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The ontogeny of *Aeschynanthus* seeds – a comparative study using scanning electron microscopy

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Seed morphology in the genus *Aeschynanthus* (Gesneriaceae) is important in sectional classification. The number and type of appendages at the hilar end of the seed, papilla structure and testa cell orientation are all significant. SEM studies of seed and appendage development, at seven-day intervals from pre-pollination to maturity, were carried out in order to investigate possible sectional and seed type relationships. Fifteen species, representing six sections, were examined. Results show that there are no significant differences pre-pollination but two patterns were evident post-pollination, for both seed and hilar appendage development. Pattern I occurred in sects *Microtrichium* and *Haplotrichium s.s.* Pattern II was seen in sects. *Aeschynanthus*, *Diplotrichium* and *Polytrichium*. Patterns were less clear in species belonging to a group not yet circumscribed and here referred to as sect. *X*, but most closely resembled Pattern II. This study shows that the orientation of the testa cells is a developmental feature, defining the two major clades in the genus. It also shows that, in the two sections possessing more than one hilar appendage, there is initial development of one appendage relative to the coma in sect. *Polytrichium* and of one appendage relative to the second in sect. *Diplotrichium*. The results of this study are discussed with respect to molecular and morphological findings, and the evolutionary significance considered.

ADDITIONAL KEY WORDS: ancestral relationships – Gesneriaceae – morphology – sectional relationships – seed appendage

INTRODUCTION

The genus *Aeschynanthus* Jack (Gesneriaceae; subfamily Cyrtandroideae; tribe Trichosporeae) comprises some 160 species distributed from the Himalayas to southern China and throughout Indo-China, and from southern India and Sri Lanka, throughout Malesia to the Solomon Islands. They are mainly epiphytic in habit and several species are cultivated for their attractive flowers. The tubular shape of the corollas, coupled with strong protandry and the production of copious nectar, suggests that *Aeschynanthus* are bird pollinated. The resulting fruits are long slender unilocular capsules containing many anatropous ovules. The apical end of each ovule points towards the base of the capsule and the hilar end towards the apex. A single appendage develops from the apical end of the ovule and one or more appendage from the hilar end. The number and appearance of the hilar appendages have been used as characters to subdivide the genus into sections *Aeschynanthus* (*Holocalyx*), *Haplotrichium*, *Diplotrichium*, *Polytrichium*, and *Microtrichium* (Bentham, 1876; Clarke, 1883; Burtt & Woods, 1975). A sixth section, *Xanthanthos*, based on corolla colour and shape, was created by Wang (1984).

Although many species can be assigned to sections on other morphological characters, this is not always accurate, and for some species the seed itself has proved problematic. A comparative SEM survey has recently been undertaken (Mendum *et al.*, 2001), in an attempt to clarify the sections. They identified two distinct seed types, A and B, each of which could be divided into subtypes that correlated well with existing sections, with the exception of sect. *Haplotrichium*. Their results are as follows:

Type A seed:

Appendages one at each end, 15 mm or less, smooth; testa cell orientation almost always spiral; papillae when present formed from a single cell. Sections are:

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Sect. *Aeschynanthus*: appendages to 15 mm, slender to base; hilar appendage often with a podium of bubble cells at the base; testa cell orientation an anticlockwise spiral; calyx tubular or campanulate with abscission layer at base; lobes almost always blunt; stamens not or very rarely exserted. Type: *A. volubilis* Jack.

Sect. *Microtrichium*: appendages to 3.5 mm, not slender to base; testa cell orientation an anticlockwise spiral, rarely straight; calyx usually deeply divided, rarely tubular or spathaceous; without abscission layer at base; stamens sometimes exserted. Type: *A. microtrichus* C.B. Clarke.

Sect. *Haplotrichium s.s.* appendages to 3.5 mm, slender to base; testa cell orientation a clockwise spiral; inflorescences long pedunculate; bracts persistent; calyx deeply divided, without abscission layer at base; stamens exserted. Type: *A. bracteatus* DC.

Type B seed:

Appendages one or more at hilar end, 10–45 mm, papillose; testa cell orientation straight; papillae formed from the raised ends of two adjacent cells; calyx without abscission layer at base. Sections are:

Sect. *X*: seed with 1 hilar appendage; calyx partly or deeply divided, lobes pointed; stamens usually exserted. This section, containing many species previously assigned to sect. *Haplotrichium,* cannot be adequately circumscribed until more material of sect. *Xanthanthos* becomes available for study. It may be equivalent to the latter but not as that section is currently described.

Sect. *Diplotrichium*: seed with 2 hilar appendages; calyx partly or deeply divided, lobes pointed; stamens exserted. Type: *A. parasiticus* (Roxb.) Wall.

Sect. *Polytrichium*: seed with few to many hilar appendages; calyx partly or deeply divided, lobes pointed; stamens exserted. Type: *A. longicaulis* R.Br.

Sect. *Xanthanthos*: Corolla white or yellow; calyx deeply divided; stamens not exserted; seed with a single hilar appendage as in sect. *X*. Type: *A. denticuliger* W.T. Wang.

Molecular studies (Denduangboripant & Cronk, 2000, and pers. comm.) show the existence of two major clades in *Aeschynanthus* defined by testa cell orientation. One has anticlockwise cell orientation, is predominantly Malesian and contains sect. *Aeschnanthus* and almost all of sect. *Microtrichium*. The other has straight or clockwise orientation, is predominantly mainland Asian and contains sects. *Microtrichium (*mainland spp.), *Haplotrichium, Diplotrichium, Polytrichium* and sect. *X*. Material from sect. *Xanthanthos* was not available for sequencing.

The purpose of this study, prompted by observations made by Saueregger & Mühlbauer (University of Vienna, unpubl.) on the seed of three *Aeschynanthus* species, was to observe seed and appendage development in *Aeschynanthus* in order to establish whether any relationship exists, in growth and developmental characters, between different seed types and sections. The sectional concepts of Mendum *et al.* (2001) are followed.

