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Research paper

Aponogeton pollen from the Cretaceous and Paleogene of North America and West Greenland: Implications for the origin and palaeobiogeography of the genus☆

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ABSTRACT

The fossil record of *Aponogeton* (Aponogetonaceae) is scarce and the few reported macrofossil findings are in need of taxonomic revision. *Aponogeton* pollen is highly diagnostic and when studied with light microscopy (LM) and scanning electron microscopy (SEM) it cannot be confused with any other pollen types. The fossil *Aponogeton* pollen described here represent the first reliable Cretaceous and Eocene records of this genus worldwide. Today, *Aponogeton* is confined to the tropics and subtropics of the Old World, but the new fossil records show that during the late Cretaceous and early Cenozoic it was thriving in North America and Greenland. The late Cretaceous pollen record provides important data for future phylogenetic and phylogeographic studies focusing on basal monocots, especially the Alismatales. The Eocene pollen morphotypes from North America and Greenland differ in morphology from each other and also from the older Late Cretaceous North American pollen morphotype, indicating evolutionary trends and diversification within the genus over that time period. The presence of *Aponogeton* in the fossil record of North America and Greenland calls for a reconsideration of all previous ideas about the biogeographic history of the family.

1. Introduction

Recent morphological and molecular based phylogenetic studies have shown that monocots are monophyletic and that they are among the earliest diverging groups of angiosperms (e.g. Bremer, 2000; Savolainen et al., 2000; Soltis et al., 2000; Chaw et al., 2004; Janssen and Bremer, 2004; Chase et al., 2006; Givnish et al., 2006). In these studies, Alismatales plot as sister to a strongly supported clade consisting of all other monocots except for Acorales, and the node separating the Alismatales (including Aponogetonaceae) from the rest of the monocots is the second most basal divergence point in the monocot tree (e.g. Chase et al., 2006; Givnish et al., 2006). Using molecular clock dating, calibrated by fossil plants, Bremer (2000) estimated the origin of monocots at ca 134 Ma, and Janssen and Bremer (2004) concluded that several extant monocot lineages must date back to the Cretaceous. According to them, the Alismatales are believed to have diverged from other monocots in the Early Cretaceous, ca 131 Ma. Within the Alismatales, further divergence is suggested to have taken place during the Early to Late Cretaceous, estimating the time of origin for Araceae to be ca 128 Ma, the Tofieldiaceae to be ca 124 Ma, the Aponogetonaceae to be ca 98 Ma, the Scheuchzeriaceae to be ca 92 Ma, the Butomaceae and Hydrocharitaceae to be ca 88 Ma, and the Juncaginaceae to be ca 82 Ma. Other families of the Alismatales are believed to have diverged in the period from around the Cretaceous–Paleocene boundary and until the middle Eocene (Janssen and Bremer, 2004). Until now only Araceae appears to have a profound and reliable Cretaceous fossil record supporting these estimations. Fossil remains documenting the occurrence of other Alismatales families only become “prominent” in the early Cenozoic or even later (e.g. Daghlian, 1981; Herendeen and Crane, 1995; Stockey, 2006).

In the Northern Hemisphere the fossil record of basal monocots (Acorales and Alismatales) extends back to the Cretaceous. The Acorales have a scattered record from the Eocene, but the Araceae (Alismatales) seem to have an extensive fossil record from the early Cretaceous. Other Alismatales, e.g. potamogetonids are also sparsely represented from the early Cretaceous, but other members of this group, e.g. Alismataceae, Butomaceae, and Hydrocharitaceae are known only from the early Cenozoic and onwards (e.g. Daghlian, 1981; Herendeen and Crane, 1995; Gandolfo et al., 2000; Stockey, 2006; Friis et al., 2011). Despite these records, fossil monocots are not commonly known from the Cretaceous, especially when compared to the rich dicot record. The reason for the

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lack or under-representation of fossil monocots is most likely related to (*i*) the dominantly herbaceous growth forms within this group, (*ii*) the fact that monocots represent only a small part of the modern angiosperm flora (ca 22%), (*iii*) that most of the monocots are insect-pollinated and produce relatively few pollen grains, and (*iv*) that macrofossils of monocots often lack characteristic universal morphological synapomorphies (e.g. Herendeen and Crane, 1995; Gandolfo et al., 2000; Friis et al., 2011). Nevertheless, when present and providing diagnostic characteristics, the fossil record of monocots can provide important data about the first occurrence of monocot lineages and their mode of diversification.

Until now the scarce fossil records of *Aponogeton* L. f. (Aponogetonaceae, Alismatales) have been questioned as most of them are fragmentary leaves of uncertain affinity and pollen grains studied using LM only (e.g. Thanikaimoni, 1985; Riley and Stockey, 2004). Even though fossil leaf parts of *Aponogeton* can be difficult to distinguish from other monocots, such as for example Potamogetonaceae (e.g. Golovneva, 1997, 2000; Riley and Stockey, 2004), pollen grains of *Aponogeton* are very characteristic and when properly studied cannot be confused with any other monocot pollen type (e.g. Zavada, 1983; Thanikaimoni, 1985; Grayum, 1992; Furness and Banks, 2010).

Today, all 52 *Aponogeton* species are aquatic and mainly distributed in the subtropical and tropical regions of Africa, Madagascar, India, Sri Lanka, Southeast Asia, New Guinea, and Australia (Table 1). The plants are herbaceous perennial, inhabiting both stagnant and running water (Table S1). They can survive dry periods by means of dormant rhizomes or tubers. The tubers produce submerged and/or floating leaves and the length of the petiole of the leaves varies with the depth of the water (van Bruggen, 1985). The inflorescences develop on long peduncles which also vary in length according to the water-depth. The inflorescences consist of one or more spikes, the flowers are usually small and sessile, and mostly spirally arranged or in rows along the rachis. The basic carpel number is 3 (and can be up to 5), the number of ovules is 2–12 per ovary, and the ripe fruit is a follicle with a distinct beak (e.g. van Bruggen, 1985). Each follicle contains many seeds, often 4–10 and sometimes 12, but rarely 2. The seeds of *Aponogeton* have easily identifiable characteristic features that are used to divide the extant species into one major group possessing seeds with a single seed coat, and one smaller group comprising seeds with a double seed coat. The seeds measure between 1 × 2 and 4 × 15 mm and have longitudinal wings or ridges. The wings and ridges are believed to have a function in hydrochoric dispersal. Single coated seeds can float on the water-surface with the help of air-filled intercellular spaces of the testa, but seeds with double coats seem to have no special floating tissue (e.g. Bouman, 1985; van Bruggen, 1985). The flowers do not produce large amounts of pollen and are insect-pollinated (Thanikaimoni, 1985). Despite the aquatic habitat of *Aponogeton* and the fact that it is often growing in or close to places where active accumulation of sediments takes place, its fossil remains are extremely rare. The lack of leaf fossils can be explained by the fact that leaf production per plant is low compared with that of woody plants, and also the leaves are very delicate and membranous and are therefore more easily destroyed when transported than hardy leaves of woody taxa, their preservation potential is very low for these reasons (cf. Ferguson, 1985). Fossil *Aponogeton* leaves may also have been misidentified as leaves of, for example, *Haemanthophyllum* or *Potamogeton* as they can look very similar (e.g. Golovneva, 1997; Riley and Stockey, 2004). The reason for the lack of fossil *Aponogeton* seeds in the palaeobotanical record could be due to the small seed size of many taxa (e.g. Bouman, 1985) for which reason they may commonly have been overlooked. On the other hand, many of the species produce larger seeds that are several mm in size and should not be missed. Misidentification should not be a problem as *Aponogeton* seeds possess very characteristic and diagnostic features (cf. Bouman, 1985; van Bruggen, 1985). Additionally, studied Cenozoic seed floras from the Northern Hemisphere are mostly of temperate to subtropical affinity and not tropical (e.g. Mai, 1995) which seems to be the optimal climate of extant *Aponogeton* (e.g. van Bruggen, 1968, 1969, 1970, 1973, 1985). The most probable explanation for the absence of *Aponogeton* in the fossil record, however, is that the seeds of *Aponogeton* are simply not preserved in the fossil record as they germinate while floating on the water surface and then sink to the bottom where they fasten their roots (e.g. Ridley, 1930; Bouman, 1985), causing the “destruction” of the seed before it would have a chance to be buried in the sediments. The lack of *Aponogeton* pollen from the palynological record is most likely the combination of two factors. Firstly, *Aponogeton* species are insect-pollinated and entomophylous plants produce considerably fewer pollen grains than wind-pollinated plants, and are consequently much rarer in palaeo-palynological assemblages (cf. Fægri and Iversen, 1989). Secondly, most previous palaeo-palynological studies used only LM to identify fossil pollen and *Aponogeton* can only be correctly determined using SEM (see Section 3.1). If *Aponogeton* pollen was present in some previously described palynofloras, which is more likely than not, it was overlooked because of its small size, rarity, and similarity to many other sulcate and/or reticulate appearing pollen grains.

In the present study we report the first unequivocal *Aponogeton* fossils worldwide, pollen grains from the late Cretaceous and Eocene of western North America and from the Eocene of West Greenland. The pollen grains are described as belonging to three different species. The origin and early divergence of *Aponogeton* is discussed in the context of the new findings and the phytogeographic history of the genus is reconsidered.

2. Material and methods

2.1. Geological and geographical background

The fossil *Aponogeton* pollen presented here originate from three different localities in North America and Greenland. The oldest pollen type is from the early Campanian Eagle Formation of the Elk Basin in Wyoming, north-western USA. The younger pollen types are from the middle Eocene Allenby Formation (Princeton Chert beds) of the Princeton Basin in British Columbia, south-western Canada, and from the middle Eocene Aumarûtigssâ Member of the Hareøen Formation on the island Hareø, West Greenland (Fig. 1).

The Elk Basin is a valley bordering the Wyoming and Montana state line in the north-western United States (Fig. 1B). Geologically, the Elk Basin is a breached and eroded anticline comprising several outcrops with both marine and terrestrial, mostly Upper Cretaceous but also lower Cenozoic, sedimentary sequences. The Eagle Formation of the Elk Basin is divided into two units. The lower unit is mostly composed of sandstones and is named the Virgelle Sandstone Member. The upper unit is named the Upper Eagle Beds and is composed of alternating sandstones, carbonaceous mudstones, siltstones, shales, clays, and lignites (Hicks, 1993; Van Boskirk, 1998). All plant macrofossils described by Van Boskirk (1998) and the sedimentary samples used for this study originate from the upper part of the Upper Eagle Beds, but from below the 1 m thick bentonite layer positioned ca 10 m from the top of the formation. Biostratigraphic and magnetostratigraphic studies of the Elk Basin by Hicks (1993) suggest that the lowest part of the Upper Eagle Beds coincides with the *Scaphites hippocrepis* III ammonite zone, and the ca. upper two third (containing fossilized plant material) of the beds lie within the *Baculites* sp. (smooth; early) zone, but both zones are characteristic of the Upper Cretaceous. Chronometric dating of the bentonite layer positioned close to the top of the Eagle Formation has resulted in an isotopic age of 81.13 ± 0.5 Ma (Hicks, 1993), suggesting that the plant bearing unit is of early Campanian age and approximately 82–81 Ma.

The Princeton Basin (Fig. 1C) is a northerly trending trough comprising various volcanic and sedimentary rock units of Eocene age that form the Princeton Group. The group is divided into two formations, the lower Cedar Formation is up to 1370 m thick and is mostly

Table 1
Species of *Aponogeton*, their distribution, pollen types, and classification.

Species	Distribution	Pollen Type (PT)	Classification of van Bruggen, 1985 sect./subsect.	Classification of Les et al., 2005 sect./subsect.	Reference for morphology and distribution
Aponogeton abyssinicus Hochst. ex A. Rich.	Democratic Republic of the Congo, Ethiopia, Kenya, Malawi, Ruanda, Somalia, Tanzania, Uganda (Africa)		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton afroviolaceus Lye	Kenya, Tanzania, Zambia, Zimbabwe (Africa)		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton angustifolius Aiton	South Africa		*Pleuranthus*/*Pleuranthus*		van Bruggen, 1985
Aponogeton appendiculatus H. Bruggen	India		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton azureus H. Bruggen	Namibia (Africa)		*Pleuranthus*/*Pleuranthus*		van Bruggen, 1985
Aponogeton bernierianus (Decaisne) Hook. f.	Eastern Madagascar	PT 4	*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton bogneri H. Bruggen	Congo		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton boivinianus Baillon ex Jumelle	Northern Madagascar		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton bullosus H. Bruggen	Atherton Tableland, northern Queensland (Australia)		*Aponogeton*/*Aponogeton*	*Flavida*/*Flavida*	van Bruggen, 1985; Hellquist and Jacobs, 1998
Aponogeton capuronii H. Bruggen	Madagascar		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton cordatus Jumelle	Madagascar		*Pleuranthus*/*Pleuranthus*		van Bruggen, 1985
Aponogeton crispus Thunb.	Southern India, Sri Lanka	PT 4	*Aponogeton*/*Aponogeton*	*Aponogeton*/*Aponogeton*	van Bruggen, 1985
Aponogeton cuneatus S.W.L. Jacobs	Queensland (Australia)				Jacobs et al., 2006
Aponogeton decaryi Jumelle	Madagascar	PT 3	*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton desertorum Zeyher ex A. Sprengel	Angola, Botswana, Democratic Republic of the Congo, Mozambique, Namibia, South Africa, Zambia, Zimbabwe	PT 5	*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton dioecus Bosser	Ankaratra Mountains (Madagascar)		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton distachyos L.f.	South Africa	PT 2	*Pleuranthus*/*Pleuranthus*	*Pleuranthus*/*Pleuranthus*	van Bruggen, 1985
Aponogeton elongatus F. Muell. ex Benth.	Queensland, New South Wales (Australia)	PT 3	*Aponogeton*/*Aponogeton*	*Flavida*/*Flavida*	Hellquist and Jacobs, 1998; Jacobs et al., 2006
Aponogeton euryspermus C.B. Hellquist & S.W.L. Jacobs	Kimberley (Australia)			*Flavida*/*Flavida*	Hellquist and Jacobs, 1998; Jacobs et al., 2006
Aponogeton fotianus Raynal	Chad (Africa)		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton gottlebei Kasselmann & Bogner	Northern Madagascar	PT 6			Kasselmann and Bogner, 2009a, 2009b
Aponogeton hexatepalus H. Bruggen	Southwest Western Australia	PT 1	*Aponogeton*/*Polystachys*	*Viridis*/*Viridis*	van Bruggen, 1985; Hellquist and Jacobs, 1998
Aponogeton jacobsenii H. Bruggen	Sri Lanka	PT 5	*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton junceus Lehm.	Angola, Lesotho, Namibia, South Africa, Zambia, Zimbabwe	PT 5	*Pleuranthus*/*Pleuranthus*		van Bruggen, 1985
Aponogeton kimberleyensis C.B. Hellquist & S.W.L. Jacobs	Western Kimberley (Australia)			*Flavida*/*Flavida*	Hellquist and Jacobs, 1998
Aponogeton lakhonensis A. Camus	India, Thailand, Cambodia, Vietnam, China, Indonesia,		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton lancesmithii C.B. Hellquist & S.W.L. Jacobs	Innisfail District, Far North Queensland (Australia)			*Flavida*/*Flavida*	Hellquist and Jacobs, 1998
Aponogeton longiplumulosus H. Bruggen	Northwest Madagascar		*Aponogeton*/*Polystachys*	*Aponogeton*/*Polystachys*	van Bruggen, 1985
Aponogeton loriae Martelli	Papua		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton madagascariensis (Mirbel) H. Bruggen	Madagascar	PT 2	*Aponogeton*/*Polystachys*	*Aponogeton*/*Polystachys*	van Bruggen, 1985
Aponogeton masoalaensis Bogner	Cap Masoala (Madagascar)	PT 1			Bogner, 2002
Aponogeton natalensis D. Oliver	Botswana, South Africa	PT 5			van Bruggen, 1985
Aponogeton natans (L.) Engl. & K. Krause	India, Sri Lanka	PT 5	*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton nudiflorus Peter	Ethiopia, Kenya, Somalia, Tanzania (Africa)		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton proliferus C.B. Hellquist & S.W.L. Jacobs	Northern Queensland (Australia)			*Flavida*/*Flavida*	Hellquist and Jacobs, 1998
Aponogeton queenslandicus H. Bruggen	Northeast Northern Territory and Queensland (Australia)	PT 3	*Aponogeton*/*Aponogeton*	*Flavida*/*Flavida*	van Bruggen, 1985; Hellquist and Jacobs, 1998
Aponogeton ranunculiflorus Jacot Guill. & Marais	Lesotho and South Africa		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton rehmannii D. Oliver	Botswana, Kenya, Mozambique, Namibia, South Africa, Zambia, Zimbabwe		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton rigidifolius H. Bruggen	Sri Lanka		*Aponogeton*/*Aponogeton*	*Aponogeton*/*Aponogeton*	van Bruggen, 1985
Aponogeton robinsonii A. Camus	Central and South Vietnam	PT 2	*Pleuranthus*/*Pleuranthus*	*Pleuranthus*/*Pleuranthus*	van Bruggen, 1985
Aponogeton satarensis Raghavan, Kulkarni & Yadav	Satara District (India)		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton stuhlmannii Engl.	Democratic Republic of the Congo, Kenya, Namibia, South Africa, Tanzania, Zambia, Zimbabwe		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton subconjugatus Schum. & Thonn.	Cameroun, Chad, Congo, Ghana, Mali, Nigeria, Senegal, Sudan, Uganda		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton tenuispicatus H. Bruggen	Antalaha (Madagascar)		*Aponogeton*/*Aponogeton*		van Bruggen, 1985

