74	1	1.5 mm	Flip outwards
<i>Sarracenia oreophila</i>	3.89	1.3	3.06 mm	Half outwards

\* Data presented here are just based on a few typical sized anthers, not of a statistical study

Seeds of the three genera are very different in shape, but their core parts are roughly in the same size. Seeds of *Darlingtonia californica* are oblate, straight or curved, 1.3 mm in diameter and about 2 mm long, consisting of a 0.6~0.8 mm long bottom part with longitudinal ridges and a top part with elongated spines that are round-tipped and up to 500  $\mu\text{m}$  long and about 70  $\mu\text{m}$  in diameter (see Figure 24). The spines appear to be hollow and can be flattened. Seeds of *Sarracenia purpurea* are oblate or kidney-shaped, 1.6~2.1 mm long and 1~1.2 mm wide, reticulate-tuberculate, and winged on one side (see Figure 23). Each tubercle can be about 60~100  $\mu\text{m}$  in diameter, with reticulate bumpy meshes, each about 30 $\times$ 50  $\mu\text{m}$ . The wing area has cells arranged more or less in radial rows (see Figure 23b). *Heliampora* sp. has winged seeds (see Figures 25-26). Similar to *S. purpurea* seeds, the core part is in the same shape and similar size, and with similar sized tubercles (see Figure 26) but the surface is relatively smooth (see Figure 25). The wing is around the meridian plane edge, up to 1 mm wide, and consists of radially arranged, elongated cells; each can be up to 55  $\mu\text{m}$  wide and 750  $\mu\text{m}$  long (see Figure 26).



Figures 23-26: Sarraceniacean seeds. 23, *Sarracenia purpurea* seeds in different magnifications. 24, *Darlingtonia californica* seeds. 25-26, *Heliampora* sp. seeds.

Our morphological results are generally matched with previous reports, but demonstrated with much better photos. Seed size and morphology concur with previous reports (McDaniel 1971; Maguire 1978; Godfrey & Wooten 1981; Ellison 2001; Schnell 2002). *Heliamphora* sp. does not have tricolporate pollen as that of *H. heterodoxa*, but mostly have four apertures and thus differ from those of *H. nutans* and *H. tyleri* (= *H. tatei*), both are mostly pentocolporate (Thanikaimoni & Vasanthi 1972). Anthers of *Heliamphora* sp. are pretty long, much longer than *H. minor* (4 mm) and *H. ionasii* (3.5 mm), but in the same length of *H. hispida* (Nerz & Wistuba 2000). So, the specimen from Wistuba could be *H. hispida*.

As we mentioned earlier, this study is a preliminary comparative study of sarraceniacean anthers, pollen, and seeds, for finding similarities and differences to test relationships among the three genera. Traditionally, *Heliamphora* is thought to be primitive for having the simplest pitcher structures, but its flowers are the mostly derived in the whole family (Renner 1989). *Sarracenia* and *Darlingtonia* had been considered to be closer to each other because they share many characters and they are endemic to North America (Maguire 1978; Juniper *et al.* 1989). However, recent molecular data suggest that *Darlingtonia* is sister to a *Heliamphora-Sarracenia* clade (Albert *et al.* 1992; Bayer *et al.* 1996; Neyland & Merchant 2006).

As we have observed, anthers of the three genera are very different in length, shape, thickness, outer and inner surface structures, so they do not display clues of their affinity. On the other hand, their pollen grains are so highly similar to each other, and thus cannot indicate a closer relationship between any two of them. Although *Darlingtonia* and *Heliamphora* share slight similarities in having elongated and bumpy anthers, with 4-5 colporate pollen grains mostly, these are not significant enough to imply a closer inter-genera relationship. The seeds of the three genera appear to be very different, as they are spiny (*Darlingtonia*), winged (*Heliamphora*), or reticulate-tuberculate (*Sarracenia*). However, with a close examination, seeds of the latter two are found much more similar to each other. *Sarracenia* seeds have a narrow wing on one side, and the wing has cells arranged in more or less radial rows. *Heliamphora* seed has wings around the primary plane, and wings with radially elongated cell tiers. Both genera have the seed core parts in the similar size and with reticulate-tuberculate surface (even the tubercles are in the similar size and shape), although *Heliamphora* sp. seeds have fewer tubercles. *Darlingtonia* seeds do not have any reticular tubercles, but elongate ridges and spines. Therefore, seed morphology suggests that *Heliamphora* and *Sarracenia* could be related to each other more closely than to *Darlingtonia*, thus supporting the hypothesis that *Darlingtonia* is sister to the *Heliamphora-Sarracenia* clade.

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## BOOK REVIEW

By Jan Schlauer

Catalano, M. “Nepenthes della Thailandia – Diario di viaggio” (*Nepenthes* of Thailand – Travel Diary, text in Italian, descriptions of new taxa in Latin/English) 207 pp., 209 col. photos, 1 line drawing, 13 maps, hard cover, published February 2010 by the author (Marcello Catalano, via Ronchi 2, 20134 Milano, Italy, <rafflesiana@yahoo.com>); 30 € (ca. 40 US\$)

In a narrative style that only Italians master (“se non è vero, è ben trovato”), the author describes his remarkable experience from roughly a decade of training and research both at herbaria and in the field. The present work is understood as a contribution to the knowledge of the genus *Nepenthes* in the whole region of Indochina, so there is potential for future supplements covering Burma (Myanmar), Laos, Vietnam, and Cambodia. Taxonomy is presented here as learning by travel, observation, and discovery, and the author also does not spare the reader the frustrating experience when herbarium/literature-based expectations were disappointed by an adverse field reality.

The diversity of the genus in Thailand as featured in the present book is far greater than expressed by any previous author. Five new taxa are described (*N. andamana*, *N. chang*, *N. kerrii*, *N. mirabilis* var. *globosa*, *N. suratensis*), and one further is introduced as a *nomen nudum* (“*ined.*”) as its formal description is credited to yet unpublished work by Martin Cheek (Kew Gardens, UK).

According to recent research at Kew, *N. smilesii* (type from Thailand or Laos; so far the type locality “Baw Saw” does not seem to have been located precisely, and “Northern Siam” covers territory of both contemporary countries) is the correct name for the species that has been called *N. anamensis* (type from Vietnam) before. Most of the Thai taxa are said to be close relatives of the recently described *N. bokorensis* (type from Cambodia) that is intermediate between *N. smilesii* and *N. thorelii* (type from Vietnam). It is thus most unfortunate that neither *N. bokorensis* nor *N. thorelii* is featured in the present book in detail, but they apparently have not been found in Thailand so far. Differences in indumentum, leaf shape, peristome width, presence/absence of bracteoles in the inflorescence, and androphore length are used to distinguish individual taxa in this complex aggregate.

Of the five new taxa, one is classified as a variety of *N. mirabilis* with large ovoid pitchers. This *N. mirabilis* var. *globosa* is the plant that has informally been called “Viking” in cultivation.

An English translation of this book is announced (in <http://www.carnivorousplants.it/indochina.htm>) to be published in 3-4 years.