MATERIAL AND METHODS

Fifteen species from the living collection at the Royal Botanic Garden Edinburgh (see Appendix) were selected to represent the different sections as follows.

Type A Seed:

Sect. *Aeschynanthus*: *A. javanicus*; *A. parvifolius*; *A. tricolor*

Sect. *Microtrichium*: *A. buxifolius*; *A. irigaensis*; *A. magnificus*; *A. oxychlamys*

Sect. *Haplotrichium s.s.*: *A. bracteatus*

Type B Seed:

Sect. *X*: *A. gracilis*; *A. humilis*; *A. pseudohybridus*

Sect. *Diplotrichium*: *A. lineatus*; *A. sikkimensis*

Sect. *Polytrichium*: *A. albidus*; *A. longicaulis*

Unfortunately living material of sect. *Xanthanthos* was not available.

PRE-POLLINATION DEVELOPMENT

Ovaries of small, medium and large buds were dissected out and measured, as were the male and female stages of unpollinated flowers. *Aeschynanthus* flowers exhibit strong protandry; the anthers ripen several days before the gynoecium lengthens and the stigma matures, by which time the anthers are withered; therefore both stages were examined. In order to limit the effects of variation in ovule size, samples were routinely taken from the mid region of the ovary and fixed in Formalin–Acetic acid–Alcohol (FAA) overnight prior to Critical Point Drying. They were then mounted on aluminium stubs using carbon disks or tempfix; coated with gold/palladium using an Emscope SC500 sputter coater; viewed using a Zeiss DSM 962 Scanning Electron Microscope and micrographs taken.

POST-POLLINATION DEVELOPMENT

Adult flowers were selfed, date tagged and collected at intervals of seven days. Ovaries were removed and measured prior to dissection. The appearance of the seed was noted before removal, and lengths of both

seed and appendages measured prior to fixation with FAA. They were then prepared for scanning electron microscopy as before, and viewed on the SEM. Micrographs were taken at standard magnifications and further measurements made.

Ripe seed was collected; mounted on tempfix coated stubs; sputter coated; viewed and measured as before.

RESULTS

PRE-POLLINATION

Rates of ovary growth varied between species, but overall there was little growth from small bud to female flower stage. The ovary at this stage was, however, always longer than that at the male stage in any one species.

The initial development of unpollinated ovules was found to be very similar in all species examined. Projections from the placenta formed funicles that supported the ovules as they grew. Integuments gradually enveloped the nucellus from the apical to the hilar end where the micropyle developed (Fig. 1).

The rate of development was found to vary along the length of the ovary in all species examined, with the most advanced ovules situated towards the centre. Development rates also varied between species, for example, ovules of *A. irigaensis* developed more rapidly relative to the corolla stage than those of *A. magnificus* (both in sect *Microtrichium*), although the ovaries were similar in size. In *A. sikkimensis* the ovary doubled in size between the medium and large bud stages, but little ovule development was observed. This suggests that rates of growth and development of ovules and ovary are not necessarily linked. Similarly, ovule growth need not be reflected in its pattern of development – *A. gracilis* ovules, for example, changed in shape and micromorphology from the earliest stages although there appeared to be only a marginal increase in their overall size (Fig. 7). The ovules of flowers at the female stage were larger than those at the male stage in all of the species examined, although maximum length was consistently less than 200 μm and the extent of development varied between species. There did, however, appear to be some similarities between species of the same section. In sect. *X* all three species studied exhibited some development of both apical and hilar appendages prior to pollination. Section *Polytrichium* species *A. longicaulis* and *A. albidus* exhibited only apical appendage development (Fig. 2), with hilar appendage development apparent by seven days after pollination (Fig. 3). Species in sects. *Aeschynanthus* and *Haplotrichium s.s.* exhibited no pre-pollination appendage development. In sect. *Microtrichium*, both *A. buxifolius* and *A. magnificus* exhibited apical and hilar development prior to pollination. However, in *A. oxychlamys* the apical appendage was not apparent until 14 days and the hilar appendage not obvious until 28 days post-pollination. In sect. *Diplotrichium* the unpollinated ovules of *A. lineatus* exhibited both apical and hilar appendage development (Fig. 17), whereas those of *A. sikkimensis* showed apical development only; the hilar appendages were not apparent until seven days after pollination (Fig. 18).

POST-POLLINATION

Ovary Growth

There was steady growth over the 4–5 weeks after pollination followed by a plateau lasting 3–4 weeks. The fleshy ovary walls then dried and became papery prior to dehiscence. This pattern was observed in most species studied with the exception of *A. bracteatus* (sect. *Haplotrichium*) and *A. humilis* (sect. *X*) in which the ovary walls remained firm.