(continued on next page)

Table 1 (*continued*)

Species	Distribution	Pollen Type (PT)	Classification of van Bruggen, 1985 sect./subsect.	Classification of Les et al., 2005 sect./subsect.	Reference for morphology and distribution
Aponogeton tofus S.W.L. Jacobs	Northern Territory and Arnhem Land (Australia)				Jacobs et al., 2006
Aponogeton troupinii Raynal	Chad, Zaire (Africa)		*Aponogeton*/*Aponogeton*		van Bruggen, 1985
Aponogeton ulvaceus Baker	Madagascar	PT 6	*Aponogeton*/*Polystachys*	*Aponogeton*/*Polystachys*	van Bruggen, 1985
Aponogeton undulatus Roxb.	India, Bangla Desh, Burma, Thailand, Malaysia, Indonesia	PT 4	*Aponogeton*/*Aponogeton*	*Aponogeton*/*Aponogeton*	van Bruggen, 1985
Aponogeton vallisnerioides Baker	Cameroun, Central African Republic, Chad, Ethiopia, Ivory Coast, Kenya, Nigeria, Rwanda, Senegal, Sierra Leone, Sudan		*Pleuranthus*/*Monostachys*		van Bruggen, 1985
Aponogeton vanbruggenii C.B. Hellquist & S.W.L. Jacobs	Eastern Northern Territory and Queensland (Australia)			*Flavida*/*Flavida*	Hellquist and Jacobs, 1998
Aponogeton viridis Jumelle	Madagascar		*Aponogeton*/*Polystachys*		van Bruggen, 1985
Aponogeton womersleyi H. Bruggen	Papua	PT 3	*Aponogeton*/*Aponogeton*		van Bruggen, 1985

of volcanic origin, and the upper Allenby Formation is up to 2100 m thick and constructed of various clastic and organic sedimentary rock units (McMechan, 1983; Read, 1987, 2000). The uppermost major unit of the Allenby Formation is the Ashnola Shale, it comprises among others the Princeton Chert beds, but they are positioned in the uppermost part of the shale unit (Read, 2000; Mustoe, 2011). The samples used for this study originate from chert-bed 43, from the upper most quarter of the Princeton Chert beds. The exact age of the Princeton Chert beds is difficult to pinpoint, but several studies based on fossil mammals, fish and plants (e.g. Russell, 1935; Gazin, 1953; Rouse and Srivastava, 1970; Wilson, 1977, 1982; Cevallos-Ferriz et al., 1991; Pigg and Stockey, 1996; Stockey, 2001) suggest that they are of Eocene age. Chronometric dating indicates that most of the volcanic rocks of the Cedar Formation and the sedimentary rocks of the lower to middle part of the Allenby Formation are 53–48 Ma (e.g. Mathews, 1963, 1964; Hills and Baadsgaard, 1967; Church and Brasnet, 1983; Moss et al., 2005), and are therefore early Eocene in age. The upper part of the Allenby Formation is dated to ca 46 Ma (Read, 2000), suggesting that the Princeton Chert beds are of early middle Eocene age.

The small island of Hareø (Fig. 1D) is mostly built by extrusive lavas that are part of a much larger volcanic igneous province covering most of the Disco–Nuussuaq–Svartenhuk Halvø area in West Greenland (e.g. Hald and Pedersen, 1975; Dam et al., 2009). The strata on Hareø are divided into two formations, the Maligât Formation and the overlying Hareøen Formation. On Hareø the Maligât Formation is composed of two subsequent lava successions, the Nûluk Member (at the base) and the overlying Kanísut Member (e.g. Hald, 1976, 1977). On the southeast part of Hareø the lavas of the Kanísut Member are overlain by sediments belonging to the Aumarûtigssâ Member (Hareøen Formation). The sediments are up to 20 m thick, and composed of various conglomerates, sandstones, and siltstones, often of tuffaceous origin. Lignites are also present with frequent occurrence of retinite inclusions (e.g. Hald, 1976, 1977). The plant macrofossils from Hareø described by Heer (1883) and Nathorst (1885) originate from this formation and so do the samples we used for pollen analyses. The fossiliferous sediments are overlain by lavas of the Talerua Member. The lavas of the Talerua Member are dated to ca 39 Ma (see Schmidt et al., 2005), suggesting that the underlying sediments and fossils are slightly older. Preliminary results based on pollen analyses (Grímsson et al., unpublished data) suggest a Lutetian age for the plant bearing sediments, and that they are ca 44–40 Ma.

2.2. Preparation of sediments and segregation of fossil pollen

Sedimentary rock samples were washed and dried and the Cretaceous material from Wyoming and the Eocene material from Hareø were then hand ground in a mortar with a pestle, but the material from the Eocene of Princeton was so hard it was broken into small pieces with a hammer. The resulting powder of the Wyoming Cretaceous material and the Eocene material from Hareø was boiled in concentrated HCl for 5 min. After decanting most of the HCl liquid, the remainder was boiled for ca 10 min in HF. The Princeton Chert material was treated directly with 200 ml of cold HF for three days. The solutions were then transferred to 4 L beakers filled with water. After settling, the liquid was decanted and the remainder was boiled in HCl for 5 min. After cooling and settling most of the HCl was decanted, the remaining solution centrifuged and the deposit washed 3–4 times with water. The sample then was acetolyzed, washed again with water, and centrifuged up to 4 times. The Princeton Chert samples were so rich in organic material (cuticle parts, wood fragments, etc.) that they were also heated in a sodium perborate (PBS) solution to decrease the amount of organic substances. The final remaining organic material was mixed with glycerine and stored in small sample tubes.

The fossil *Aponogeton* pollen grains were investigated both by LM and SEM, using the single grain technique (Zetter, 1989; Hesse et al., 2009). This technique has proved to be very useful for studying particular fossil pollen/spore and complete palynofloras, compared with using LM only. This method gives an additional detailed SEM study of the sculpturing elements of the same individual pollen/spore studied under LM and therefore allows for a more accurate systematic identification to be made (e.g. Grímsson et al., 2008; Denk et al., 2010; Grímsson et al., 2011a,b; Grímsson and Zetter, 2011; Denk et al., 2012; Grímsson et al., 2012a,b). Drops from the sample tubes were transferred to glass slides and single *Aponogeton* pollen grains were picked out by using a preparation needle with a human nasal hair mounted on it. The grains were placed on a separate slide within fresh drops of glycerine for photography in an LM. The *Aponogeton* pollen grains then were transferred to SEM stubs by using the preparation needle and were washed with drops of absolute ethanol to remove the remaining glycerine. The stubs then were sputter coated with gold and the pollen photographed in a SEM (JEOL 6400). Each fossil pollen type is based on 3–25 individual pollen grains (depending on richness in sample) that were studied in LM and SEM.

2.3. Pollen of extant species

Flowers of extant *Aponogeton* species were obtained from the herbariums of the University of Vienna (Institute of Botany), Austria (WU), the Royal Botanical Gardens, Kew, UK (K), The National Botanical Collection in Munich (M), and the National Herbarium of the Netherlands, Leiden (NHN-L). The flowers and/or anthers were studied under a dissecting microscope. Mature anthers were removed from the flowers and transferred into large drops of acetolyzing liquid on a glass slide. The anthers then were broken gently by squeezing them with a dissecting needle to free the pollen. The slides were heated over a candle until the cell contents were removed and the pollen wall had acquired the desirable colour for photography. Some pollen grains then were transferred into drops of glycerine on new slides and photographed

Fig. 1. Geographical maps indicating positions of the 3 sample sites bearing fossil *Aponogeton* pollen. US Interstate highways are marked IH. Cities and towns are marked with black squares and fossil localities are pointed out with dark-grey pollen like symbols. For precise coordinates of outcrops see information on type locality within corresponding species descriptions.

in a LM. Other pollen grains from the same sample were transferred onto SEM stubs, sputter coated with gold, and photographed with a SEM.

2.4. Conservation of studied material

Parts of the original sedimentary rocks, all sample tubes, and SEM stubs produced under this study are stored in the collection of the Department of Palaeontology, University of Vienna, Austria (holotypes and paratypes: nos. IPUW 2012-0008 to 2012-0014).

2.5. Assessment of phylogenetic position of modern and fossil Aponogeton species

So far there has only been a single study (Les et al., 2005) that, based on selected morphological characters and two molecular regions (ITS region of the nuclear-encoded 35S rDNA cistron, i.e. the region comprising the internal transcribed spacer 1 [ITS1], the 5.8S rRNA gene [5.8S rDNA], and internal transcribed spacer 2 [ITS2]; part of the plastid *trn*K intron including the *mat*K gene), inferred an explicit molecular phylogenetic hypothesis for the genus. The sampling was, however, focussed on Australian species, for the most part described after the completion of van Bruggen's (1985) monograph, and only include one African (*Aponogeton distachyos* L. f.; out of 17 spp. in van Bruggen, 1985) and three out of 13 Madagascan species (*Aponogeton madagascariensis* (Mirbel) H. Bruggen, *Aponogeton longiplumulosus* H. Bruggen, *Aponogeton ulvaceus* Baker; File S1). Four out of nine S./S.E. Asian species were covered, but no data were included on the type species of the genus, *Aponogeton natans* (L.) Engler & Krause, from India.

The morphological matrix used here is based on the morphological matrix in Les et al. (2005), supplemented with all modern species described in van Bruggen (1968, 1969, 1970, 1973, 1985), Hellquist and Jacobs (1998), Bogner (2002), Jacobs et al. (2006), and Kasselmann and Bogner (2009a,b). Character [15], tepal colour, was rescored from (0) white/pink, (1) yellow, regarded as synapomorphy of the N. Australian sect. *Flavida* according to Les et al. (2005), and (2) green, autapomorphy of S. Australian *Aponogeton hexatepalus* H. Bruggen, to (0) white/purplish and (1) yellowish. A number of species have purple and/or violet tepals, which can be regarded as intensification of the white/pink basic type, and was scored as such by Les et al. (2005) in the case of three Madagascan species: *Aponogeton longiplumulosus* (pink or violet tepals), *Aponogeton madagascariensis* (white or violet tepals; van Bruggen, 1968, 1985), and *Aponogeton ulvaceus* (white, creamish, or violet; van Bruggen, 1985). One species from Namibia, *Aponogeton azureus* H. Bruggen, has been reported to have luminous light blue flowers; it was tentatively scored as "0". Yellow, greenish yellow and light green tepals are, however, not confined to Australian species but common outside of Australia. For instance van Bruggen (1973, 1985) reports yellow flowers for half of the African species, and one of the two New Guinean species, not covered by Les et al. (2005), is said to have greenish yellow flowers and the other yellow (van Bruggen, 1970, 1985). Thus, the separate scoring as (putative) synapomorphy and (putative) autapomorphy is problematic, and all species with yellow to green flowers were scored as "1". We scored two additional characters following van Bruggen (1968–1985): [18] filaments, widened/thickened to base (0), not thickening (1); [19] Flowers bisexual (0), unisexual (1).

The two molecular matrices include all ITS and *trn*K/*mat*K accessions available in gene banks per June, 1st, 2012, which, for the most part, represents the data assembled by Les et al. (2005). NCBI GenBank flat files were processed with GBK2FAS (Göker et al., 2009), aligned with MAFFT (Katoh et al., 2005) using the default alignment strategy, and the resultant FASTA-formatted files were read-in into MESQUITE v. 2.75 (Maddison and Maddison, 2011) for further handling.

The molecular matrices were used to infer phylogenetic trees and branch support (via non-parametric bootstrapping, Felsenstein, 1985) under maximum likelihood, using RAxML-HPC v. 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2008). The -f a option was used, that allows for fast optimisation of tree topology and substitution parameters under the per site rate model (originally "CAT" model, Stamatakis, 2006; re-labelled to PSR in newer RAxML versions) with a final slow optimisation step under the general time reversible model (GTR; Rodriguez et al., 1990) that accounts for unequal substitution rates and allowing for variation of substitution rates between sites (GTR + Γ; option -m GTRCAT). We used the fast bootstrap implementation (option -x) with the number of necessary bootstrap (pseudo)replicates determined by the extended majority rule criterion (Pattengale et al., 2009) implemented in RAxML (option -# autoMRE). In addition, distance-based phylogenetic networks (neighbour-net splits graphs; Bryant and Moulton, 2002, 2004) were reconstructed based on the morphology, ITS, and *trn*K intron matrices using SPLITSTREE4 (Huson and Bryant, 2006). In particular, the morphological data matrix shows extreme levels of homoplasy that result in largely incompatible signals, and render any tree-based inference highly problematic (see Section 4.2). In such cases, the traditionally used maximum parsimony-based tree inference typically results in a high number of equally parsimonious trees, which then are represented in the form of essentially unresolved, comb-like strict consensus trees (Les et al., 2005, p. 509, fig. 2); and would totally collapse if slightly less parsimonious solutions, e.g. all topologies requiring just one more change than the most parsimonious solutions, would be considered. On the contrary, phylogenetic networks, either based on distance matrices or tree samples, have been designed visualize and handle incompatible signal (reviewed in Huson and Bryant, 2006; see e.g. Denk and Grimm, 2009; Friis et al., 2009 for studies using morphology-based distances). For means of comparison, also the original morphological matrix by Les et al. (2005) was treated in the same way.

All used matrices and inferences are included in an online supplementary archive hosted at www.palaeogrimm.org/data/Gms13_SI.zip.

3. Systematic taxonomy

3.1. Morphological characteristics of Alismatales pollen and distinction between genera

Pollen morphologies of families and genera of the Alismatales, based on combined LM, SEM and TEM studies, have been well documented in recent years (e.g. Zavada, 1983; Chanda et al., 1988; Grayum, 1992; Furness and Banks, 2010). In the 13 families composing the Alismatales (cf. APG, 2009) various different aperture types (monosulcate, disulcate, ulcerate (monoporate), diporate, pantoporate, inaperturate, diaperturate) and sculpturing forms (psilate, perforate, granulate, microechinate, reticulate, fossulate, rugulate, etc.) have been described (see Tables 1 and 2 in Chanda et al., 1988; and Table 2 in Furness and Banks, 2010). The combinations of these characters are quite diverse, but conservative within genera and families, therefore allowing for a clear distinction between groups/taxa of the Alismatales based solely on pollen morphology. *Aponogeton* pollen is as a result easily differentiated from other pollen types of this order. *Aponogeton* and *Butomus* (Butomaceae) are the only two Alismatales genera producing monosulcate pollen. Since the sculpturing of the *Aponogeton* pollen is reticulate and the muri are mostly broad and always with a microechinate to echinate suprasculpture, but the *Butomus* pollen is reticulate (heterobrochate) and with narrow and psilate muri (e.g. Zavada, 1983; Thanikaimoni, 1985; Grayum, 1992; Furness and Banks, 2010), they are easily distinguished.

3.2. Basis for pollen descriptions

The pollen descriptions presented here include the most diagnostic features observed both in LM and SEM. The terminology follows mostly Punt et al. (2007) and Hesse et al. (2009). The classification down to family level and author names of orders and families follows the APG III (2009). The three new fossil pollen species described in this paper are arranged by their stratigraphic age, the older fossils being listed first. The descriptions contain no synonyms as there are no fossil records of these pollen taxa prior to this study. Authors of generic and extant species names discussed in the text are cited according to most recent publications dealing with the particular taxon, i.e. van Bruggen (1985), Hellquist and Jacobs (1998), Bogner (2002), Jacobs et al. (2006), Kasselmann and Bogner (2009b).

3.3. Systematic palaeobotany

3.3.1. Alismatales R.Br. ex Bercht. & J.Presl

3.3.2. Aponogetonaceae Planch

3.3.3. Aponogeton L.f

Generic pollen diagnosis: The genus, *Aponogeton*, was originally described by Carl Linnaeus the Younger (Linnaeus fil, 1781; Pl. 32). The description of the type species *Aponogeton natans* (Krause and Engler, 1906) does not include an account of its pollen, and the pollen of this species has not been described independently by later authors. The following descriptions of fossil *Aponogeton* pollen grains are therefore based on the combination of pollen descriptions (combined LM and SEM, sometimes also TEM) from several extant *Aponogeton* species described and figured by Zavada (1983), Thanikaimoni (1985), Sun et al. (2002), Kasselmann and Bogner (2009a), and Furness and Banks (2010), and our own investigations of extant species observed or re-investigated especially for this study (Table 2). Additional measurements and/or characters obtained from the fossil species are also included. We agree mostly with the descriptions provided by previous studies with the following additions and adjustments: Pollen monad, heteropolar, oblate to spheroidal, circular, elliptic to boat-shaped in equatorial view; equatorial diameter 16–35 μm wide, polar axis 12–28 μm; sulcate; exine 0.7–1.5 μm thick, nexine thinner than sexine (LM), footlayer thin, pluricolumellate, columellae short, varying in thickness, tectum thick (TEM); semitectate, sculpturing reticulate in LM, reticulate, microreticulate to perforate, and microechinate to echinate in SEM; muri narrow to broad, flattened to convex in cross-section, straight to sinuous, 0.5–2.5 μm across (SEM); lumina circular to polygonal or irregular in outline, 0.2–2.8 μm across, sometimes with free-standing columellae or echini (SEM); echini positioned on intersection of muri, mostly centred, 4–9 echini around each lumen, at regular to irregular intervals, sometimes forming groups, echini 0.2–1.5 μm long, conical in shape, sometimes fused; aperture membrane microechinate (SEM).

