

CYTOTAXONOMIC OBSERVATIONS IN THE GENUS *AESCHYNANTHUS* (*GESNERIACEAE*)

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This study is a contribution to the further understanding of cytological patterns in *Aeschynanthus* (*Gesneriaceae*). Chromosome numbers are reported for 12 species from six sections; nine of these are new counts. Two basic numbers, $x=16$ and $x=15$, are generally encountered. *Aeschynanthus gracilis* proved to be of exceptional interest, as its rare somatic number, $2n=28$, confirms the occurrence of a third basic number, $x=14$, in the genus. Variation in chromosome number in relation to seed morphology is examined.

Keywords. *Aeschynanthus*, chromosomes, cytotaxonomy, *Gesneriaceae*, sectional relationships.

INTRODUCTION

Aeschynanthus Jack (*Gesneriaceae*, subfamily *Cyrtandroideae*, tribe *Trichosporeae*) is a genus of some 150 species of perennial, usually epiphytic, subshrubs distributed from India and China and throughout SE Asia to the Solomon Islands. The tribe *Trichosporeae* (consisting of *Aeschynanthus*, *Agalmyla* (including *Dichrotrichium*), *Loxostigma*, *Lysionotus*, and the doubtful monotypic genus *Micraeschynanthus*) is distinguished by the possession of appendages, often hair-like, at each end of the seed. The apical appendage is always single, but in *Aeschynanthus* the number and form of the hilar appendages are taxonomically important. Bentham (1876) first proposed a sectional classification of *Aeschynanthus* based almost entirely on seed appendages, and recognized four sections: *Polytrichium*, *Diplotrichium*, *Haplotrichium*, and *Holocalyx* (now sect. *Aeschynanthus*). Clarke (1883) added sect. *Microtrichium*. Schlechter (1923) added sect. *Anisocalyx* but this was subsumed under *Microtrichium* by Burt & Woods (1975), while Wang (1984) created sect. *Xanthanthos*, based not on seed but on corolla characters, to accommodate a single Chinese species. Recent studies particularly of seed and appendage morphology (Mendum *et al.*, 2001) identified two major groups within the genus, with each group subdividing into sections. Species with Type A seeds encompass sects *Microtrichium*, *Aeschynanthus* and *Haplotrichium* *sens. str.*; species with Type B seeds comprise sects *Polytrichium*, *Diplotrichium*, *Xanthanthos*, and a section consisting of many of the species previously placed in sect. *Haplotrichium*. The last group cannot be adequately circumscribed until further studies, particularly on sect. *Xanthanthos*, are complete

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so is provisionally referred to as sect. *X*. This classification has been adopted for this study.¹

The first cytological studies on *Aeschynanthus* were made by Rogers (1954); since then 25 species, two unnamed taxa and two synthetic hybrids have been counted. The basic numbers for the genus appear to be $x=16$ and $x=15$ (Ratter, 1975), which as Kiehn & Weber (1997) point out do not neatly correlate with sectional circumscription. The unusual number of $2n=28$ occurs in *A. longicaulis* (Eberle, 1956) as a variant within a species which also shows $2n=30$ (Rogers, 1954; Ratter & Prentice, 1964). This has been the only deviation from the pattern of $x=16$ and 15. Polyploidy has been recorded from all examined sections except *Diplotrichium* and *Haplotrichium* sens. str. In the light of increased taxonomic knowledge, the present investigation has been undertaken to seek further elucidation of cytological relationships between sections.

MATERIALS AND METHODS

The Royal Botanic Garden Edinburgh has an extensive living collection of *Aeschynanthus*. From this, three species from sect. *X*, two each from sects *Aeschynanthus* and *Diplotrichium*, and one each from sects *Haplotrichium* sens. str., *Polytrichium* and *Microtrichium* were selected. In addition, two species of sect. *Microtrichium* previously regarded as anomalous, *A. magnificus* Stapf and *A. vinaceus* P. Woods, were chosen to provide chromosomal results which might assist in further clarifying their status. The species are listed in the Appendix. Material of species of sect. *Xanthanthos* was not available. Root tips, cotyledons and ovules were used as sources of meristematic tissue.

Root tips were harvested from cuttings grown in perlite in a propagator unit with bottom heat; usable roots were produced in 3–4 weeks. For ovules, ovaries from very young flower buds were cut in half longitudinally before pretreatment and subsequent processing. Seedlings of most Old World gesneriads develop unequal cotyledons after germination, and the basal meristem of the larger one is a good source of dividing cells. Seed was germinated on filter paper in a growth chamber; 10–14-day-old seedlings were pretreated and fixed whole, and enlarging cotyledons squashed after staining (after Jong & Möller, 2000).