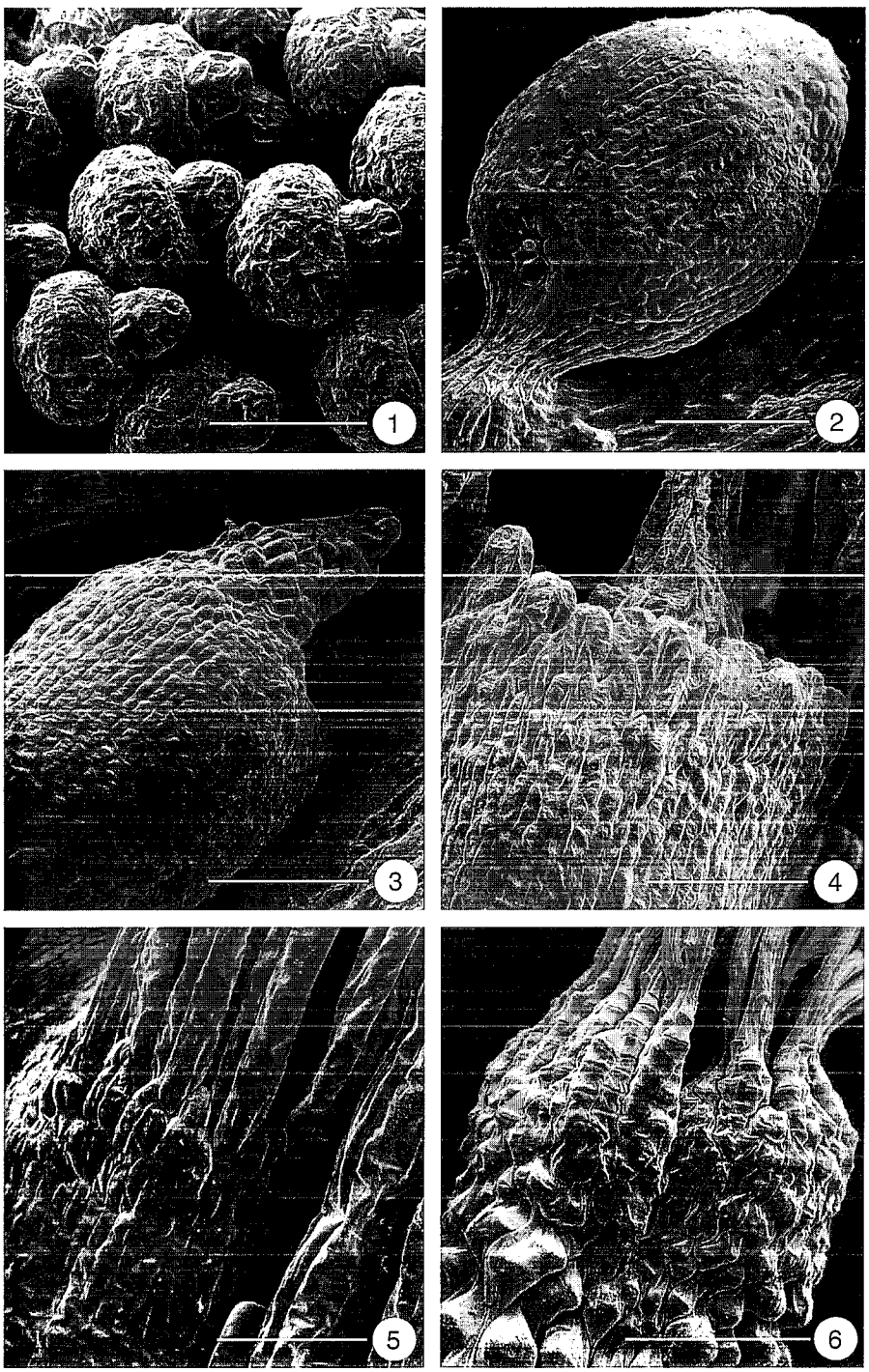
Seed Growth and Development

Two distinct patterns emerged from the results:

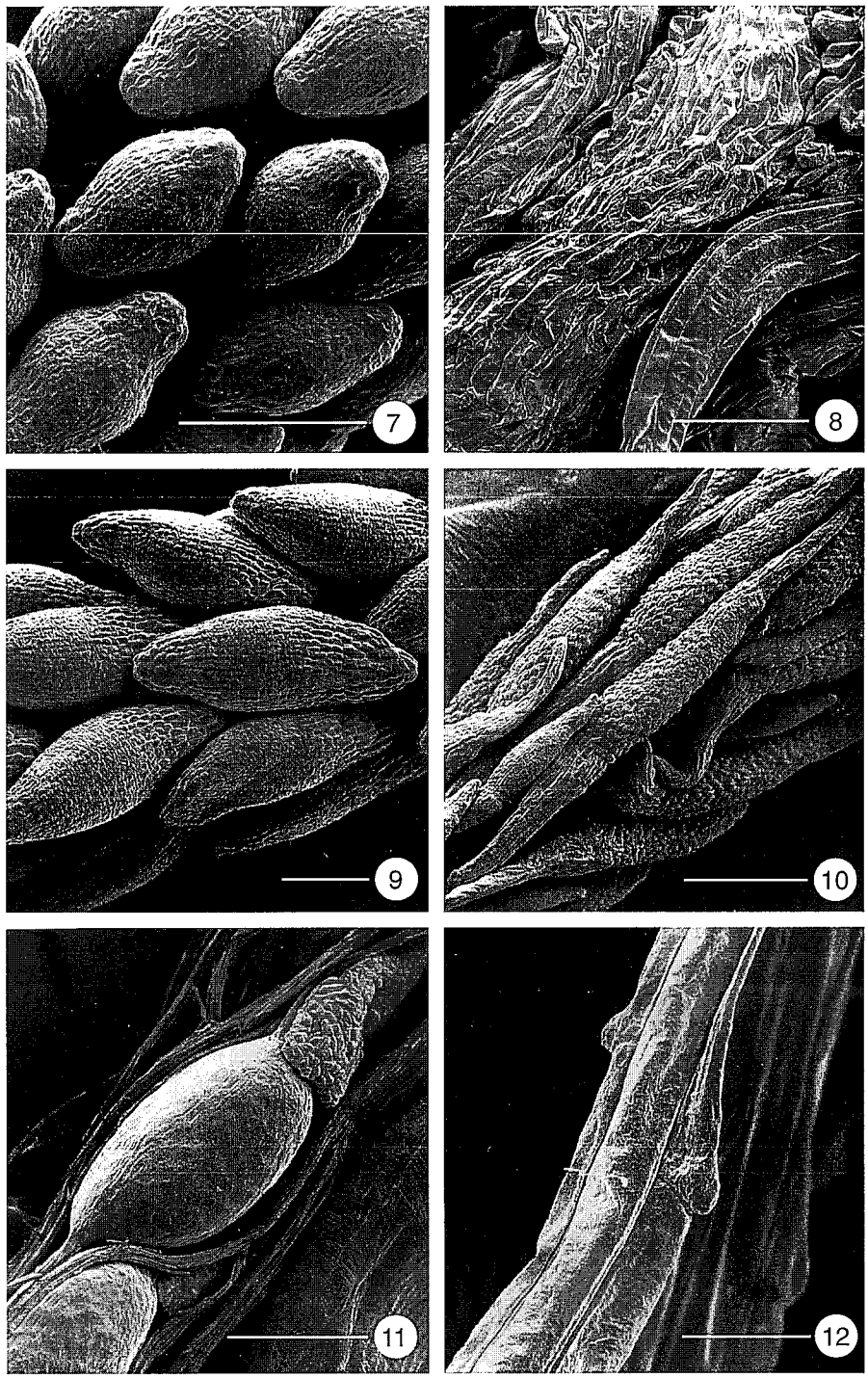
Pattern I (Fig. 19):	Rapid development phase Peak of growth Rapid decline in size to dehiscence;
Pattern II (Fig. 20):	Long slope of development Plateau to dehiscence.