3.3.3.1. Aponogeton harryi sp. nov. (Plate I, 1–6)

Holotype: IPUW 2012–0008 (Plate I, 1–3).
Paratype: IPUW 2012–0009 (Plate I, 4–6).
Type locality: VB9608 (Van Boskirk, 1998), Park County, Wyoming, USA (ca 44°59′N, 108°52′W).
Stratigraphy: Upper Eagle Beds, Eagle Formation, Montana Group, Elk Basin (e.g. Hicks, 1993; Van Boskirk, 1998).
Age: Late Cretaceous (Lower Campanian), 82–81 Ma (Hicks, 1993).
Etymology: The fossil species is named after the late Harry W. E. van Bruggen (06.12.1927–08.02.2010), a Dutch amateur botanist, in recognition of his excellent work on the morphology, species diversity, and geographical distribution of extant Aponogetonaceae.
Description: Pollen monad, heteropolar, oblate, elliptic in equatorial and polar views (LM); equatorial diameter 22–24 μm wide in LM, 21–23 μm in SEM, polar axis 16–17 μm long in LM, 15–16 μm in SEM; sulcate; exine 0.8–1.0 μm thick, nexine thinner than sexine; semitectate, sculpturing reticulate in LM, reticulate to microreticulate and microechinate to echinate in SEM, muri broad and flattened in cross-section, muri psilate to irregularly granulate, mostly straight, 0.7–1.3 μm across, pluricolumellate, columellae short, lumina circular to polygonal, 0.6–1.7 μm across (long axis), sometimes with free-standing columellae, nexine perforate, echini positioned on intersection of muri, 5–7 around each lumen, at regular interval, echini 0.8–1.2 μm long, mostly around 1 μm long, conical in shape.
Comparison: The *Aponogeton harryi* pollen grains fit within the general size documented for various *Aponogeton* species (Table 2), they show the same outline and shape, aperture type, wall thickness, and ornamentation (muri, lumina, echini). The most distinct character differentiating this fossil pollen species from the up to now investigated extant taxa is the slightly longer echini. This fossil pollen type corresponds best to extant pollen that can be found in Pollen Type 3 (see Section 4.1).
Remarks: This is the earliest fossil record of *Aponogeton* from North America, and also the only reliable Mesozoic (Upper Cretaceous; lower Campanian) fossil record of this genus worldwide.

3.3.3.2. Aponogeton longispinosum sp. nov. (Plate I, 7–12)

Holotype: IPUW 2012–0010 (Plate I, 7–9).
Paratype: IPUW 2012–0011 (Plate I, 10–12).
Type locality: Has been referred to as Location I (Boneham, 1968), the Ashnola Chert (Wilson, 1980) and the Princeton Chert locality (most authors; e.g. Pigg and Stockey, 1996; Stockey, 2001). Outcrop positioned ca 8–9 km south of Princeton, along the east bank of the Similkameen River, British Columbia, Canada (ca 49°22′N, 120°32′W).
Stratigraphy: Princeton Chert beds, Ashnola Shale, Allenby Formation, Princeton Basin (e.g. McMechan, 1983; Pigg and Stockey, 1996; Stockey, 2001; Mustoe, 2011).
Age: Lutetian, early middle Eocene, ca 46 Ma (Read, 2000).
Etymology: The name given to this fossil pollen type emphasizes how characteristic the long echini are for the general appearance of the grain. Long = *longus* in Latin, and echinate = thorny = *spinosus* in Latin.
Description: Pollen monad, heteropolar, oblate, elliptic in equatorial and polar views (LM); equatorial diameter 16–22 μm wide in LM, 16–20 in SEM, polar axis 12–18 μm long in LM, 11–15 μm in SEM; sulcate; exine 0.9–1.0 μm thick, nexine thinner than sexine; semitectate, sculpturing reticulate in LM, reticulate to microreticulate and microechinate to echinate in SEM, muri broad and slightly convex in cross-section, often sinuous, muri psilate to irregularly granulate and sometimes partly fossulate, muri 0.5–1.2 μm across, pluricolumellate, columellae very short, lumina rounded/triangular to polygonal, decreasing towards sulcus, lumina 0.7–1.7 μm across, sometimes with free-standing columellae, nexine perforate, echini mostly positioned on intersection of muri, 5–7 around each lumen, mostly at regular intervals, echini sometimes fused together (2–3), echini 0.9–1.5 μm long, mostly longer than 1 μm, long narrowly conical in shape (SEM).
Comparison: The *Aponogeton longispinosum* pollen grains compare well with extant pollen of studied *Aponogeton* species (Table 2). The fossil grains have a similar size range, show the same outline and shape, aperture type, wall thickness, and ornamentation (muri, lumina, echini). This fossil pollen type corresponds best to extant pollen of the Pollen Type 1 (see Section 4.1). As with the older *Aponogeton harryi*, one of the characters differentiating *A. longispinosum* from pollen of extant taxa is the long echini. The Eocene *A. longispinosum* is slightly smaller than the Cretaceous *A. harryi*. The muri of *A. longispinosum* are more convex as compared to the flattened muri of *A. harryi*. The echini of *A. longispinosum* are frequently longer than the echini of *A. harryi* and much narrower, and they are more often fused together.
Remarks: There is a high variation in the construction and configuration of echini around the lumina. In some pollen the echini are mostly originating from a single base on the intersection of the muri, but can often be composed of up to 3 fused echini bases. The number of echini seems to increase around the sulcus on many pollen grains, but in reality they are more densely packed due to the rapid decreasing size of the lumina.

This is the second oldest fossil record of *Aponogeton* from North America, and the oldest Cenozoic fossil record of this genus worldwide.

3.3.3.3. Aponogeton hareoensis sp. nov. (Plate II)

Holotype: IPUW 2012–0012 (Plate II, 1–3, 10).
Paratypes: IPUW 2012–0013 (Plate II, 4–6), IPUW 2012–0014 (Plate II, 7–9, 11).
Type locality: Aumarûtigssâ (Heer, 1883; Hald, 1977), on the southeast coast of the island Hareø, West Greenland (ca 70°24′N, 54°41′W).
Stratigraphy: Retinite Lignite Bed, Aumarûtigssâ Member, Hareøen Formation, West Greenland Basalt Group (e.g. Hald, 1976, 1977; Dam et al., 2009).
Age: Lutetian, late middle Eocene, ca 44–40 Ma (see also Schmidt et al., 2005).
Etymology: This pollen type is named after the island Hareø, as the fossils are only known from sediments of that island.
Description: Pollen monad, heteropolar, oblate, elliptic in equatorial and polar views (LM); equatorial diameter 26–28 μm wide in LM, 27–30 in SEM, polar axis 15–23 μm long in LM, 15–22 μm in SEM; sulcate; exine 0.9–1.1 μm thick, nexine thinner than sexine; semitectate, sculpturing reticulate in LM, reticulate to microreticulate and microechinate in SEM, muri broad and flattened to slightly convex in cross-section, muri psilate to irregularly granulate muri 0.7–1.2 μm across, pluricolumellate, columellae very short, lumina polygonal, decreasing towards sulcus, lumina 0.5–1.3 μm across, sometimes with short free-standing columellae, echini mostly positioned on intersection of muri, 5–7 around each lumen, at regular intervals, echini rarely fused together (2), echini 0.7–1.0 μm long, conical in shape (SEM).
Comparison: This fossil pollen type corresponds best to extant pollen of the Pollen Type 2 (see part 4.1). *Aponogeton hareoensis* has similar muri as *Aponogeton longispinosum*, but the echini are shorter than in both *Aponogeton harryi* and *A. longispinosum*, and the echini are also broader and with a much wider base.
Remarks: Pollen grains of this type show a considerable variation in the width of the lumen. There is general trend for smaller lumen on the distal side compared to the proximal side, and the lumen becomes gradually smaller towards the sulcus.

4. Morphological and molecular differentiation in modern species of *Aponogeton*

4.1. Pollen types in modern species

The separation of *Aponogeton* species based on pollen using LM is hindered by the low magnification (max. 1000–1500) and the low

Table 2
Comparison between fossil and extant *Aponogeton* pollen.

	Cretaceous	Eocene		Extant			
	USA	Canada	West Greenland	S & E Africa	Southern Africa		
Taxa	*A. harryi*	*A. longispinosum*	*A. hareoensis*	*A. desertorum*	*A. junceus*	*A. natalensis*	*A. distachyos*
Aperture	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus
Shape	Oblate	Oblate	Oblate	Oblate	Oblate	Oblate	Oblate
Outline in p.v.	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Size in LM (eq. d./p.a.; μm)	22–24/16–17	16–22/12–18	26–28/15–25	23–29/16–19	23–29/13–23	24–27/13–20	22–36/17–27
Size in SEM (eq. d./p.a.; μm)	21–23/15–16	16–20/11–15	27–30/15–22	18–24/11–16	14–25/10–16	20–24/11–16	31–34/21–25
Exine thickness in LM (μm)	0.8–1.0	0.9–1.0	0.9–1.1	0.7–1.1	0.8–1.1	0.7–1.2	0.7–1.3
Nexine vs. Sexine	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner
Sculpturing in LM	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Sculpturing in SEM	Reticulate to microreticulate, microechinate to echinate	Reticulate, microechinate to echinate	Microreticulate	Microreticulate, microecinate	Microreticulate, microecinate	Microreticulate, microecinate	Microreticulate, microechinate
Muri (SEM)	Short, wide, flattened	long, narrow, angular at intersections	Short, slightly convex	Short, convex	Short, convex	Short, convex	Short, slightly convex
Lumina (SEM)	Rounded to angular	Angular to polygonal		Irregular, small	Irregular, small	Irregular, small	Irregular
Nr. of echini around lumina (SEM)	5–7	5–7	5–6	5–7	5–7	4–7	5–8
Position of echini (SEM)	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred
Form of echini on muri (SEM)	Long conical, rarely fused (2)	Long, narrow conical, sometimes fused (2–3)	Long, wide base	Short	Short	Short	Long, wide base, sometimes fused (2)
Length of echini in SEM (μm)	0.8–1.2	0.9–1.5	0.7–1.0	0.2–0.3	0.2–0.3	0.2–0.3	0.8–1.1
Pollen Type	(PT 3)	(PT 1)	PT 2	PT 5	PT 5	PT 5	PT 2

	Extant					
	Madagascar					
Taxa	*A. bernierianus*	*A. decaryi*[2]	*A. gottlebei*	*A. madagascariensis*[2]	*A. ulvaceus*	*A. masoalaensis*
Aperture	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus
Shape	Oblate	Oblate	Oblate	Oblate	Oblate	Oblate
Outline in p.v.	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Size in LM (eq. d./p.a.; μm)	18–26/12–18	22–27/20–22	17–26/	27–35/19–23	17–23/14–17	18–21/
Size in SEM (eq. d./p.a.; μm)	18–24/10–16	22–25/18–20	16–19/13–14	17–20/	14–18/14–16	16–18/
Exine thickness in LM (μm)	0.9–1.2	1.0	0.9–1.3	1.0–1.2	0.7–0.9	0.9–1.1
Nexine vs. Sexine	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner
Sculpturing in LM	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Sculpturing in SEM	Microreticulate, microechinate	Reticulate to microreticulate, microechinate	Microreticulate, microechinate	Microreticulate, microechinate	Microreticulate, microechinate	Reticulate, microechinate
Muri (SEM)	Short, convex	Short, wide, flattened	Very short, markedly convex, columellae spreading	Short, wide, slightly convex	Very short, markedly convex, columellae spreading	Long and narrow, straight, flattened, angular at intersections
Lumina (SEM)	Irregular	Rounded to angular	Irregular	Irregular	Irregular	Angular to polygonal
Nr. of echini around lumina (SEM)	4–7	5–6	5–9	5–7	5–9	5–8
Position of echini (SEM)	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred
Form of echini on muri (SEM)	Medium length, sometimes fused (2)	Medium length	Short	Medium length, wide base	Short	Medium length, rarely fused (2)
Length of echini in SEM (μm)	0.4–0.7	0.6–0.8	0.2–0.4	0.6–0.8	0.2–0.3	0.4–0.6
Pollen Type	PT 4	PT 3	PT 6	PT 2	PT 6	PT 1

	Extant					
	India/Sri Lanka			India/SE Asia	Vietnam	Papua
Taxa	*A. crispus*[1,2]	*A. natans*	*A. jacobsenii*[2]	*A. undulatus*	*A. robinsonii*	*A. womersleyi*
Aperture	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus	Sulcus
Shape	Oblate	Oblate	Oblate	Oblate	Oblate	Oblate
Outline in p.v.	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic	Elliptic
Size in LM (eq. d./p.a.; μm)	16–22/12–17	17–23/13–17	18–25/16–17	20–23/15–21	18–24/12–19	20–27/20–22
Size in SEM (eq. d./p.a.; μm)	16–20/14–17	15–21/	19–22/	18–24/12–14	15–21/	17–19/13–16
Exine thickness in LM (μm)	0.8–1.0	0.7–1.2	1.0	0.6–0.9	1.0–1.3	0.8–1.2
Nexine vs. Sexine	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner	Nexine thinner
Sculpturing in LM	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Sculpturing in SEM	Microreticulate, microechinate	Microreticulate, microechinate	Microreticulate, microechinate	Microreticulate, microechinate	Microreticulate, microechinate to echinate	Reticulate to microreticulate, microechinate
Muri (SEM)	Short, markedly convex	Very short, markedly convex	Very short, markedly convex	Short, wide, convex	Short, wide, slightly convex	Short, wide, flattened
Lumina (SEM)	Irregular	Irregular	Irregular	Irregular	Irregular, slit-like	Rounded to angular
Nr. of echini around lumina (SEM)	5–8	5–7	5–9	5–6	5–7	5–9
Position of echini (SEM)	Intersection of muri, centred	Intersection of muri, centred,	Intersection of muri, centred,	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred
Form of echini on muri (SEM)	Short	Short	Short	Medium length	Long, sometimes fused (2–3)	Medium length, sometimes fused (2–3)
Length of echini in SEM (μm)	0.4–0.5	0.2–0.4	0.3–0.5	0.4–0.7	0.8–1.2	0.6–0.8
Pollen Type	PT 4	PT 5	P T 5	PT 4	PT 2	PT 3

	Extant		
	Australia		
Taxa	*A. hexatepalus*	*A. elongatus*[2]	*A. queenslandicus*
Aperture	Sulcus	Sulcus	Sulcus
Shape	Oblate	Oblate	Oblate
Outline in p.v.	Elliptic	Elliptic	Elliptic
Size in LM (eq. d./p.a.; μm)	24–28/15–20	20–30/17–20	21–25/16–20
Size in SEM (eq. d./p.a.; μm)	23–27/	18–29/	19–25/12–19
Exine thickness in LM (μm)	0.9–1.2	0.8–1.0	1.0–1.3
Nexine vs. Sexine	Nexine thinner	Nexine thinner	Nexine thinner
Sculpturing in LM	Reticulate	Reticulate	Reticulate
Sculpturing in SEM	Reticulate, microechinate	Reticulate to microreticulate, microechinate	Reticulate to microreticulate, microechinate
Muri (SEM)	Long and narrow, straight, flattened, angular at intersections	Short, wide, flattened	Short, wide, flattened
Lumina (SEM)	Angular to polygonal	Rounded to angular	Rounded to angular
Nr. of echini around lumina (SEM)	5–8	5–9	5–7
Position of echini (SEM)	Intersection of muri, centred	Intersection of muri, centred	Intersection of muri, centred
Form of echini on muri (SEM)	Medium length, sometimes fused (2–3)	Medium length	Medium length
Length of echini in SEM (μm)	0.5–0.9	0.4–0.8	0.6–0.8
Pollen Type	PT 1	PT 3	PT 3

Note: Numbers accompanying names of extant species refer to previously figured material. 1 = Zavada (1983); 2 = Thanikaimoni (1985); 3 = Sun et al. (2002). Please note that the quantity of measurements/pollen behind every value varies considerably (from 5 to 25), and can be combined from different sources. All measurements of published species are original or calculated using scale bars (only) on figured material. LM = light microscope; SEM = scanning electron microscope; p. v. = polar view; eq. d. = equatorial diameter; p.a. = polar axis.

resolution as noticed in the work by Thanikaimoni (1985; see table 2, p. 12). The echini are too narrow to be visible with LM and other “measurable” characters (polar axis, diameter, exine thickness, size of lumina, and width of muri) are often overlapping. Also, pollen size is rarely a distinguishing character within genera because of the often large size range observed within species. We have grouped the *Aponogeton* pollen based on distinguishing characters observable under SEM only. The groups are defined based on the size, form, and arrangement of the lumina/muri and the echini (Tables 1 and 2).