Two pretreatment chemicals were normally used concurrently, α -bromonaphthalene for 2–3h at room temperature (20°C) and 0.002M 8-hydroxyquinoline for 4–6h at 13°C, the latter consistently yielding better chromosome spreads than the former. The material was then fixed in fresh Farmer's Fluid (3:1, ethanol:glacial

¹ *Aeschynanthus* classification according to Mendum *et al.* (2001). Type A seed: testa cell orientation almost always spiral, papillae formed from single cells, appendages short, not papillose. Subtypes equivalent to sects *Haplotrichium* sens. str., *Microtrichium* and *Aeschynanthus*. Type B seed: testa cell orientation straight, papillae formed from raised ends of two adjacent cells, appendages long, slender, always papillose. Subtypes equivalent to sects *Polytrichium*, *Diplotrichium*, *Xanthanthos*, and those species with Type B seed that were previously assigned to sect. *Haplotrichium*, now placed in a provisional sect. *X*.

acetic acid) and stored until required. Cotyledons and ovules were stained with lacto-propionic orcein (after Dyer, 1963) after softening in 5M HCl for 15min. The preferred protocol for root tips was hydrolysis in 5M HCl for 30–50min, washing in several changes of water, and staining with Feulgen Reagent (after Fox, 1969). Additional softening in a 1:1 enzyme mixture of 4% cellulase and 4% pectinase for 20–40min at 35°C greatly improved squashing. Mounting in 0.4% aceto-carmine after Feulgen staining, and viewing under phase-contrast optics, greatly increased visibility of the chromosomes.

All the photomicrographs were taken on 35mm Kodak Technical Pan film. Permanent slides were prepared according to a modified quick-freeze method (after Conger & Fairchild, 1953, in Jong, 1997).

Voucher herbarium specimens and permanent slides are lodged at the Royal Botanic Garden Edinburgh.

RESULTS AND DISCUSSION

All counts from this study are listed in Table 1. Most counts were based on root tips, a few on ovules, and some confirmatory ones on cotyledons. Apart from those for *A. boschianus* (sect. *Aeschynanthus*) with $2n=64$, and *A. lineatus* (sect. *Diplotrichium*) and *A. longicaulis* (sect. *Polytrichium*), both $2n=30$, agreeing with already published data, all are first reports. Previously published chromosome numbers in *Aeschynanthus* are summarized in Table 2.

The results of the present study, together with previously published counts

TABLE 1. Chromosome counts from *Aeschynanthus* taxa, obtained in the current study

Taxon	Accession	Country of origin #	2n	Fig. 2
<i>A. angustifolius</i> (Bl.) Steud.	19881452	Sumatra	30	a,b
<i>A. arctocalyx</i> Mendum & Madulid	19922776	Philippines	32	c
<i>A. boschianus</i> de Vriese	19570134	Cult. origin	64†	
<i>A. bracteatus</i> Wall. ex DC.	19970165	Vietnam	32*	d,e
<i>A. buxifolius</i> Hemsley	19970178	Vietnam	32	
<i>A. ceylanicus</i> Gardner	19850904	Sri Lanka	32	f
<i>A. gracilis</i> Parish ex. C. B. Clarke	19821972	Bhutan	28	g
	19802575	Cult. origin	28	h,k
	19802720	Cult. origin	28	
	19821969	Bhutan	28	
	19821970	Bhutan	28	
<i>A. hookeri</i> C. B. Clarke	19892128	Nepal	32	i
<i>A. lineatus</i> Craib	19970163	China	30*†	
<i>A. longicaulis</i> Wall. ex R.Br.	19621423	Pen. Malaysia	30†	
<i>A. magnificus</i> Stapf	19812958	Sabah	32	j
<i>A. vinaceus</i> P. Woods	19672118	Sarawak	32	l

* Counts from ovules; † recounts; # for further details see Appendix.