Pattern I was evident in sects. *Microtrichium* and *Haplotrichium s.s.* (seed Type A). Pattern II was evident in sects. *Aeschynanthus* (seed Type A); *Diplotrichium* and *Polytrichium* (seed Type B). Patterns in sect. *X* species (seed Type B) were less clear but closest to Pattern II.

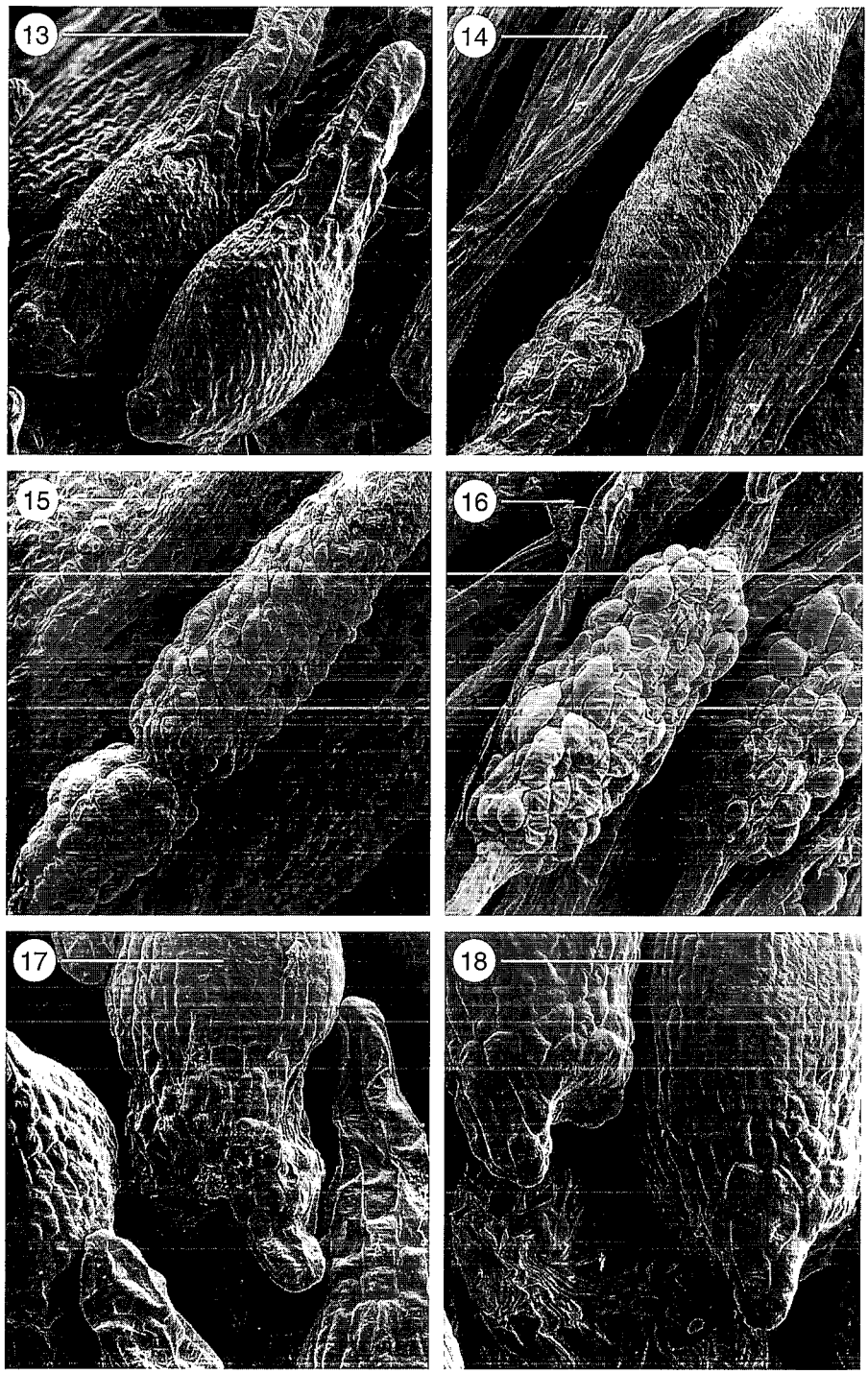
The developing seeds were translucent and surrounded by mucilage that was present in the ovary until the seed and appendages were fully formed, at which point the ovary and contents slowly began to dry prior to dehiscence. Development was marked by a series of processes that appeared to follow the same order. Spiral testa cell orientation was observed within seven days in most seed Type A species, although not until 21 days in *A. bracteatus* (Fig. 8). This developmental feature is present long before drying and shrinkage of the seed occurs. Seed size did not appear to increase until after the initiation of appendage development. It also varied significantly between species, as larger seeds took more time to develop. The small seeds of, for example, *A. buxifolius* (sect. *Microtrichium*), stopped growing after 42 days whereas seeds of sect. *Polytrichium* species, which are the largest so far seen in the genus, did not reach full size until 70 days or more. Testa papillae in most cases did not develop until around the time when maximum seed size was reached. Drying of the seed prior to



Figures 1–6. SEMs of *Aeschynanthus albidus*. Figs 1–5, scale bars = 50 μm; Fig. 6, scale bar = 200 μm. Fig. 1. Initial development of unpollinated ovules: integuments envelop the nucellus from the apical to the hilar end prior to the formation of the micropyle (hilar end to right). Fig. 2. Little apical and no hilar appendage growth in unpollinated ovules (hilar end lower left). Fig. 3. Hilar appendage development at 7 days post-pollination (hilar end upper right). Fig. 4. A fringe of projections developing at the hilar end at 14 days post pollination (hilar end uppermost). Fig. 5. Hilar appendages developing outwards from the centre at 21 days post pollination (hilar end uppermost). Fig. 6. Coma development ceased at 70 days post-pollination (hilar end uppermost).