Pollen Type 1: The muri are long and narrow, straight, flat, and angular at intersections, forming a reticulate sculpturing. Form of the lumina is angular, tetragonal to polygonal, and lumina are large and closely spaced. Echini are 4–9 µm long. Species with this type of pollen include *Aponogeton hexatepalus* (Plate III, 1–6) and *Aponogeton masoalaensis* Bogner (Plate III, 7–12).

Pollen Type 2: The muri are short and wide and slightly convex, forming a microreticulate sculpturing. Lumina are irregularly shaped, often slit-like. Echini are 6–1.2 µm long and with a very wide base. Species with this type of pollen include *Aponogeton distachyos* (Plate IV, 7–12), *Aponogeton robinsonii* A. Camus (Plate IV, 1–6), and *Aponogeton madagascariensis* (see plate 13, Figs. 254–256 in Thanikaimoni, 1985).

Pollen Type 3: The muri are short and very wide and flat, forming a reticulate to microreticulate sculpturing. Lumina are rounded to angular and they are wide apart and large. Echini are 0.6–0.8 µm long. Species with this type of pollen include *Aponogeton decaryi* Jumelle (see plate 9, Figs. 237–238 in Thanikaimoni, 1985), *Aponogeton elongatus* F. Muell. ex Benth. (see plate 12, Figs. 248–251 in Thanikaimoni, 1985), *Aponogeton queenslandicus* H. Bruggen (Plate V, 1–6), and *Aponogeton womersleyi* H. Bruggen (Plate V, 7–12).

Pollen Type 4: The muri are very short and wide and markedly convex, forming a microreticulate sculpturing. Lumina are irregularly shaped and they are small. Echini are 0.5–0.7 µm long. Species with this type of pollen include *Aponogeton bernierianus* (Decaisne) Hook. f. (Plate VI, 1–6), *Aponogeton crispus* Thunb. (see plate 9, Figs. 233–236 in Thanikaimoni, 1985), *Aponogeton undulatus* Roxb. (Plate VI, 7–9).

Pollen Type 5: The muri are very short and wide and markedly convex, forming a microreticulate to foveolate sculpturing. Lumina are irregularly shaped and they are very small. Echini are short, 0.2–0.5 µm

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Plate I. Fossil Cretaceous and Eocene *Aponogeton* pollen. LM (1, 4, 7, 10) and SEM (2, 3, 5, 6, 8, 9, 11, 12) micrographs of dispersed pollen.

1–3. *Aponogeton harryi* sp. nov. Late Cretaceous, Wyoming, USA. Holotype: IPUW 2012–0008.
4–6. *Aponogeton harryi* sp. nov. Late Cretaceous, Wyoming, USA. Paratype: IPUW 2012–0009.
7–9. *Aponogeton longispinosum* sp. nov. Early middle Eocene, British Columbia, Canada. Holotype: IPUW 2012–0010.
10–12. *Aponogeton longispinosum* sp. nov. Early middle Eocene, British Columbia, Canada. Paratype: IPUW 2012–0011. Scale bar 10 µm in panels 1, 4, 7, 10, and 1 µm in 2, 3, 5, 6, 8, 9, 11, 12.

Plate II. Fossil Eocene *Aponogeton* pollen. LM (1, 4, 7) and SEM (2, 3, 5, 6, 8, 9–11) micrographs of dispersed pollen. (see on page 172)

1–3, 10. *Aponogeton hareoensis* sp. nov. Middle Eocene, Hareø (Hareoen), Western Greenland. Holotype: IPUW 2012–0012.
4–6. *Aponogeton hareoensis* sp. nov. Middle Eocene, Hareø (Hareoen), Western Greenland. Paratype: IPUW 2012–0013.
7–9, 11. *Aponogeton hareoensis* sp. nov. Middle Eocene, Hareø (Hareoen), Western Greenland. Paratype: IPUW 2012–0014. Scale bar 10 µm in panels 1, 4, 5, 7, 8, and 1 µm in 2, 3, 6, 9, 10, 11.

Plate III. Extant *Aponogeton* pollen. Pollen Type 1. (see on page 173)

1–6. *Aponogeton hexatepalus*, D. den Hartog, No. 48635 (NHN-L).
7–12. *Aponogeton masoalaensis*, J. Bogner, No. 2087 (M). Scale bar 10 µm in panels 1, 4, 5, 7, 10, and 1 µm in 2, 3, 6, 8, 9, 11, 12.

Plate IV. Extant *Aponogeton* pollen. Pollen Type 2. (see on page 174)

1–6. *Aponogeton robinsonii*, P.C. Boyce, No. 1189 (K).
7–12. *Aponogeton distachyos*, Sennen, Acq. J. No. 2022 (WU). Scale bar 10 µm in panels 1, 4, 7, 8, 10, 11, and 1 µm in 2, 3, 5, 6, 9, 12.

Plate V. Extant *Aponogeton* pollen. Pollen Type 3. (see on page 175)

1–6. *Aponogeton womersleyi*, Womersley & Havel, No. 1717 (M).
7–12. *Aponogeton queenslandicus*, H.S. Mckee, No. 10350 (K). Scale bar 10 µm in panels 1, 4, 7, 10, and 1 µm in 2, 3, 5, 6, 8, 9, 11, 12.

Plate VI. Extant *Aponogeton* pollen. Pollen Type 4. (see on page 176)

1–6. *Aponogeton bernierianus*, Hildebrandt, No. 2645 (WU).
7–12. *Aponogeton undulatus*, Collector unknown, Acq. J. No. 737 (WU). Scale bar 10 µm in panels 1, 4, 7, 10, and 1 µm in 2, 3, 5, 6, 8, 9, 11, 12.

Plate VII. Extant *Aponogeton* pollen. Pollen Type 5. (see on page 177)

1–3. *Aponogeton desertorum*, R. Schlechter, Acq. J. No. 2693 (WU).
4–6. *Aponogeton junceus*, Collector unknown, Acq. J. No. 1367 (WU).
7–9. *Aponogeton natalensis*, R. Schlechter, Acq. J. No. 1608 (WU).
10–12. *Aponogeton natans*, F.R. Fosberg & M.-H. Sachet, No. 52850 (K). Scale bar 10 µm in panels 1, 4, 7, 10, and 1 µm in 2, 3, 5, 6, 8, 9, 11, 12.

Plate VIII. Extant *Aponogeton* pollen. Pollen Type 6. (see on page 178)

1–6. *Aponogeton gottlebei*, C. Kasselmann, No. 955 (M).
7–12. *Aponogeton ulvaceus*, J. Bogner, No. 2700 (M). Scale bar 10 µm in panels 1, 4, 7, 10, and 1 µm in 2, 3, 5, 6, 8, 9, 11, 12.

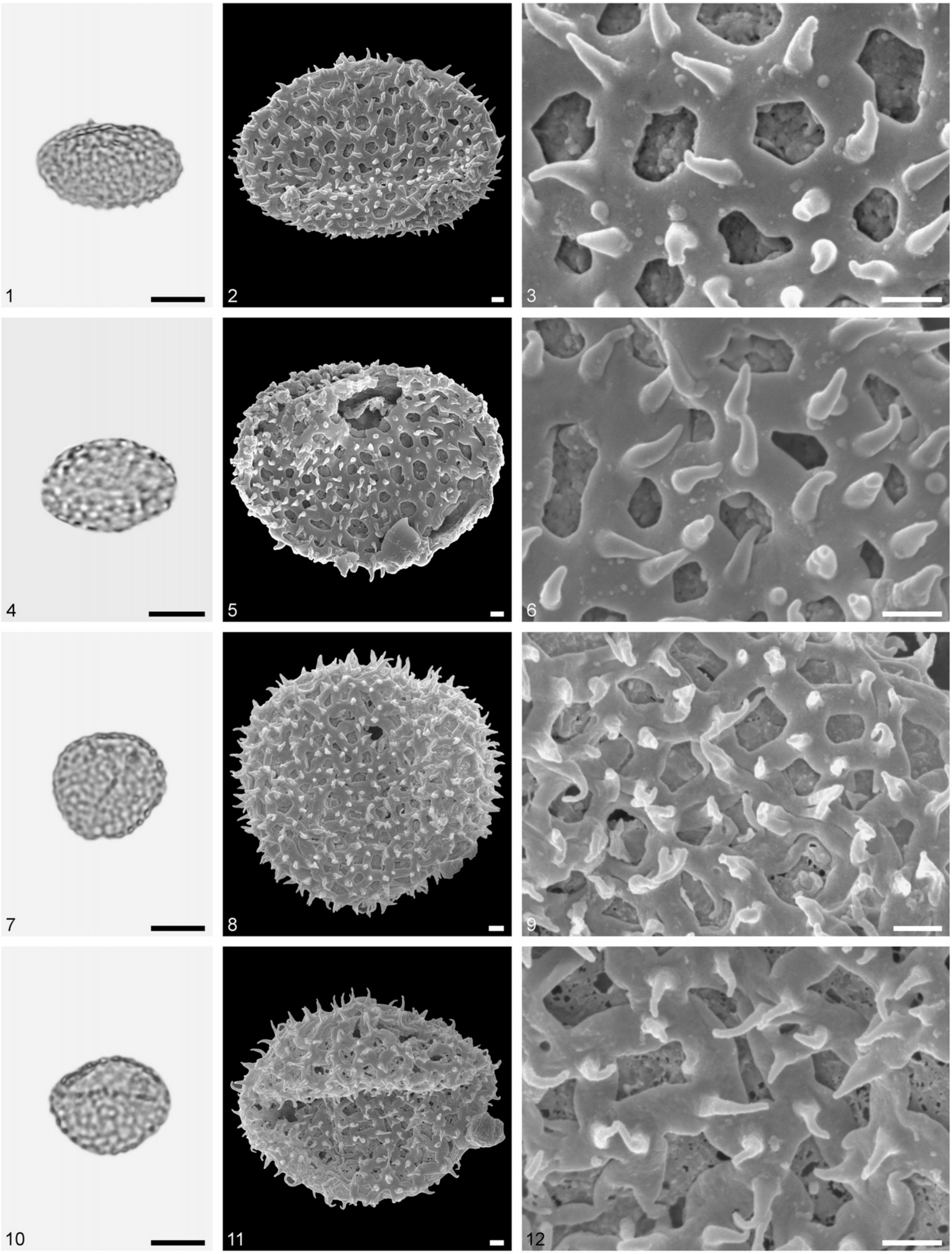

Plate I.

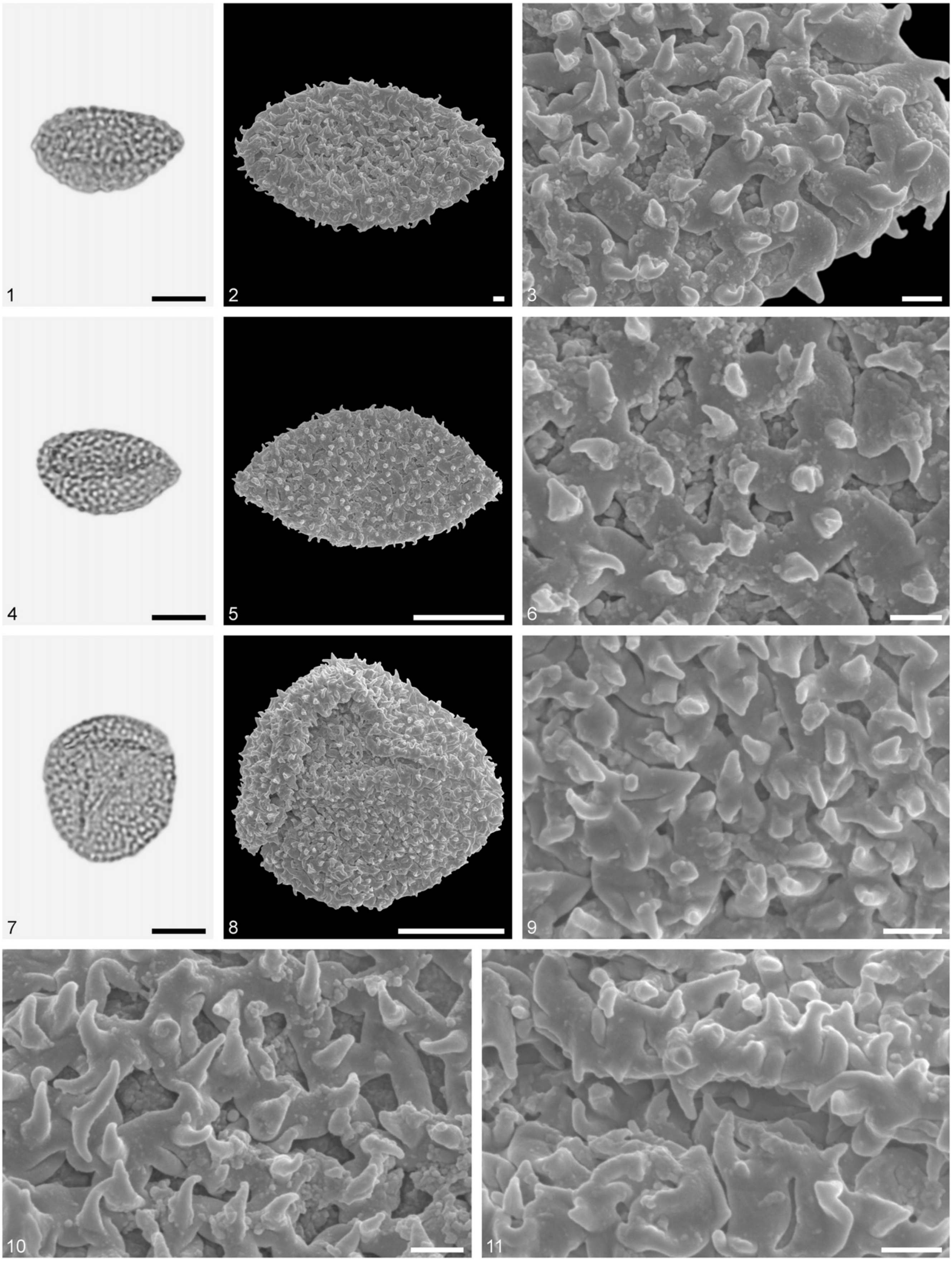

Plate II (*caption on page 170*).

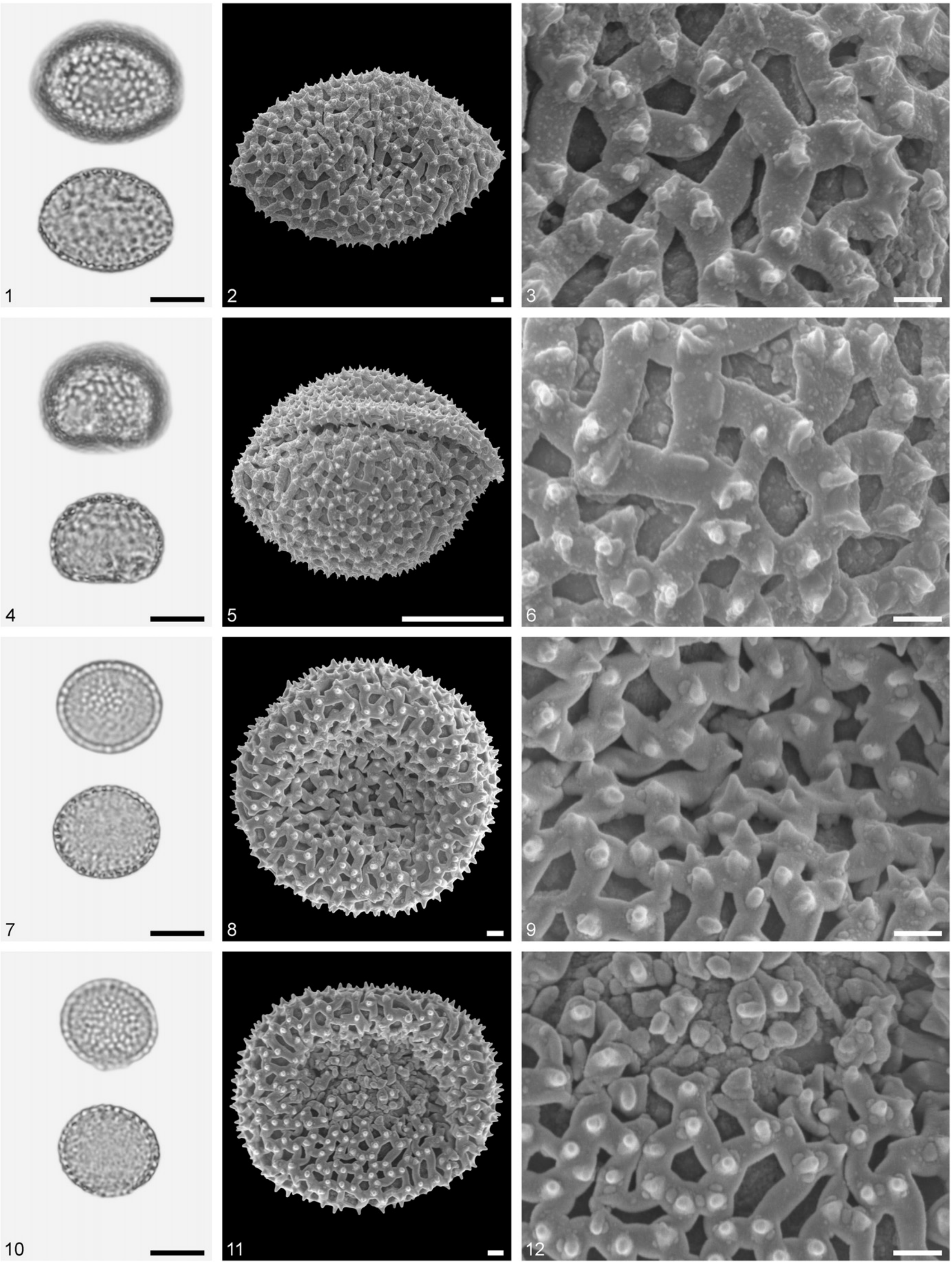

Plate III (*caption on page 170*).