TABLE 2. Previously published chromosome numbers in *Aeschynanthus*

Taxon	Distribution	n	2n	Reference
<i>A. albidus</i> (Bl.) Steud.	W Malesia, Borneo		30	Milne, 1975
<i>A. boschianus</i> de Vriese (as <i>A. lamponga</i> Miq.)	Java	32		Eberle, 1956
<i>A. ellipticus</i> Lauterb. & K. Schum.	New Guinea		32	Milne, 1975
			64	Ratter, 1963 (3 stocks)
			96	R & P, 1964
<i>A. fecundus</i> P. Woods	Thailand, Pen. Malaysia	16		R & M, 1970
<i>A. guttatus</i> P. Woods	New Guinea		32	Milne, 1975
<i>A. horsfieldii</i> R.Br.	Java		32	Milne, 1975
<i>A. hosseussii</i> Pellegr	Thailand, Vietnam		32	Ratter, 1963
<i>A. javanicus</i> Hook. (as <i>A. javanicus</i> Hort. Rollisson ex Hook.)	Java	32		Eberle, 1956
<i>A. lineatus</i> Craib	China, N Thailand		30	Milne, 1975
<i>A. longicaulis</i> Wall. ex R.Br. (as <i>A. marmoratus</i> T. Moore) (as <i>A. marmoratus</i> T. Moore) (as <i>A. marmoratus</i> T. Moore)	China, Indo-China Pen. Malaysia	14		Eberle, 1956
			30	Rogers, 1954
			30	R & P, 1964
			30	Fussell, 1958
<i>A. longiflorus</i> DC (as <i>A. perakensis</i> Ridl.)	W Malesia		30	Ratter, 1963
			30	R & P, 1964, also polysomatic 2n = 21 and 2n = 28
			30	Milne, 1975
<i>A. myrmecophilus</i> P. Woods	Pen. Malaysia		64	Milne, 1975

TABLE 2. (continued)

Taxon	Distribution	n	2n	Reference
<i>A. nummularius</i> (Burk. & S. Moore) K.Sch.	New Guinea		64	Ratter, 1963
			64	R & M, 1970 (2 stocks)
<i>A. obconicus</i> C. B. Clarke	W Malesia	16		R & P, 1967
<i>A. obovatus</i> C. B. Clarke				
(as <i>A. papuanus</i> (Schltr.) B. L. Burtt)	Borneo, New Guinea		32	Milne, 1975
<i>A. parasiticus</i> (Roxb.) Wall.	E India	16		Malla <i>et al.</i> , 1978
(as <i>A. grandiflorus</i> (D. Don) Spreng.)		16		Eberle, 1956
(as <i>A. grandiflorus</i> (D. Don) Spreng.)			30	Rogers in Lee, 1962
<i>A. parviflorus</i> (D. Don) Spreng.	NE India to SW China		32	Ratter, 1963
<i>A. parvifolius</i> R.Br.	W Malesia, Borneo		64	R & M, 1970 (5 stocks)
			64	K & W, 1997
			32	K & W, 1997 (2 stocks)
		32	64	Hellmayr, 1989
(as <i>A. lobbianus</i> Hook.)		32		Eberle, 1956
<i>A. praelongus</i> Kraenzl.	Borneo	16		R & M, 1970
<i>A. pulcher</i> (Blume) G. Don	Java		60	Rogers, 1954
		32		Eberle, 1956
			64	Ratter, 1963
<i>A. radicans</i> Jack	W Malesia, Borneo, Thailand	15		R & M, 1970 (1 stock)
			32	R & M, 1970 (2 stocks)
		16	32	K & W, 1997 (1 stock)
			32	Hellmayr, 1989

TABLE 2. (continued)

Taxon	Distribution	n	2n	Reference
<i>A. rhododendron</i> Ridl. (as <i>A. longicalyx</i> Ridl.) (as <i>A. longicalyx</i> Ridl.)	Pen. Malaysia		32	K & W, 1997 (3 stocks)
			32	Milne, 1975
			32	Hellmayr, 1989
<i>A. sikkimensis</i> Stapf	NE India		32	Ratter, 1963
			32	R & M, 1970 (2 stocks)
<i>A. speciosus</i> Hook.	W Malesia, Borneo	32		Eberle, 1956
<i>A. tricolor</i> Hook.f.	Borneo	16		Eberle, 1956
			32	R & M, 1970
UNNAMED TAXA				
<i>A. sp.</i> G260		15		Lee, 1962*
<i>A. sp.</i>	New Guinea		60	Borgmann, 1964
HYBRIDS				
<i>A. tricolor</i> Hook.f. × <i>A. parvifolius</i> R.Br.	Synthetic hybrid		48	R & M, 1970
<i>A.</i> × <i>splendidus</i> T. Moore (<i>A. speciosus</i> × <i>A. parasiticus</i>) (as <i>A. parasiticus</i>)	Synthetic hybrid		32	R & P, 1964**
			32	Ratter, 1963

*, received as *A. micranthus* C. B. Clarke(?) in Lee; **, repetition of count on same accession. Key to abbreviated names: K & W, Kiehn & Weber; R & M, Ratter & Milne; R & P, Ratter and Prentice.