Figures 7–12. SEMs of *Aeschyanthus* species. Fig. 7. Unpollinated ovules of *A. gracilis*, illustrating shape and micromorphology but little apparent growth (hilar end lower left). Scale bar = 100 μm. Fig. 8. Testa cell orientation showing clockwise spiral twist, apparent at 21 days post-pollination in *A. bracteatus* (hilar end lower left). Scale bar = 100 μm. Fig. 9. Growth and development at 7 days post-pollination—*A. buxifolius* (hilar end to right). Scale bar = 100 μm. Fig. 10. Growth and development at 14 days post-pollination—*A. buxifolius* (hilar end lower left). Scale bar = 500 μm. Fig. 11. Thickened placenta observed at 56 days post-pollination—*A. humilis* (hilar end upper right). Scale bar = 500 μm. Fig. 12. Appendage papillae at 21 days postpollination—*A. lineatus*. Scale bar = 20 μm.



Figures 13–18. Fig. 13. Testa cell orientation showing anticlockwise spiral twist, apparent at seven days post-pollination in *A. parvifolius* (hilar end lower left). Scale bar = 100 μm. Fig. 14. Bubble cell formation at the base of the hilar appendage at 21 days post-pollination—*A. parvifolius* (hilar end lower left). Scale bar = 100 μm. Fig. 15. Bubble cell and testa papillae formation at 28 days post-pollination—*A. parvifolius* (hilar end lower left). Scale bar = 100 μm. Fig. 16. Testa papillae formation at 28 days post-pollination—*A. tricolor* (hilar end upper right). Scale bar = 100 μm. Fig. 17. Appendage development in unpollinated ovules of *A. lineatus*—note that one hilar appendage is longer than the other (hilar end lowermost). Scale bar = 50 μm. Fig. 18. Appendage development at seven days post-pollination in *A. sikkimensis*, RBGE 19611984, again illustrating the unequal growth of the hilar appendages (hilar end lowermost). Scale bar = 50 μm.

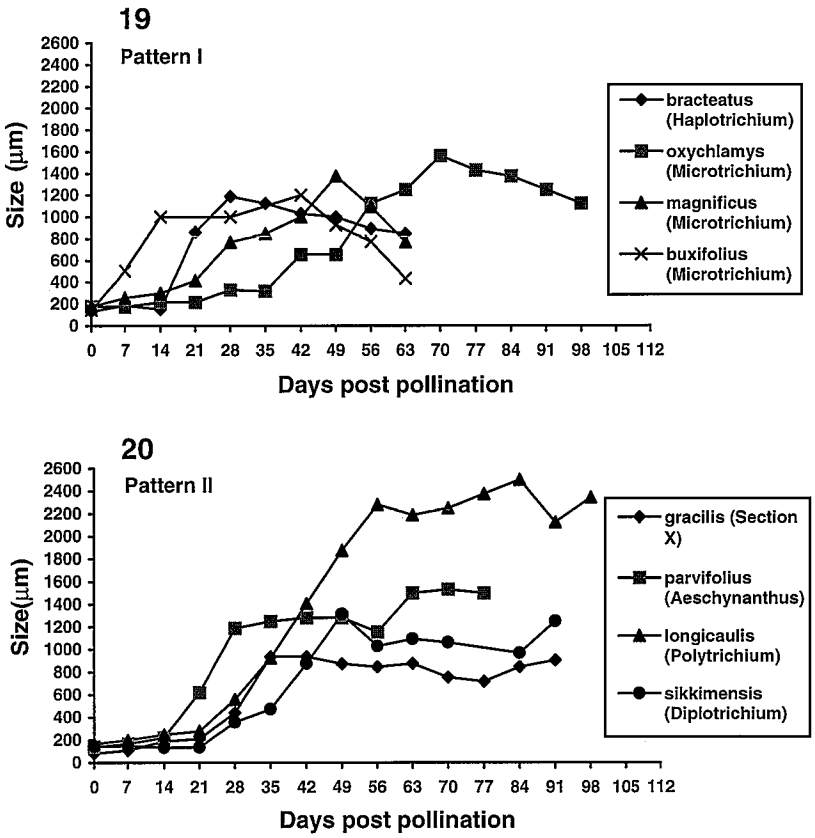


Figure 19. Seed growth Pattern I, evident in sections *Microtrichium* and *Haplotrichium s.s.*
Figure 20. Seed growth Pattern II, evident in sections *Aeschynanthus*, *Diplotrichium*, *Polytrichium* and section *X*.

dehiscence resulted, in Pattern I species, in a decrease in size but in Pattern II species a decrease was not evident. In both *A. parvifolius* and *A. tricolor* (sect. *Aeschynanthus*; Pattern II), drying and shrinkage of the ovary walls, but little reduction in seed size, resulted in the formation of what appeared to be constriction marks on the surfaces of some seeds.

Appendage Growth and Development

Two patterns of growth were observed, reflecting the development of both apical and hilar appendages:

Pattern I (Fig. 21): Rapid growth from 14 to 21 days followed by a period of more gradual growth (short appendage pattern);

Pattern II (Fig. 22): Steady growth from seven to 14 days post-pollination (long appendage pattern).

Pattern I was seen in *Microtrichium* and *Haplotrichium s.s.* although the period of greatest growth in *A. buxifolius* (sect. *Microtrichium*) was between seven and 14 days and it produced much shorter appendages than the other species studied (Figs 9 and 10). The slowest initiation of appendage development was seen in *A. oxychlamys*, with apical development not observed until 14 days and hilar development from 28 days post-pollination; thereafter growth was rapid. Pattern II was exhibited by species in sects. *Aeschynanthus*, *Diplotrichium*, *Polytrichium* and sect. *X*.