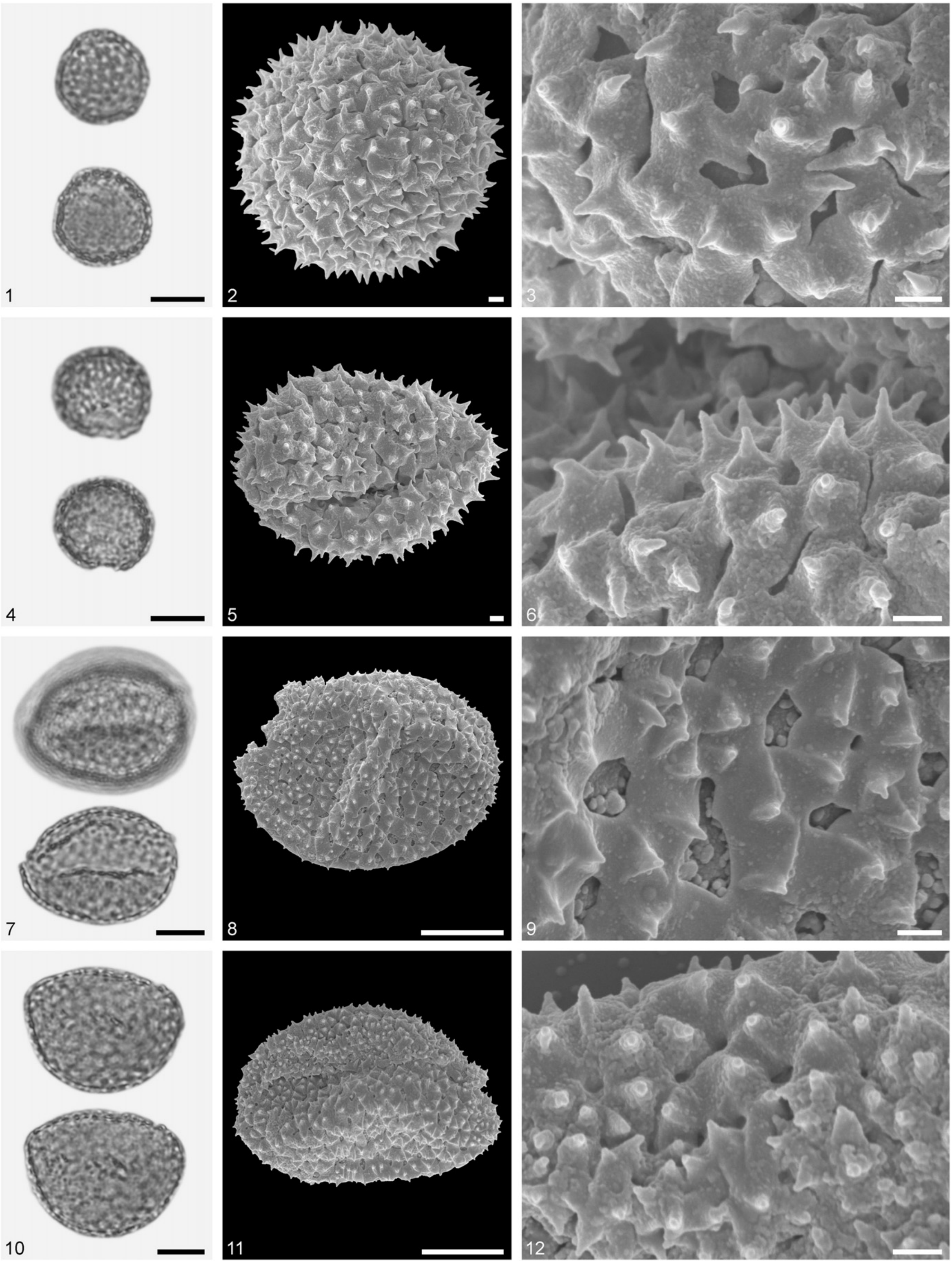

Plate IV (*caption on page 170*).

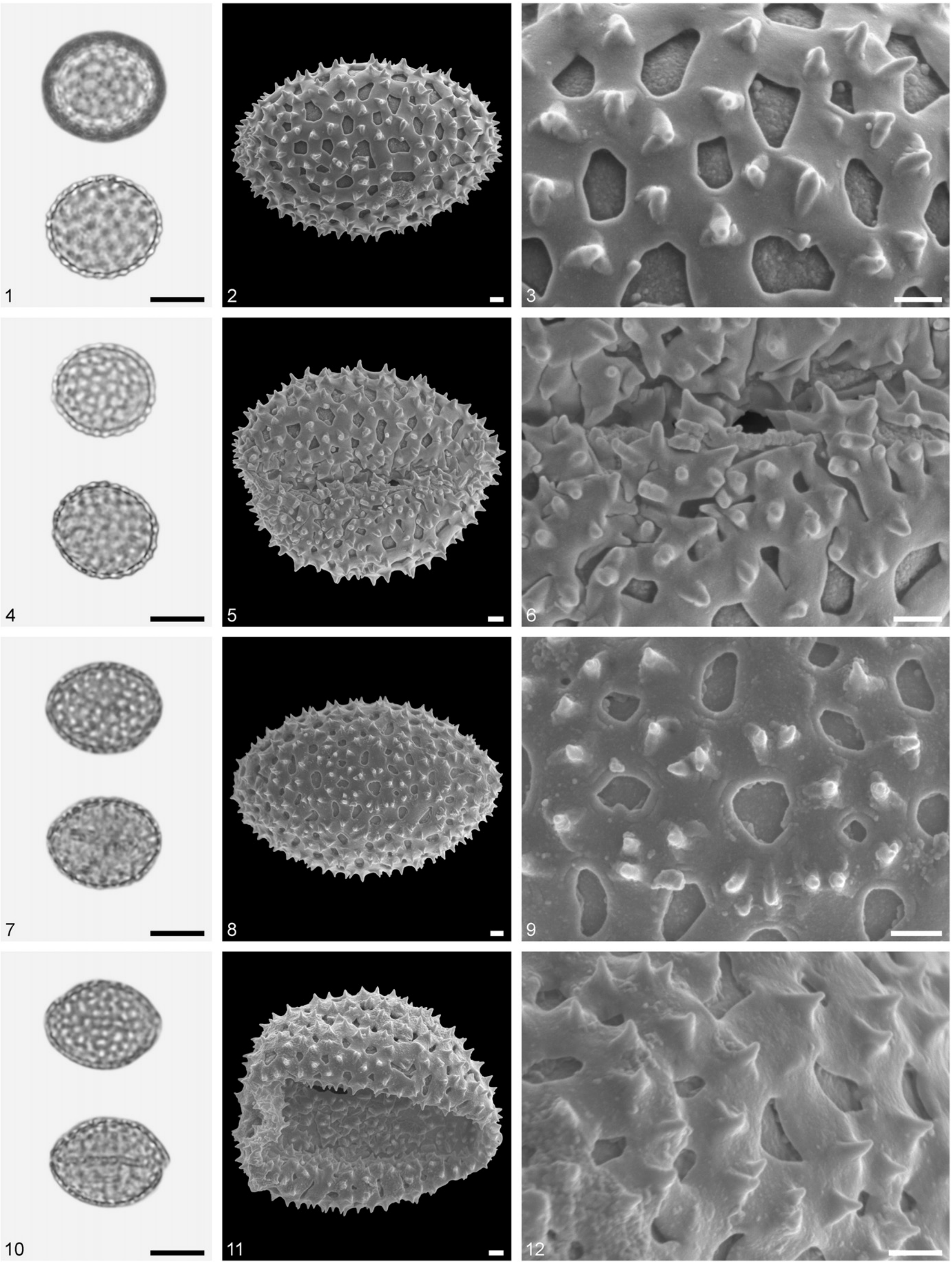

Plate V (*caption on page 170*).

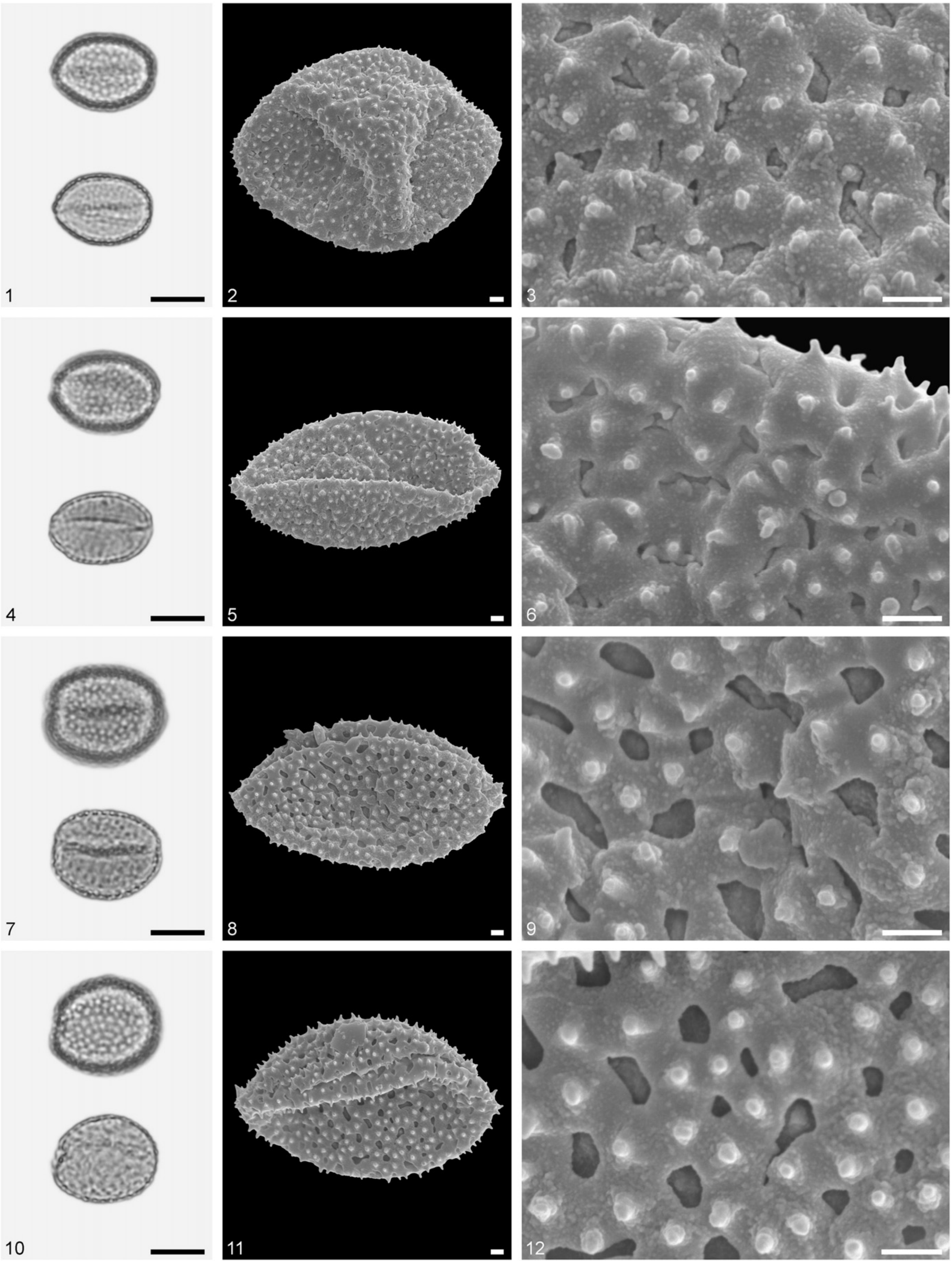

Plate VI (*caption on page 170*).

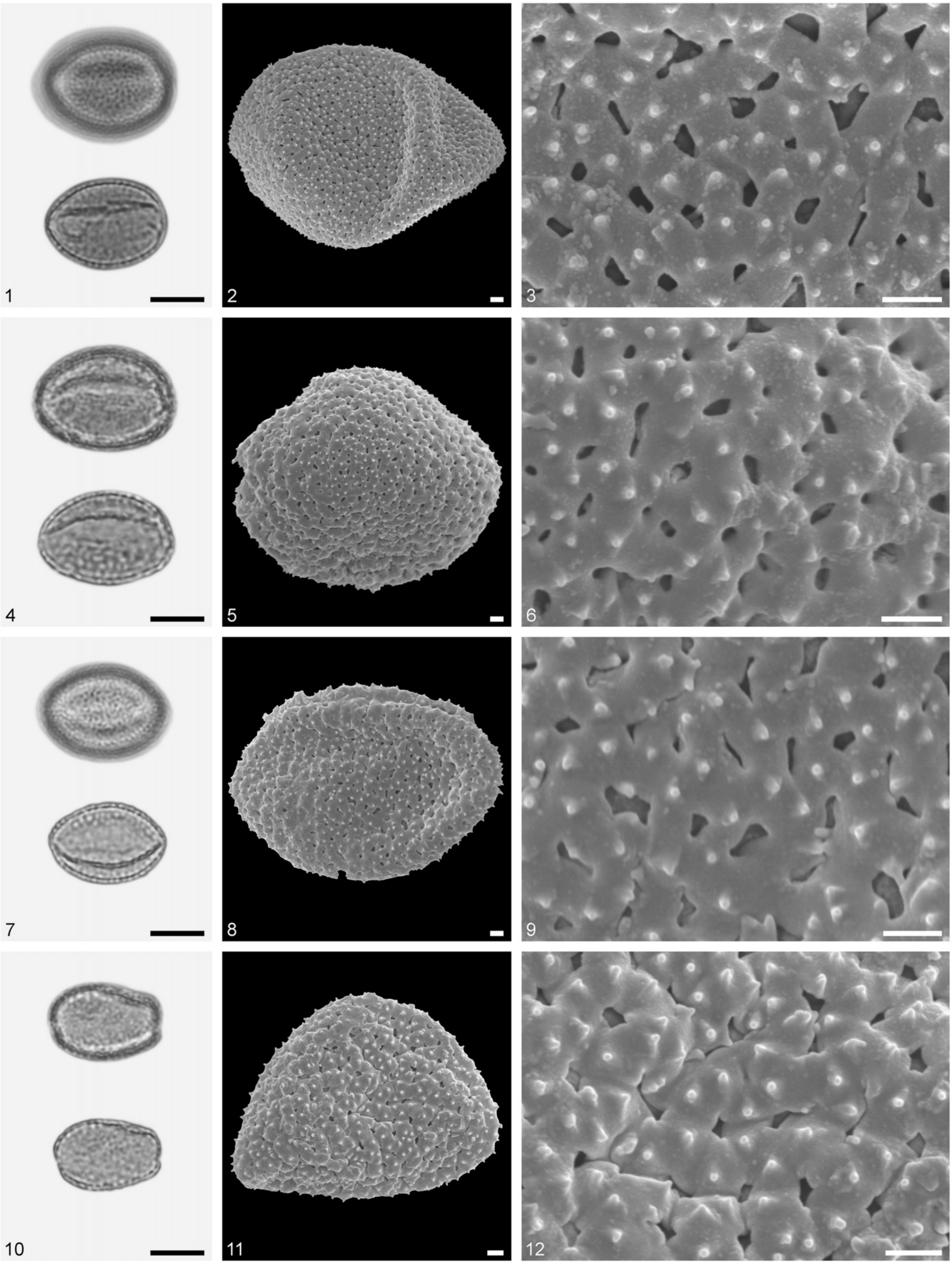

Plate VII (*caption on page 170*).

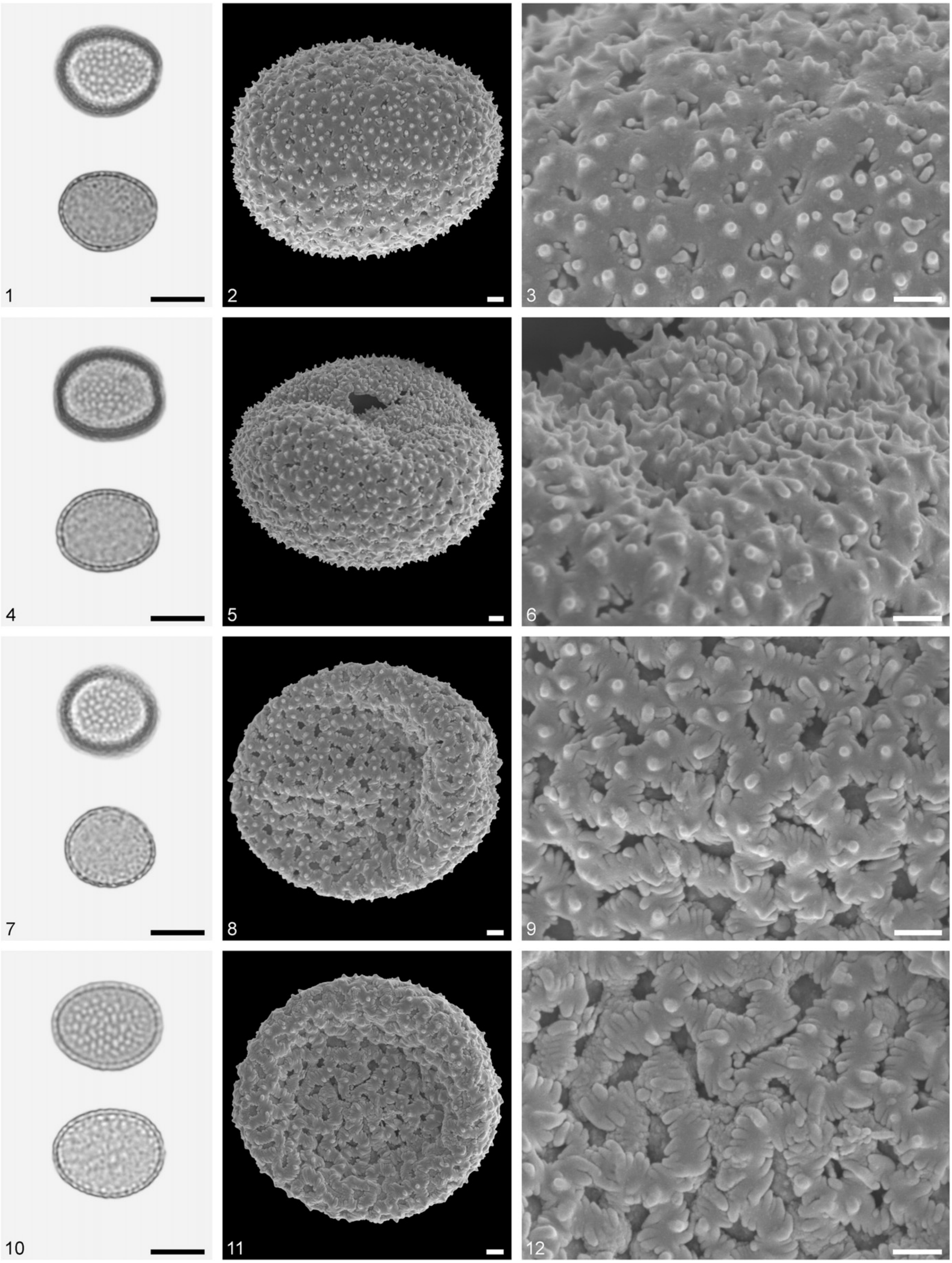

Plate VIII (*caption on page 170*).

long. Species with this type of pollen include *Aponogeton desertorum* Zeyher ex A. Sprengel (Plate VII, 1–3), *Aponogeton natalensis* D. Oliver (Plate VII, 7–9), *Aponogeton junceus* Lehm. (Plate VII, 4–6), *Aponogeton jacobsenii* H. Bruggen (see plate 13, Figs. 252–253 in Thanikaimoni, 1985), and *Aponogeton natans* (Plate VII, 10–12).

Pollen Type 6: The muri are very short and markedly convex, forming a microreticulate sculpturing. There are numerous columellae spreading out from the sides of the muri. Form of the lumina is irregular and they are small to very small. Echini are short, 0.2–0.4 μm long.

Species with this type of pollen include *Aponogeton gottlebei* Kasselmann & Bogner (Plate VIII, 1–6) and *Aponogeton ulvaceus* (Plate VIII, 7–12).

4.2. Morphological homoplasy in modern Aponogeton

Fig. 2 shows the level of incompatible signal due to the high level of homoplasy in the morphological data used by Les et al. (2005) and how it correlates to molecular data (Fig. 2B, cf. Section 4.3) and the here defined pollen types (see Section 4.1). The graph is generally spider web-like, with the tropical Australian taxa (sect. *Flavida* according Les et al., 2005) placed apart from the Asian species and the temperate species *Aponogeton distachyos* from S.W. South Africa (Cape Province; Pollen Type 2) and *Aponogeton hexatepalus* from S.W. Australia (Pollen Type 1), which agrees to molecular data of these species. Two of the Madagascan species, including *Aponogeton madagascariensis* with pollen of the same type as found in *A. distachyos*, are phylogenetically close to the Asian taxa (Pollen Type 3) based on their morphology, whereas the third species, *Aponogeton ulvaceus*, nests within sect. *Flavida*, which is in stark contrast to its molecular-systematic affinities. Morphological divergence within section *Flavida* is higher than within their putative Asian and Madagascan sister groups. If all extant species of *Aponogeton* are added to the matrix, the phylogenetic structure of the morphological signals becomes even less clear (Fig. 3). The clear differentiation between the tropical N. Australian species of section *Flavida* and their putative Asian and Madagascan relatives (Fig. 2B) dissolves: species from continental Africa, Madagascar, the Indian subcontinent, and S.E. Asia are nested within the group comprising all members of section *Flavida* according to Les et al. (2005; Group A in Fig. 3). Group A also includes both New Guinean species of *Aponogeton* and *Aponogeton lakhonensis* A. Camus widespread in S.E. Asia (Indochina; van Bruggen, 1985), all with yellow tepals. Most tropical continental African species are clustered on one side of the graph (Group B in Fig. 3), with several species being morphologically close to section *Flavida* s.l. Among them, species from Africa with yellow tepals, a

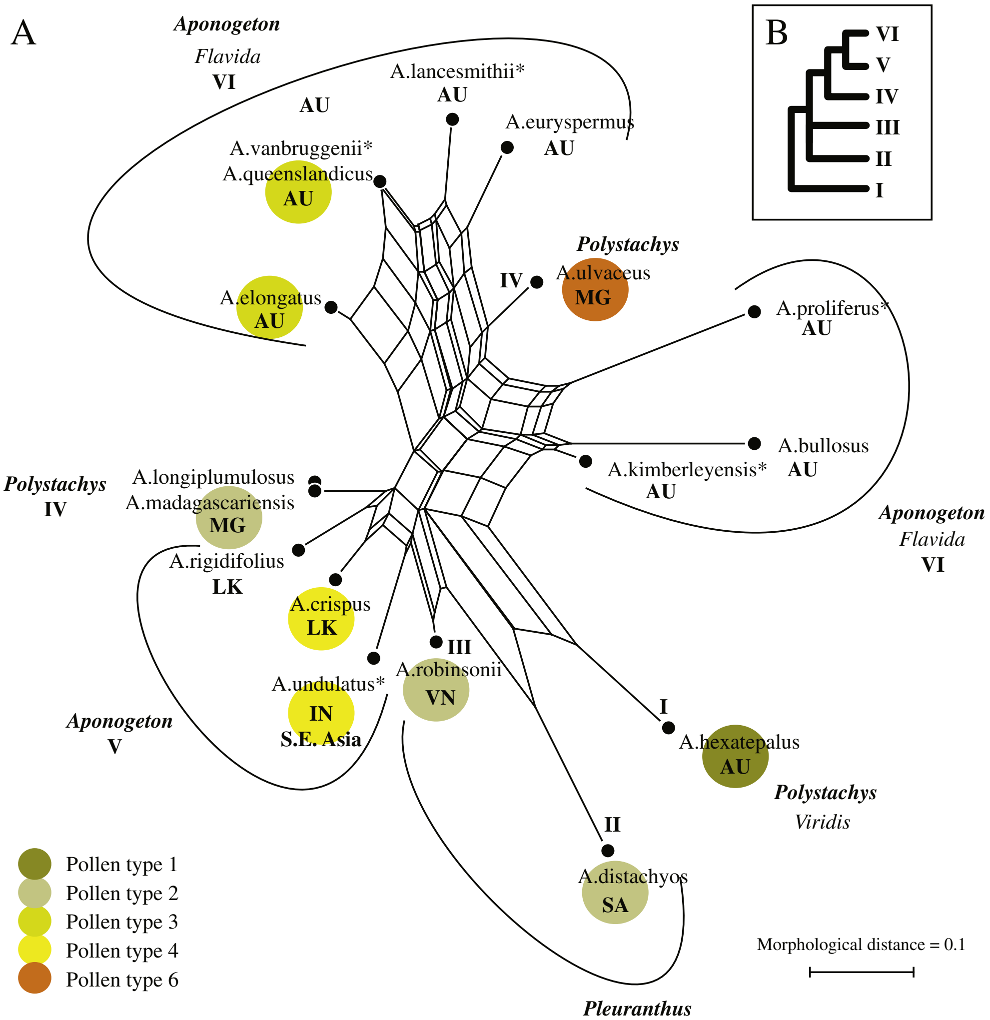

Fig. 2. A, Phylogenetic network based on mean morphological distances inferred from the morphological matrix used by Les et al. (2005). Indicated are the general pollen types (see Section 4.1) and the molecular phylogenetic affinities of species (B). Roman numerals refer to molecular-defined lineages. Countries of provenance are abbreviated following ISO standard 3166-1: AU, Australia; IN, India; LK, Sri Lanka; MG, Madagascar; SA, South Africa; VN, Vietnam. B, Cladogram depicting the molecular-based relationships according to Les et al. (2005). *, incl. *A. stachyopyros* of the original study which was scored as identical to *A. undulatus*, confirming van Bruggen (1985) who considered this taxon as con-specific with *A. undulatus* and placed it in synonymy under the latter. Les et al. (2005) treated it as a distinct taxon in their analyses because two out of three ITS clones showed sequences distinct from their sampled specimen of *A. undulatus* (cf. Fig. 4).

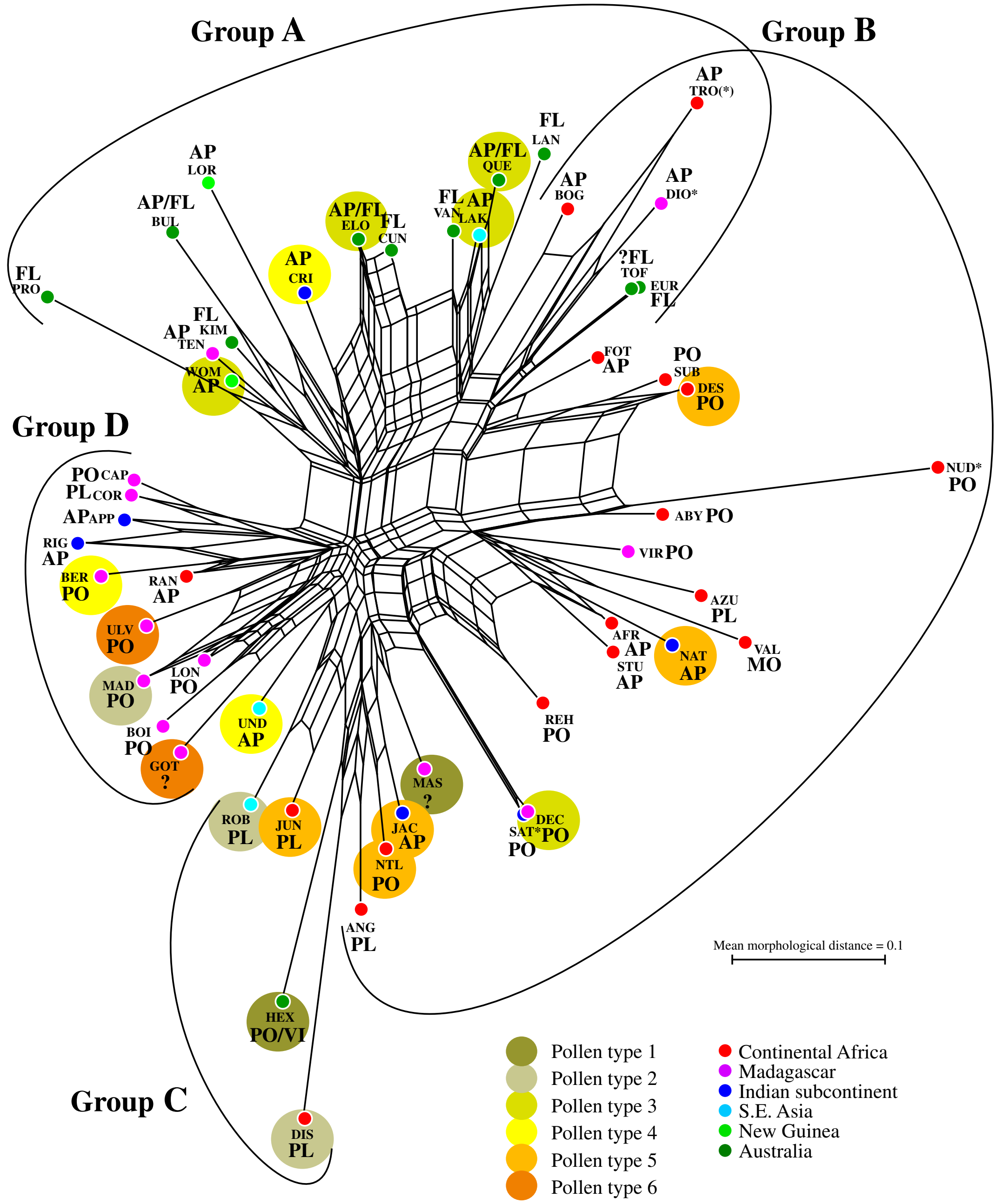

Fig. 3. Phylogenetic network based on mean morphological distances. The high level of morphological convergence is illustrated by the general lack of tree-like portions in the graph. Pollen types and geographic provenance of species are indicated by colouring. The groups refer to reconstructions by Les et al. (2005) based on morphological and molecular data. Group A spans the morphological variation in the newly erected sect. *Flavida* according to Les et al. based on morphological and genetic data, which includes also non-Australian species not covered by that study. Group B spans the morphological variation in most tropical continental African species (except for *A. ranunculiflorus*), which includes two of the (tropical) Australian species and the type species *A. natans* from the Indian subcontinent; no genetic data is available for species of this group. Group C comprises the genetically most distinct taxa within the genus *Aponogeton* (*A. distachyos*, *A. hexatepalus*, *A. robinsonii*). Group D includes most Madagascan species of *Aponogeton*, and all three sequenced taxa (see Figs. 4, 5). All species names are abbreviated by the first three letters except for: NTL, *A. natalensis*. Asterisks indicate species with unisexual flowers. Two letter abbreviations indicate the section and/or subsection according to van Bruggen (1985) and Les et al. (2005): AP, sect. *Aponogeton* subsect. *Aponogeton*; FL, sect. *Flavida*; MO, sect. *Pleuranthus* subsect. *Monostachys*; PL, sect. *Pleuranthus* subsect. *Pleuranthus*, PO, sect. *Aponogeton* subsect. *Polystachys*; VI, sect. *Virides*.

character that has been suggested as synapomorphy of section *Flavida* by Les et al. (2005), but also two morphologically derived potentially dioecious species (*Aponogeton troupinii* Raynal, *Aponogeton dioecus* Bosser) with unisexual flowers and inflorescences. The third species with unisexual flowers, *Aponogeton satarensis* Raghavan, Kulkarni & Yadav from the Indian subcontinent, is also included in Group B as well as the type species of the genus, *Aponogeton natans*. The genetically most isolated and distinct taxa of *Aponogeton*, *A. distachyos*, *A. hexatepalus*, and *Aponogeton robinsonii* (see Section 4.3), are also grouped (Group C in Fig. 3). *Aponogeton distachyos* and *A. hexatepalus* are characterized by unique morphological traits, reflected in their long terminal branches in the graph, and are the only two modern species extending into temperate climates (Cfb and Csb climates according Köppen–Geiger classification; Köppen, 1936; Kottek et al., 2006). Most Madagascan, three Asian species, and *Aponogeton ranunculiflorus* Jacot Guill. & Marais from southern Africa (Lesotho; Group D in Fig. 3) are morphologically relatively close to each other contrasting the pattern seen elsewhere in the genus. This group includes all genetically analysed Madagascan species, which share virtually indistinguishable ITS and *trn*K/*mat*K sequence types (Les et al., 2005; see following paragraph), but also the genetically distinct Asian species *Aponogeton rigidifolius* H. Bruggen and *Aponogeton undulatus*. No strict correlation is found between possible morphologically defined phylogenetic clusters in Fig. 3 and the general pollen types that can be distinguished within modern species of *Aponogeton*. Fig. 3 further demonstrates the variation of morphologies in African taxa and Asian taxa not covered by molecular data so far.