Species in sects *Microtrichium* and *Haplotrichium s.s.* had the shortest, thickest appendages at less than 3.5 mm long. Those in sect. *Diplotrichium* and sect. *X* had the longest, in some cases measuring >40 mm although there was a wide variation between species. Appendages in sect. *Aeschynanthus* measured 8–15 mm, and in sect. *Polytrichium* measured 20–25 mm. In *A. gracilis* and *A. humilis* (sect. *X*) appendage length and growth patterns resembled those in sect. *Aeschynanthus*, although their development took longer. In *A. humilis* the placenta, to which the developing seeds are attached, thickened from 21 days and developed into substantial growths (Fig. 11); a feature not observed in other species in this study. *A. pseudohybridus* (sect. *X*) had longer appendages than the other two in this section and they continued to grow for a longer period. Growth and development was more complex in sects. *Diplotrichium* and *Polytrichium* due to the presence of more than one hilar appendage. In sect. *Diplotrichium*, apical growth started prior to pol-

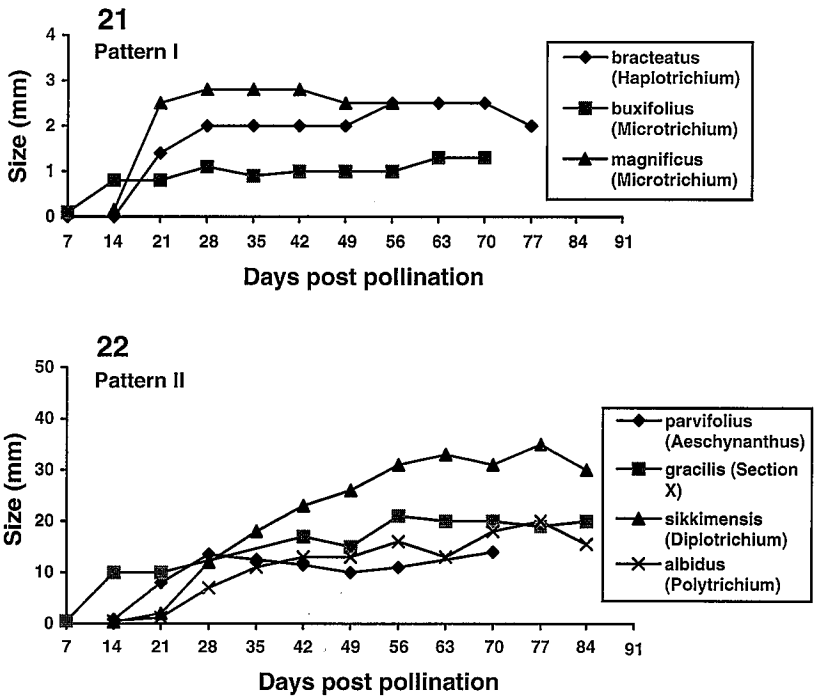


Figure 21. Hilar appendage growth Pattern I, evident in sections *Microtrichium* and *Haplotrichium s.s.*
Figure 22. Hilar appendage growth Pattern II, evident in sections *Aeschynanthus*, *Diplotrichium*, *Polytrichium* and section *X*.

lination and in *A. lineatus* a little hilar development was also seen, with one appendage marginally longer than the other (Fig. 17). In *A. sikkimensis* hilar development was not observed until seven days post-pollination, although once again one appendage appeared to be longer than the other (Fig. 18). In both species appendage papillae (Fig. 12) formed seven days prior to testa papillae and 14 days before seed growth stopped. The sect. *Polytrichium* species *A. albidus* and *A. longicaulis* were alike in appendage development. Apical growth started prior to pollination with a single hilar appendage emerging within seven days post-pollination (Fig. 3). In *A. albidus* the apical appendage developed much faster than the hilar appendage. Within 14 days the hilar end had, in addition, developed a fringe of projections (Fig. 4) which by 21 days became a fused mass of cells radiating outwards from the centre (Fig. 5). By 28 days these had started to separate and by the 35th day coma formation had started. The coma continued to develop until the 70th day (Fig. 6), after which there was little change in the size or appearance of the seed until dehiscence at 13 weeks. *A. longicaulis* seeds are larger than those of *A. albidus* and took longer to develop although their appendage lengths were comparable. At 14 days there was little hilar development and the apical appendage had altered only slightly. Rapid development was, however, seen at both ends of the seed between 14 and 21 days. During this period hilar appendages developed outwards from the region surrounding the micropyle although at 21 days some had yet to emerge from the outer edge of the seed. By the 49th day hilar appendage growth had almost ceased while rapid apical growth continued. At 70 days the coma was fully developed and, as with *A. albidus*, there were no further changes in either the seed or appendages until dehiscence at 16 weeks.

Overall, seed and appendage development appeared to follow the same order for species within sections. Some species appeared to follow a similar time scale, as *A. parvifolius* and *A. tricolor* (sect. *Aeschynanthus*) illustrate. Both apical and hilar appendage development started within seven days of pollination, when the spiral orientation of the testa cells also became apparent (Fig. 13). Bubble cells, never found on *A. tricolor*, were observed on *A. parvifolius* at 21 days (Fig. 14) with testa papillae forming on both species at around 28 days, prior to maturation (Figs 15 and 16). Seed of other species within sections, when compared, followed the same order but not necessarily at the same rate. For example, in *A. albidus* and *A. longicaulis* (sect. *Polytrichium*) apical and hilar appendage development started at the same time after pollination as did coma and appendage papillae development.

However, testa papillae were first apparent in *A. albidus* at 42 days but not seen in *A. longicaulis* until 56 days.

Maturation (Ripening)

Maturation was measured as the time between the last recorded increase in seed size and dehiscence, as a percentage of the total period from pollination. This was not found to be of great significance although it may reflect the complexity of the seed. Species with small seeds developed over a shorter period and took longer to ripen than those with more elaborate seeds. For example, *A. bracteatus* seed developed over 29% and ripened over 71% of the total period, whereas the more complex *A. albidus* developed over 69% and ripened over 31% of the period to dehiscence. No mucilage remained in the ovary during maturation.