4.3. Molecular differentiation patterns in Aponogeton and their correlation to general pollen types

The plotting of general pollen types on phylograms available via the PhyLoTa Browser (release 1.5; www.phylota.net; Fig. S1) showed some conflict between the signal from the ITS and *trn*K/*mat*K data regarding evolutionary interpretation of pollen types. Therefore, we refrained from concatenating the ITS and *trn*K/*mat*K data for our re-analysis. Based on ITS and *trn*K/*mat*K data, Pollen Type 2 represents a plesiomorphic state: it is found in three species that are genetically and ecologically most distinct to each other (Figs. 4 and 5) and geographically isolated since the Paleogene. Pollen Type 6 appears to be derived from Type 2: based on available genetic data, *Aponogeton ulvaceus* (Pollen Type 6) is virtually indistinguishable from other Madagascan species. Regarding Pollen Types 3 and 4, the currently available genetic data is inconclusive: based on the ITS data, both types may be directly derived from Pollen Type 2 (Fig. 4), whereas the *trn*K/*mat*K data suggests that Pollen Type 3 is derived from Pollen Type 4 (Fig. 5). Based on the putative position of the genus' root according to Les et al. (2005), Pollen Type 1, found in the S.W. Australian *Aponogeton hexatepalus*, but also a newly described species of *Aponogeton* from Madagascar, may either be derived from Type 2, or vice versa. Species showing other pollen types are genetically most distinct to *A. hexatepalus*. Genetic data do however not cover the type species of the genus, *Aponogeton natans*, widespread on the Indian subcontinent, and African species sharing Pollen Type 5, and those Madagascan and Asian species that are macromorphologically and/or based on their pollen types close to two Australian lineages. The Madagascan species are clearly undersampled and the question of on-culture hybridisation (red labels in Fig. 4) raises doubts about the genetic indifference of Madagascan species with different pollen types (Clade IV in Figs. 4, 5; Pollen Types 2 and 6).

5. Evolution and distribution of pollen types of *Aponogeton* in space and time

Based on (*i*) the geographical distribution of modern taxa (van Bruggen, 1985; Table 1), (*ii*) their morphological and molecular differentiation patterns (see Sections 4.2, 4.3; Figs. 3–5), (*iii*) the mapping of general pollen types (Section 4.1) on phylogenetic reconstructions based on available morphological and molecular data (Figs. 2–5), and (*iv*) the incorporation of fossil pollen (see Section 3.3) in the obtained modern differentiation framework, a first evolutionary hypothesis regarding the pollen types of the genus can be put forward (Fig. 6). Pollen Types 1, 2 and 3 are represented in the fossil record of western N. America and western Greenland (Fig. 7B, C). All three types show a highly disjunct modern distribution pattern covering (temperate) southwestern Australia (Type 1), Madagascar (all types), and Southeast Asia–tropical Australasia (Types 2 and 3; Fig. 7F). Morphologically and genetically, species with these pollen types comprise all of the divergence observed in the modern genus (Figs. 3–5). Thus, we postulate that these lineages (corresponding to molecular clades I, II, III, and IV + V + VI in Figs. 4, 5), or lineages ancestral to the modern species, were already diverged and widespread by the onset of the Eocene

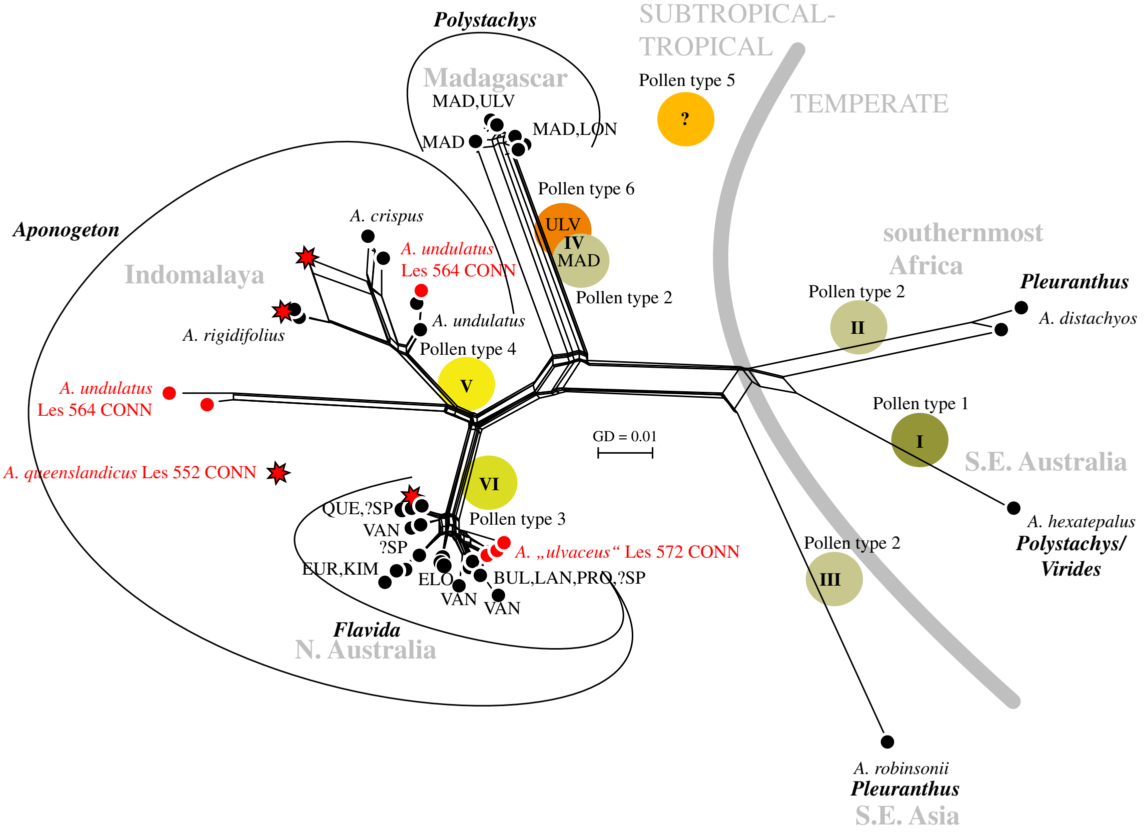

Fig. 4. Phylogenetic network based on cloned ITS sequences. General Pollen Types 1–6 are indicated for each major molecular clade (labelled with roman numerals I–VI). Species names are abbreviated by the first three letters in the case of Madagascan (clade IV) and tropical Australian species (sect. *Flavida*; clade VI). Position of specimens (annotated with voucher numbers) with ambiguous signal indicated by red font/signatures. No genetic data is available for subtropical-tropical African and S. Asian species of sect. *Aponogeton* (subsects *Aponogeton* and *Polystachys*) having Pollen Type 5. The black triangle indicates the putative position of the genus' root according to Les et al. (2005). * The edge lengths to/from *A. hexatepalus* are likely underestimated: only ITS2 has been made available for the species, the sequence structure of the generally more variable ITS1 is unknown.

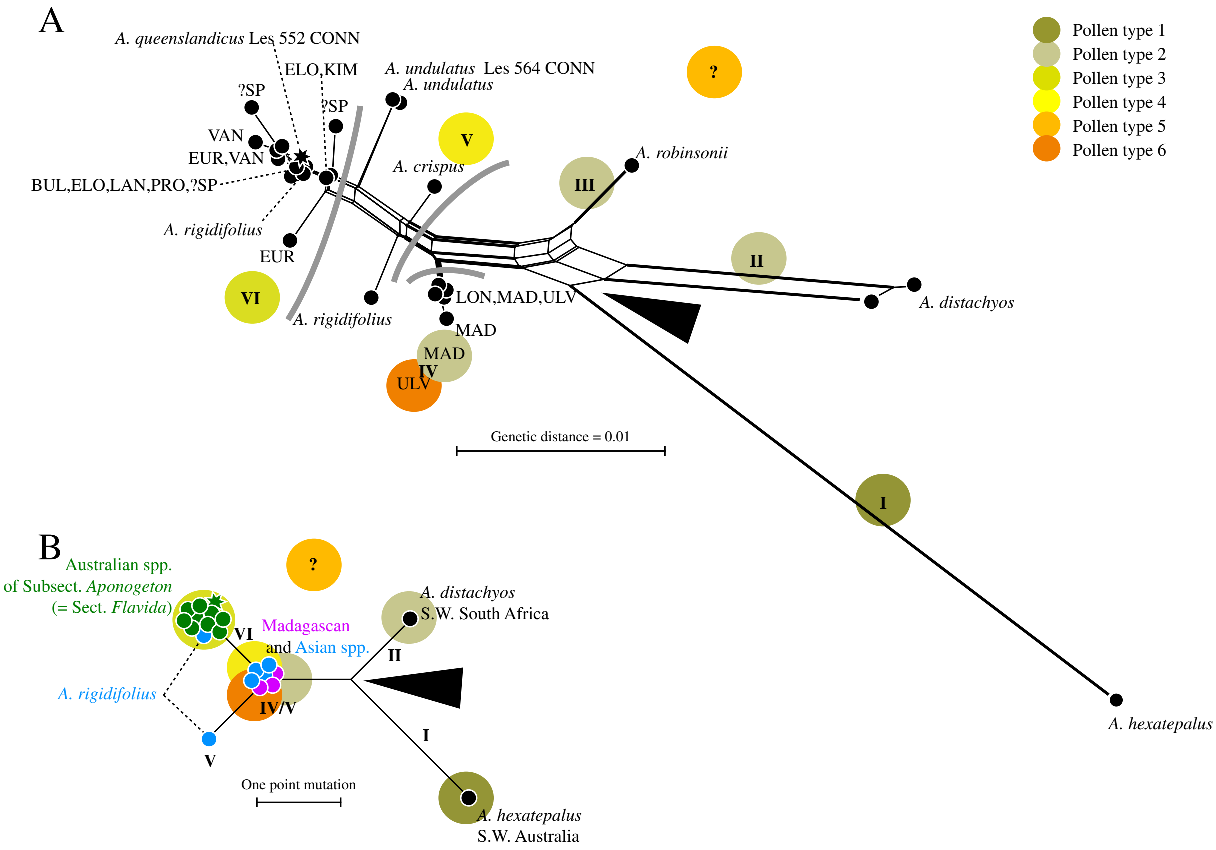

Fig. 5. Phylogenetic relationships based on directly sequenced portions of the *trn*K intron/*mat*K gene. General Pollen Types 1–6 are indicated for each major molecular clade (I–IV, VI)/grade (V). A. Phylogenetic network based on the *trn*K intron upstream of the *mat*K gene. Species names are abbreviated by the first three letters in the case of Madagascan (clade IV) and tropical Australian species (sect. *Flavida*; clade VI). B. Median network based on 1538 nucleotides from the 5′ part of the *mat*K gene. No genetic data is available for subtropical–tropical African and S. Asian species of sect. *Aponogeton* (subsects *Aponogeton* and *Polystachys*) having Pollen Type 5. The black triangle indicates the putative position of the genus' root according to Les et al. (2005).

(Fig. 7C). They represent generally more plesiomorphic types; the stock from which the other (apomorphic) Pollen Types 4, 5, and 6 could have evolved. Pollen Types 4 and 6 may have evolved from Pollen Type 2, a scenario favoured by genetic data. Alternatively, Pollen Type 4 may represent a derived variant of Pollen Type 3, which further evolved into Pollen Types 5 and 6. Such a scenario finds support from non-molecular evidence, in particular gradients of intraspecific variation seen in the pollen of *Aponogeton bernierianus*/*Aponogeton undulatus* (Plate VI), *Aponogeton desertorum*/*Aponogeton junceus*/*Aponogeton natans*/*Aponogeton natalensis* (Plate VII), and *Aponogeton gottlebei*/*Aponogeton ulvaceus* (Plate VIII). The modern distribution of Pollen Types 3, 4, 5 and 6 would be in good agreement with tectonic and climate-triggered radiation and divergence of a once pan-palaeotropical lineage spanning from Africa to Indomalaya (Fig. 7).

6. Discussion

6.1. Critical evaluation of the published fossil record of Aponogeton

Aponogeton has been reported from the Upper Cetaceous of Argentina/Chile (South America; Selling, 1947), the Paleocene of Scotland (Europe; Simpson, 1961), and the Oligocene to Miocene of Kazakhstan (Central Asia; Zhilin, 1974a,b; Pneva, 1988). The earliest alleged fossils were "supposedly" flowering and fruiting structures from Argentina/Chile mentioned by Selling (1947). These fossils have never been described nor figured, and van Bruggen (1985) writes that the original material appears to be lost from the collections of the Swedish Museum of Natural History in Stockholm; however, this might not be the case. Specimens believed to represent the original specimens (S165482 to S165484; Plates S1 and S2) can be found in the collection, categorized as Angiosperm inflorescences. After careful comparison to reproductive structures of extant *Aponogeton* species we conclude that the fossils are not *Aponogeton* remains.

The Paleocene reports from the Isle of Mull and the Ardnamurchan peninsula, western Scotland, by Simpson (1961) are pollen grains investigated using LM. Simpson (1961) describes and illustrates four species; *Aponogeton parvi-pollenites*, *Aponogeton magni-pollenites*, *Aponogeton herbridicum*, and *Aponogeton pre-basalticum*. The diagnostic pollen features used by Simpson (1961) consist of the four following characters; single germinal furrow (= sulcate), reticulate sculpturing, oval in outline, and the pollen is between 22 and 55 × 13 and 30 µm. Pollen of *Aponogeton* seen under LM looks the same or very similar to pollen from a number of other taxa that are also monosulcate and reticulate (see Section 3.1). In his LM-based descriptions, Simpson (1961) never mentions any echini or similar sculpturing elements which can be regarded as echini, from any of his pollen types. The only way to be sure if particular dispersed pollen is truly an *Aponogeton* grain is to verify the identifications using a SEM, because only then do the narrow echini become clearly visible. Based on what we observe from the figured material in Simpson's (1961) paper, one of two pollen figured as *A. parvi-pollenites* clearly has an ulcus and could belong in Sparganiaceae or Typhaceae. The muri of the pollen figured as *A. herbridicum* are also very untypical for *Aponogeton*, they appear to be much too narrow, but we cannot say to which group this pollen belongs. The pollen figured as *A. magni-pollenites* has no real reticulum and could be some type of a folded spore. The pollen figured as *A. pre-basalticum* is clearly

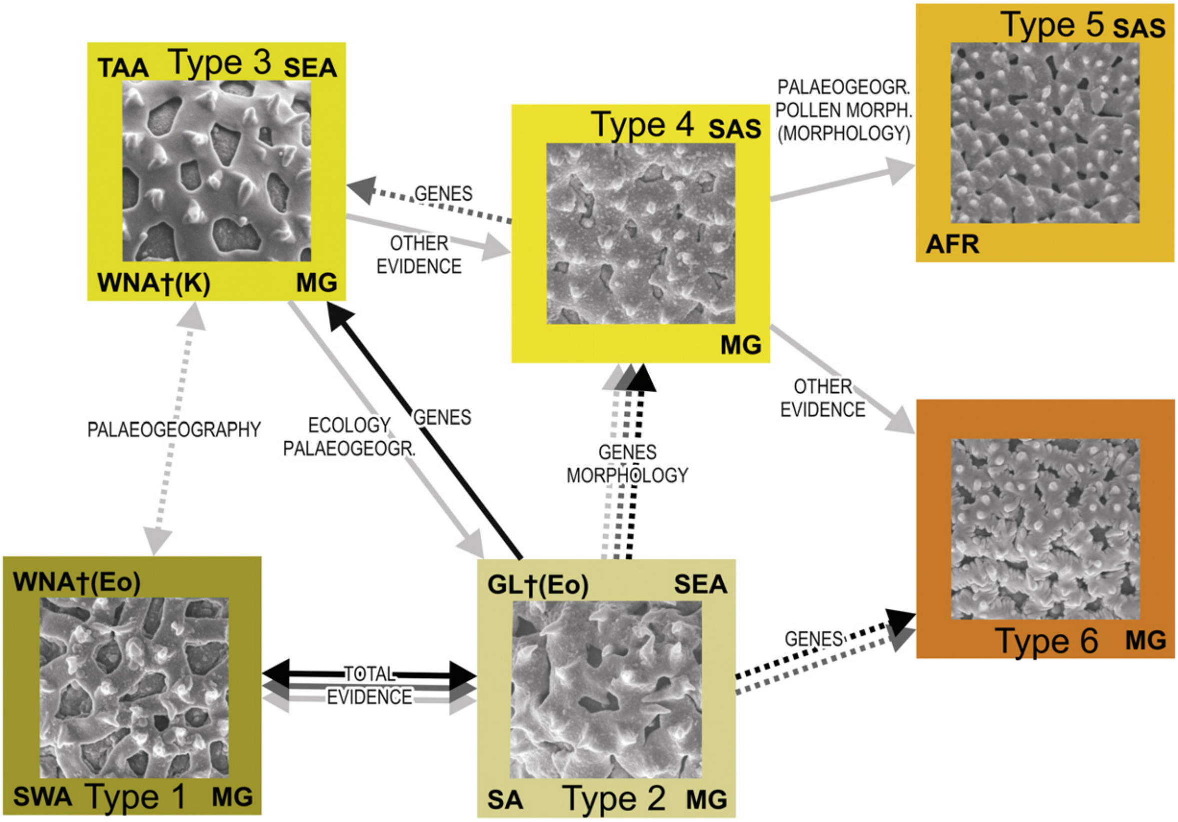

Fig. 6. Evolution and distribution of *Aponogeton* Pollen Types in space (corner labels) and time (in brackets: K, late Cretaceous; Eo, Eocene). Here preferred pathways are given with solid lines, alternative pathways shown with broken lines. Black: pathways favoured by ITS data; dark grey, pathways favoured by *trn*K/*mat*K data; light grey, pathways inferred based on non-molecular evidence (fossil pollen and palaeogeography, ecology and modern distribution patterns, gross and pollen morphology). Geographic identifiers are abbreviated as follows: AFR, (continental) Africa; SAS, South Asia; SEA, Southeast Asia; SWA, (temperate) southwestern Australia; TAA, tropical Australasia; WNA, western N. America (†); countries/territories using two-letter codes (cf. Fig. 2): GL, Greenland (†); MG, Madagascar; SA, South Africa.

tricolpate and is no *Aponogeton* pollen. Based on the material figured by Simpson (1961) the presence of *Aponogeton* in the Paleocene of Scotland is doubtful.

The late Oligocene to early Miocene *Aponogeton* reports from Kazakhstan are composed of leaf compressions. Both fossil taxa, *Aponogeton tertiarius* (Zhilin, 1974a,b) and *Aponogeton chilinii* (Pneva, 1988), are based on very few fragmentary specimens. Golovneva (1997) combined the Oligocene specimens originally described as *A. chilinii* by Pneva (1988) with other similar fossils from Kazakhstan and concluded that they belong in *Haemanthophyllum*. Riley and Stockey (2004) agreed with Golovneva that this specimen showed similarities to *Haemanthophyllum*, but also stated that its taxonomic affinity was uncertain. The early Miocene specimen described as *A. chilinii* by Pneva (1988) and originally identified as *Aponogeton* sp. by Zhilin (1974b) was considered too fragmentary and without any diagnostic characters for a generic/species identification by both Golovneva (1997) and Riley and Stockey (2004). The late Oligocene specimens described by Zhilin (1974a,b) as *A. tertiarius*, include basal, central, and upper parts of leaves, and of the existing macrofossil material these specimens are the most similar to extant *Aponogeton*. Both Andreev (1991) and Golovneva (2000) seem to agree with the identification of Zhilin (1974a,b), but Riley and Stockey (2004) conclude that these specimens show affinity to both extant Aponogetonaceae and Potamogetonaceae, and consider them of uncertain affinity.

6.2. Palaeo-phytogeography of Aponogeton

6.2.1. Previous hypotheses

As the fossil record of *Aponogeton* is highly problematic the phytogeographic history of the genus has until now been based solely on the present distribution of its species and their alleged relationships. Two theories have been put forward concerning the place of origin of *Aponogeton*. Based on the genus' present absence in the Americas and the presence of closely related taxa in Africa, Madagascar, Sri Lanka, and India, Thanikaimoni (1985) suggested that the family originated on Madagascar in the mid Cretaceous and directly dispersed into nearby mainland Africa, India and Antarctica-Australia (Fig. 7A, B). Thanikaimoni (1985) assumed that all Asian and African species, and the temperate Australian *Aponogeton hexatepalus*, were derived from the Madagascan stock, following the Gondwana break-up and later collision of the Indian subcontinent with Eurasia. He believed that the tropical Australian and Papuan taxa, in contrast to *A. hexatepalus*, further evolved from Malaysian plants coming from India during the Miocene (Fig. 7D), particularly *Aponogeton lakhonensis*. Les et al. (2005) proposed a very similar historical biogeography of the genus, but, in contrast to Thanikaimoni, concluded that *Aponogeton* originated in Australia under temperate conditions and radiated from there into the tropics of Africa and via Madagascar/proto-India into Asia based on the inferred position of *A. hexatepalus* as sister to the rest of the genus. Les et al. (2005) do not assign their migration events to any geological time periods nor do they suggest any particular ages for these divergence steps. With the tectonic evolution of the Earth in mind (Fig. 7), Les et al.'s alternative scenario requires that *Aponogeton* must have evolved in the Lower Cretaceous at high latitudes in Antarctica-Australia (Fig. 7A), which experienced temperate climate conditions at that time and provided appropriate aquatic environments (e.g. McLoughlin et al., 2002). Furthermore, *Aponogeton* must then have migrated into the expanding tropics of Africa and Madagascar–India during the middle Cretaceous, at times of increasing temperatures and high atmospheric CO_2 levels (e.g. Caldeira and Rampino, 1991; Huber

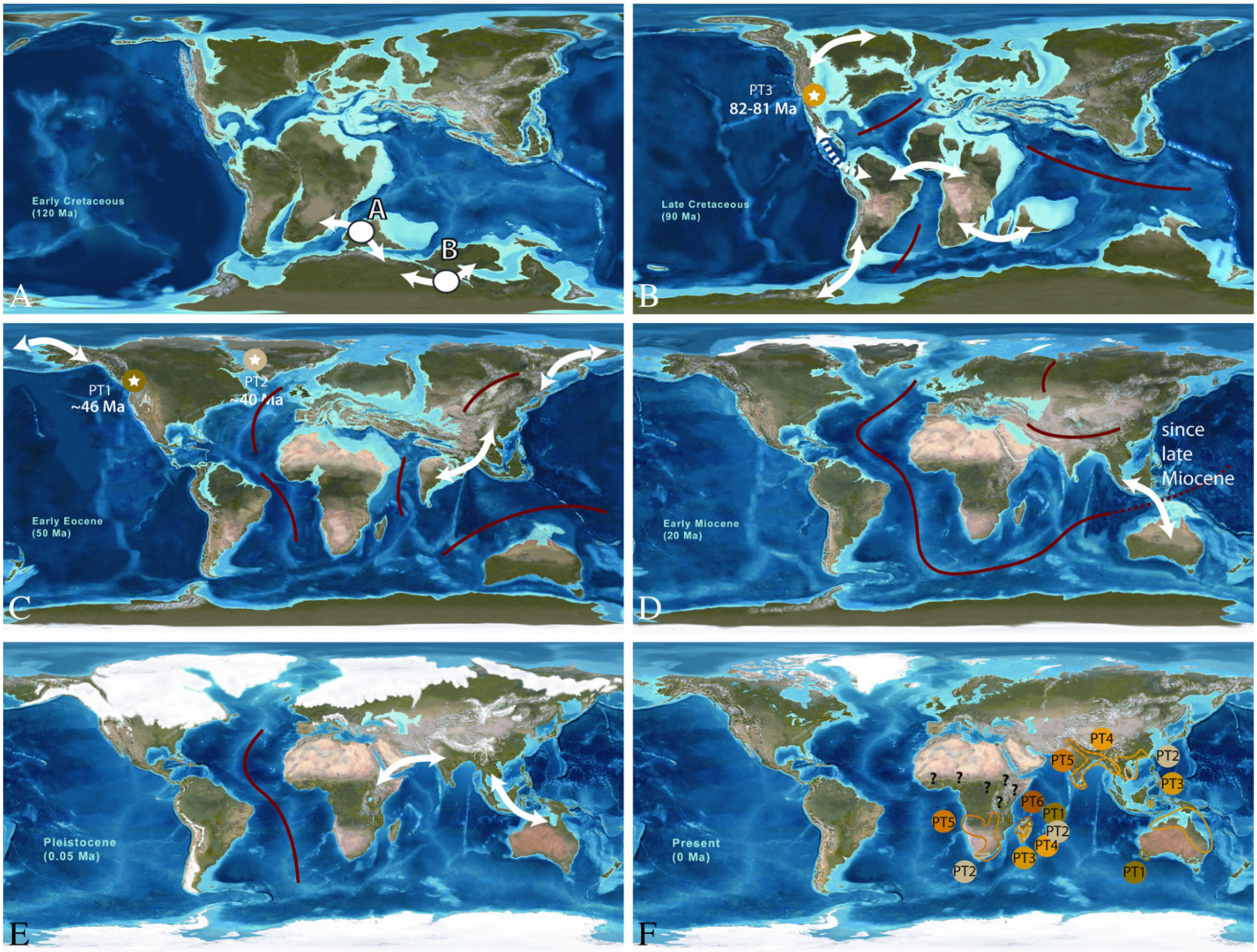

Fig. 7. Placement of pollen types of *Aponogeton* in a phylogenetic and palaeogeographic context. Palaeomaps (A, 120 Ma; B, 90 Ma; C, 50 Ma; D, 20 Ma; E, 50 ka, F, Present) are Mollweide views, projected through the prime meridian. Red lines, geographic barriers, white arrows, potential migration corridors. A, Early Cretaceous, indicated are the two possible points of origin according to the hypotheses put forward by Thanikaimoni (1985; "A") and Les et al. (2005; "B"). B, Late Cretaceous, showing the occurrence of *A. harryi*. C, Early Eocene, showing the occurrences *A. longispinosum* and *A. hareoensis*. D, Early Miocene. E, During glacial maximums of the last glacial period, the Weichselian (NW Europe Stage) ca 110–12 ka ago, the globally changed precipitation patterns opened new corridors in southwestern Asia and Malaysia for opportunistic genera. F, Modern day, the distribution ranges of species with Pollen Types (PT) 1 to 6 are indicated (maps in van Bruggen, 1985), except for PT5 all pollen types can be found on Madagascar. ?, no pollen of equatorial African species has been investigated.

et al., 2002). However, the well-studied Berriasian to early Barremian sedimentary rock formations of the Otway and Gippsland Basins (S.E. Australia), the Eromanga Basin (C. Australia), the Surat Basin (Queensland, Australia), and the Laura Basin (N. Queensland, Australia), made up of various fluvial and/or lacustrine sequences, do not yield any evidence of angiosperm presence (e.g. Douglas, 1969; Cantrill and Webb, 1987; Dettmann et al., 1992; Dettmann, 1994; Douglas, 1994; McLoughlin, 1996; Hill et al., 1999; McLoughlin et al., 2002). Also, the earliest Australian records of angiosperms date only to the latest Barremian/earliest Aptian and Albian times (e.g. Taylor and Hickey, 1990; Dettmann, 1994; Pole, 1999), and so do the earliest southern South American records (e.g. Barreda and Archangelsky, 2006; Archangelsky et al., 2009).

6.2.2. New ideas based on combined evidence

Based on the fossil pollen, the SEM-based extant pollen types, and the re-investigation of available morphological and molecular data on the genus, it is clear that *Aponogeton* had already a wider and/or entirely different distribution in the late Cretaceous and early Cenozoic than today, suggesting a possible early Cretaceous origin of the genus (Fig. 7A). Such an age would agree with the molecular dating by Janssen and Bremer (2004), which inferred that the Alismatales diverged from other monocots already in the early Cretaceous, ≥131 Ma, and the Aponogetonaceae at ≥98 Ma; i.e. minimum divergence ages that are comfortably older than our oldest fossil pollen of *Aponogeton* (82–81 Ma).

The late Cretaceous North American fossil shares a pollen type (Pollen Type 3) with a purely tropical, Indomalayan–Australasian lineage leading to section *Flavida* that includes at least one Madagascan and one S.E. Asian species (Figs. 3–5; Fig. 7B, F), but no African species. Furthermore, the pollen types of two modern temperate species, *Aponogeton distachyos* and *Aponogeton hexatepalus* (Pollen Types 1 and 2) are not restricted to these species, but are also found in tropical Madagascan and S.E. Asian species (Fig. 7F), and middle Eocene North American and Greenland fossil assemblages (Fig. 7C) thriving under humid warm temperate (or subtropical) to tropical-like conditions (e.g. Boyd, 1990 [Greenland]; Graham, 1999 [North America]). Extant *Aponogeton* species are hydrochorically dispersed, tolerant against seasonal draught, but non-tolerant against salty water and low temperatures, hence, widening seaways and high mountain ranges are likely the primary barriers to the dispersal of the genus (red lines in Fig. 7). Therefore, the combined evidence indicates that the modern temperate species, thriving in mild Mediterranean climates (Csb according Köppen–Geiger), genetically unique based on the current sample, could well have evolved from largely extinct tropical and subtropical lineages (thriving in As, BSh and/or Csa climates) long after its Cretaceous origin and radiation. The current genetic data must not be overinterpreted regarding the genus' root. The genus *Aponogeton* represents an isolated lineage within its larger molecular clade, the Alismatales (e.g. Soltis et al., 2000; Janssen and Bremer, 2004; Givnish et al., 2006; Li and Zhou, 2009); hence, any outgroup selected from the Alismatales may inflict long-branch attraction (LBA) with part of the ingroup based on the current taxon and gene sampling. Outgroup-inflicted LBA will unavoidably place the extremely long-branched *A. hexatepalus* (Pollen Type 1), and the long-branched *Aponogeton robinsonii* and *A. distachyos* (Pollen Type 2; see Figs. 4, 5), as sister to the remainder of the genus.

Pollen ornamentation unambiguously connects the tropical Australian species with the Papuan and *Aponogeton lakhonensis* (Pollen Type 4), all species show the yellow tepals that Les et al. (2005) defined as synapomorphy for their section *Flavida*. Pollen Type 3, found in two Asian sister species of section *Flavida* (Figs. 4, 5), may be derived from Pollen Type 4 or represent the sister lineage of the latter group (Fig. 6). Current genetic data agree with the assumption that species with Pollen Types 3 and 4 share a common origin, and this lineage can be traced back to the late Cretaceous (*Aponogeton harryi*; Fig. 7B). Being tropical but tolerant regarding precipitation regimes, members of this lineage may have migrated and exchanged genetic material until very recent times (Fig. 7E, F). Les et al. (2005) noted the potential to form hybrids between species of different provenances (red labelled accession in Fig. 4), hence, it would not be surprising to find “Asian” ITS or plastid sequences (note the position of the Sri Lankan *Aponogeton rigidifolius* accessions in Fig. 5) in Australian species or vice versa even in material collected from the wild as a result of relatively recent hybridisation and incomplete lineage sorting. Today, the dense tropical rainforests of Malesia separate *A. lakhonensis* from their potential Australian relatives. During the Pleistocene climate fluctuations, this ecological barrier was possibly broken by periodically dry woodlands and savannah-like landscapes that expanded during the glacial maxima at the expense of rainforests (e.g. Cannon et al., 2009 for the last glacial maximum).

Species with Pollen Types 5 and 6 may belong to the same lineage, but comprehensive molecular data would be needed to test the alternatives shown in Fig. 6. The fossil pollen further proves that modern disjunct and genetically distinct lineages of *Aponogeton* ones thrived in western North America and Greenland (Pollen Types 1–3; Fig. 7) and that its present absence from that continent must be related to post-Eocene events such as for instance climate cooling, loss of habitat, or competition. For a final phylogenetic interpretation of the data on *Aponogeton distachyos*, *Aponogeton robinsonii* and *Aponogeton hexatepalus*, it is obligatory to screen all species for according pollen types and to further analyse those species that show the putatively or potentially plesiomorphic Pollen Types 1 and 2. The occurrence of Pollen Types 1 and 2 in the lower Eocene of western North America, Madagascar, Vietnam and the summer-dry, temperate regions of S.W. South Africa and S.W. Australia may indicate the relict nature of this type with its root extending back into the Cretaceous predating the final break-up of Gondwana. On the other hand migration corridors were available for various temperate to warmth-loving angiosperms (e.g. *Acer*, *Amersinia*, *Davidia*, *Dipteronia*, *Eucommia*, *Limnobiophyllum*, *Macginitiea*, *Paliurus*, *Quereuxia*, and *Trochodendron*) during the late Cretaceous and early Cenozoic between N. America and E. Asia via Beringia (e.g. Manchester, 1999; Manchester et al., 2009). Theoretically, *Aponogeton* could have dispersed via the same passageway from North America over to Asia and then further into India, E. Africa and Madagascar (Fig. 7C–F), where they eventually were outcompeted by the today dominant group(s) and could only survive outside the main range of their congeners.

7. Conclusion

The present palynological study uses the combination of LM and SEM to document the first reliable fossil occurrences of *Aponogeton* in the palaeobotanical record. The single grain technique proves once again how important it is to apply advanced and high taxonomic resolution methods to obtain useful palaeo-palynological data when studying fossil floras and the past distribution of plant groups. The fossil pollen presented here shows that during the late Cretaceous and early Cenozoic *Aponogeton* was growing in North America and Greenland, far from its present distribution range in subtropical to tropical Africa, India, Asia, and Australia. The presence of *Aponogeton* in the fossil record of the New World challenges previous theories regarding the origin and dispersal history of the genus based solely on modern-day data.

The habitat of *Aponogeton* facilitates the fossilisation of its pollen. If scientists would study their samples in a similar way as presented here it is probable that in the future much more fossil *Aponogeton* pollen will be found from the Cretaceous and Cenozoic in different parts of the world. Final conclusion about the past distribution and origin of *Aponogeton* therefore awaits further future additional evidence. The palynological and genetic characterisation of all modern lineages and future finds of fossil *Aponogeton* pollen will be crucial to elucidate the long history of this unique lineage of water plants.

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