Days to Dehiscence

There appeared to be very little correlation between species or sections when the periods to dehiscence were compared.

DISCUSSION

Data from unpollinated ovule development or ovary growth show little to separate species within or between sections. Seed maturation time and the period from pollination to dehiscence may be of ecological importance but as indicators of seed type or section no correlation is apparent from this study. However patterns of development do emerge post-pollination. Similarities can be seen both within and between sections when the patterns of seed and appendage development are examined together.

Two groupings emerge:

(i) Sects *Microtrichium*; *Haplotrichium s.s.*—

Appendage Pattern I; Seed Pattern I

(ii) Sects Aeschynanthus; Diplotrichium; Polytrichium; sect. *X*—

Appendage Pattern II; Seed Pattern II. (Patterns are less clear in sect. *X* species.)

This study is of only a small sample of a large genus, and the conclusions are necessarily speculative. However these groupings may provide valuable clues to ancestral relationships between the sections. It is therefore useful to consider molecular and morphological evidence when attempting to interpret these patterns. Molecular studies of *Aeschynanthus* by Denduangboripant & Cronk (2000 and pers. comm.) and Denduangboripant *et al.* (2001) show the existence of two clades.

Clade I closely corresponds to the seed Type B of Mendum *et al.*, 2001). Species in this clade occur mainly in continental Asia, with dry and monsoonal wet seasons. Testa cell orientation is straight or in a clockwise spiral. Clade II correlates with seed Type A species, found predominantly in the ever-wet Malesian rain forests of the east. Species in this clade have anticlockwise spiral cell orientation. It would be reasonable to assume from the current evidence (Kiehn & Weber, 1997; Denduangboripant & Cronk, 2000; Denduangboripant, Mendum & Cronk, 2001; Mendum *et al.*, 2001) that sect. *Microtrichium* is basal within the genus, containing species to be found in both clades.

The sect. *Microtrichium* species examined in this study share the same seed and appendage development patterns. However, while *A. irigaensis*, *A. magnificus* and *A. oxychlamys* are Clade II species, *A. buxifolius* is basal in Clade I and has similar development patterns to the sect. *Haplotrichium s.s.* species *A. bracteatus*. Both *A. buxifolius* and *A. bracteatus* are mainland south-east Asian species while *A. irigaensis*, *A. magnificus* and *A. oxychlamys* are Malesian, as are the great majority of sect. *Microtrichium* species. The seed morphology and molecular and developmental similarities observed between the Clade I species *A. buxifolius* and *A. bracteatus* suggest a strong link between *Microtrichium* and *Haplotrichium s.s.*

It is interesting that species in sect. *Aeschynanthus* (seed type A; Clade II) share patterns of seed and appendage development with those in sects *Diplotrichium*, *Polytrichium* and sect. *X* (seed type B; Clade I). The groupings reflect the longer periods required for the development of the more complex seed. When the patterns are examined in a phylogenetic context (Fig. 23), it appears that the simple seed and shorter development period are the ancestral states. When external influences (altitude, country or area of origin) were considered there appeared to be no correlation with the observed patterns of development, but it must be remembered that the samples were taken from cultivated plants and the effects of these factors may be suppressed.

The early development of one hilar appendage relative to the coma in sect. *Polytrichium*, first noted in *A. fecundus* P.Woods by Saueregger & Mühlbauer (unpubl.), is confirmed by this study. The development of one primary appendage, and later secondary development of the remainder, indicates that the condition of more than one hilar appendage is a derived state. Initial development of one hilar appendage relative to the second in sect. *Diplotrichium* is further evidence for this, and for the view that these sections are derived from sect. *X* (Denduangboripant & Cronk, 2000; Mendum *et al.*, 2001). The longest hilar appendage in the genus occurs in sect. *X*; appendages are shorter in sect. *Diplotrichium* and shorter still in *Polytrichium* (Mendum *et al.*, 2001). The evolution of additional

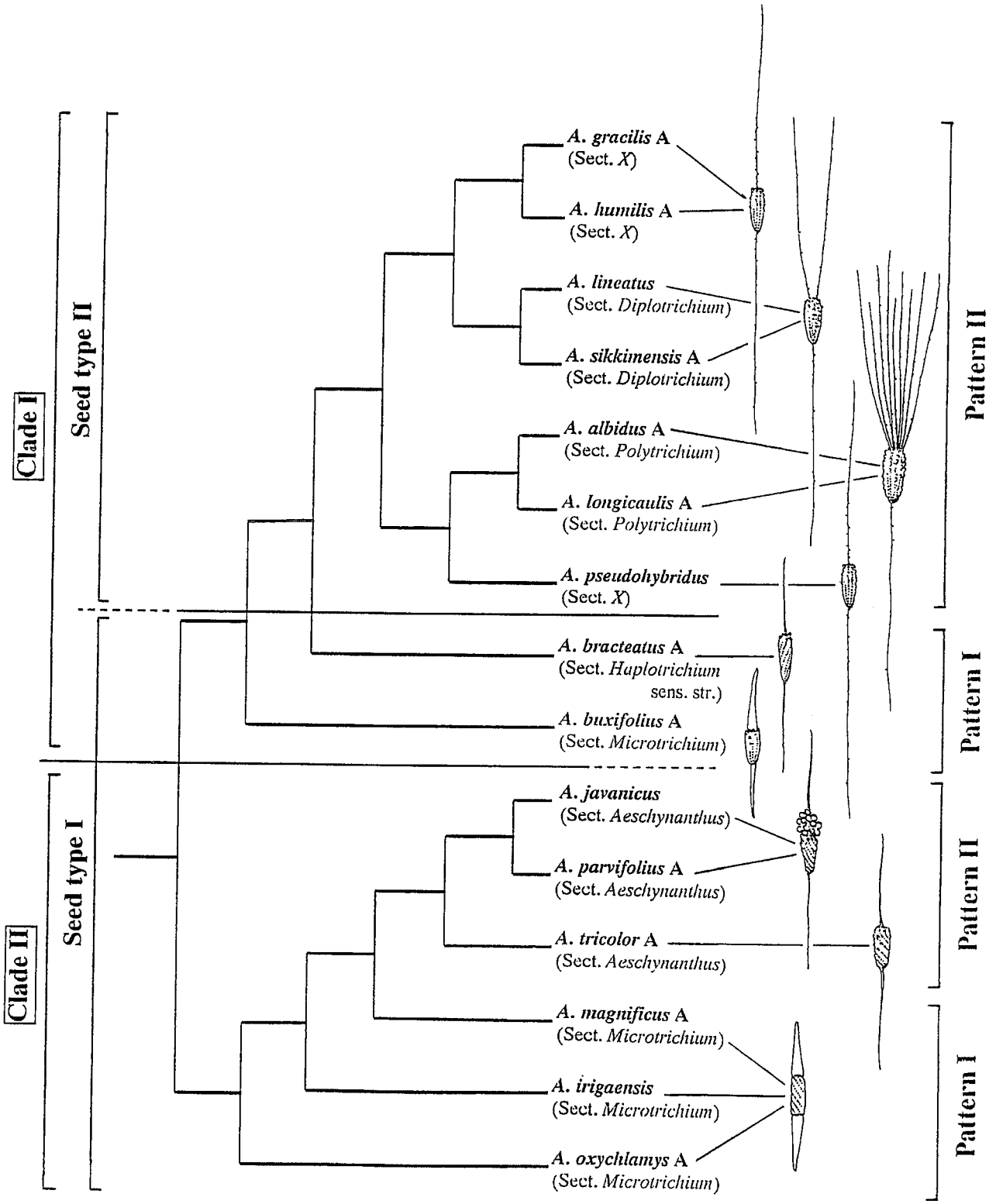


Figure 23. Data for examined species showing relationships between ontogeny, seed morphology and molecular results (analysis of data from Denduangboripant *et al.*, 2001). This tree is identical to that based on a larger set of species and rooted on *Cyrtandra* and *Lysionotus* (not shown) as presented in Denduangboripant *et al.* (2001).

dispersal aids may have reduced the significance of appendage length.

CONCLUSION

The results of this study indicate that different post-pollination patterns of growth and development do exist for different seed types and between sections. Further inferences regarding their value as 'ancestral markers' are speculative. However, it provides evidence that the condition of more than one hilar appendage is derived. It also shows that the orientation of the testa cells is a consistent developmental feature, important morphologically and linking with molecular studies. This ontogenetic study of *Aeschynanthus* therefore reinforces current theories concerning the evolution of the genus.

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REFERENCES

Bentham G. 1876. Gesneriaceae. In: Bentham G, Hooker JD, eds. *Genera Plantarum*. 2. London: 900–1025.

Burtt BL, Woods PJB. 1975. Studies in the Gesneriaceae of the Old World XXXIX: towards a revision of *Aeschynanthus*. *Notes from the Royal Botanic Garden Edinburgh* **33:** 471–489.

Clarke CB. 1883. Cytandreae. In: De Candolle A, De Candolle D, eds. *Monographiae Phanerogamarum*, 5. Paris: 18–57.

Denduangboripant J, Cronk QCB. 2000. High intraindividual variation in internal transcribed spacer sequences in *Aeschynanthus* (Gesneriaceae): implications for phylogenetics. *Proceedings of the Royal Society of London B* **267:** 1407–1415.

Denduangboripant J, Mendum M, Cronk QCB. 2001. Evolution in *Aeschynanthus* (Gesneriaceae) inferred from ITS sequences. *Plant Systematics and Evolution* **228:** 181–197.

Kiehn M, Weber A. 1997. Chromosome numbers of Malayan and other paleotropical Gesneriaceae. 11. Tribes Trichosporeae, Cyrtandreae and Epithemateae. *Beiträge Zur Biologie der Pflanzen* **70:** 445–470.

Mendum M, Lassnig P, Weber A, Christie F. 2001. Testa and seed appendage morphology in *Aeschynanthus* (Gesneriaceae): phytogeographical patterns and taxonomic implications. *Botanical Journal of the Linnean Society* **135:** 195–213.

Wang WT. 1984. *Aeschynanthus*. *Bulletin of the Botanical Laboratory of North-Eastern Forestry Institute* **4:** 26–30.

APPENDIX

SPECIES INVESTIGATED

A. albidus (Blume) Steud. Argent 84/17, cult. RBGE 19841169; Malaysia, Sabah.
A. bracteatus A. DC. cult. RBGE 19970175; Vietnam.
A. buxifolius Hemsl. Cherry 135, cult. RBGE 19970164; Vietnam.
A. gracilis C.B. Clarke: Grierson & Long 4125, cult. RBGE 19821972; Bhutan.
A. humilis Hemsl. cult. RBGE 19972045; Thailand.
A. irigaensis (Merr.) B.L. Burtt & P. Woods: cult. RBGE 19972532; Philippines, Luzon.
A. javanicus Hook: Vogel s.n., cult. RBGE 19971334; Peninsular Malaysia.
A. lineatus Craib: Wallace, Chambers & Curry 615, cult. RBGE 19970174; China.
A. longicaulis R. Br. Copenhagen University, cult. RBGE 19672218; Thailand.
A. magnificus Stapf: cult. RBGE 19801181; Malaysia, Sabah; cult. RBGE 19812958; Malaysia, Sabah.
A. oxychlamys Mendum: Mitchell & Smith 187, cult. RBGE 19930953; New Guinea.
A. parvifolius R. Br. cult. RBGE 19622831; Malaysia, Sarawak.
A. pseudohybridus Mendum: Vogel, Schuiteman & Roelfsema s.n., cult. RBGE 19971340; Malaysia, Sarawak.
A. sikkimensis (C.B. Clarke) Stapf: cult. RBGE 19611984; Montreal Botanic Garden, origin unknown; cult. RBGE 19892475; Nepal.
A. tricolor Hook. cult. RBGE 19970167; Malaysia, Sabah.