(Table 2) confirm the presence of two prevalent basic numbers, $x=16$ and $x=15$, in *Aeschynanthus*, with $2n=32$ the dominant sporophytic number (Fig. 1), and the most frequent in sects *Diplotrichium* and *Microtrichium* (Table 3). Gametophytic numbers of $n=14, 15, 16$ and 32 , and sporophytic numbers of $2n=28, 30, 32, 60, 64$ and 96 , have all been encountered. Of the hybrids examined, one gave $2n=32$ and one $2n=48$, as expected from the numbers of the parental species. It is worth pointing out that $n=11$ (for *A. parasiticus*), attributed to Malla *et al.* (1978), is most probably a typographical error (in Goldblatt, 1981) and should be ignored.

The chromosome counts listed in Table 3 show that although the sections cannot be separated on basic number, certain cytological patterns of variation occur correlated with the seed group classification proposed by Mendum *et al.* (2001).

Type A seed group, basic number $x=16$, and polyploidy

Species with Type A seed (sects *Microtrichium*, *Aeschynanthus*, and *Haplotrichium* sens. str.) are based on $x=16$, although two species show intraspecific dysploidy (counts of $2n=60$ and 64 in *A. pulcher*, and $2n=30$ and 32 in *A. radicans*). Furthermore, polyploidy appears to be relatively common in sect. *Aeschynanthus*, occurring in four out of the 10 species counted, and is also recorded in sects *Microtrichium*, *X* and *Polytrichium*. Most of the polyploids are tetraploids ($2n=64, 60$) but one stock of *A. ellipticus* (sect. *Microtrichium*) is hexaploid ($2n=96$, Ratter & Prentice, 1964). This species, together with *A. parvifolius* and *A. pulcher*, are the only examples known so far with intraspecific polyploid series. *Aeschynanthus boschianus* (sect. *Aeschynanthus*) is the only polyploid species encountered in the present study. Although cytological data are at present only available for an inadequate representation of species, it does seem that polyploidy has

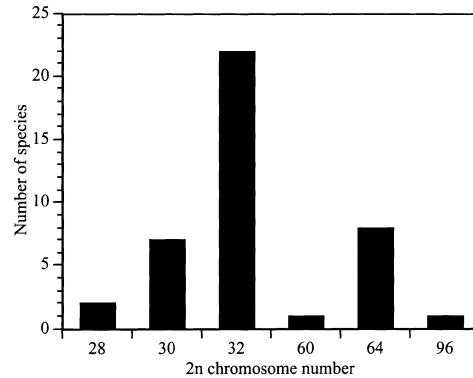


FIG. 1. Histogram showing the distribution of sporophytic chromosome numbers in all counted named species of *Aeschynanthus*. (Different cytotypes occurring in the same species have been scored separately.)

TABLE 3. Summary of all *Aeschynanthus* chromosome counts to date, relative to sectional classification (all given as 2n for ease of comparison)

Type A seed group	2n	Type B seed group	2n
SECT. <i>MICROTRICHIMUM</i>		SECT. <i>X</i>	
<i>A. buxifolius</i>	32*	<i>A. angustifolius</i>	30*
<i>A. ellipticus</i>	32, 64, 96	<i>A. ceylanicus</i>	32*
<i>A. guttatus</i>	32	<i>A. gracilis</i>	28*
<i>A. horsfieldii</i>	32	<i>A. hosseussii</i>	32
<i>A. rhododendron</i>	32	<i>A. longiflorus</i>	30 (21, 28)
<i>A. magnificus</i>	32*	<i>A. speciosus</i>	64
<i>A. nummularius</i>	64	SECT. <i>DIPLOTRICHIMUM</i>	
<i>A. vinaceus</i>	32*	<i>A. hookeri</i>	32*
SECT. <i>AESCHYNANTHUS</i>		<i>A. lineatus</i>	30*
<i>A. arctocalyx</i>	32*	<i>A. parasiticus</i>	30, 32
<i>A. boschianus</i>	64*	<i>A. parviflorus</i>	32
<i>A. javanicus</i>	64	<i>A. sikkimensis</i>	32
<i>A. obconicus</i>	32	SECT. <i>POLYTRICHIMUM</i>	
<i>A. obovatus</i>	32	<i>A. albidus</i>	30
<i>A. parvifolius</i>	32, 64	<i>A. fecundus</i>	32
<i>A. praelongus</i>	32	<i>A. longicaulis</i>	28, 30*
<i>A. pulcher</i>	60, 64	<i>A. myrmecophilus</i>	64
<i>A. radicans</i>	30, 32		
<i>A. tricolor</i>	32		
SECT. <i>HAPLOTRICHIMUM</i> SENS. STR.			
<i>A. bracteatus</i>	32*		

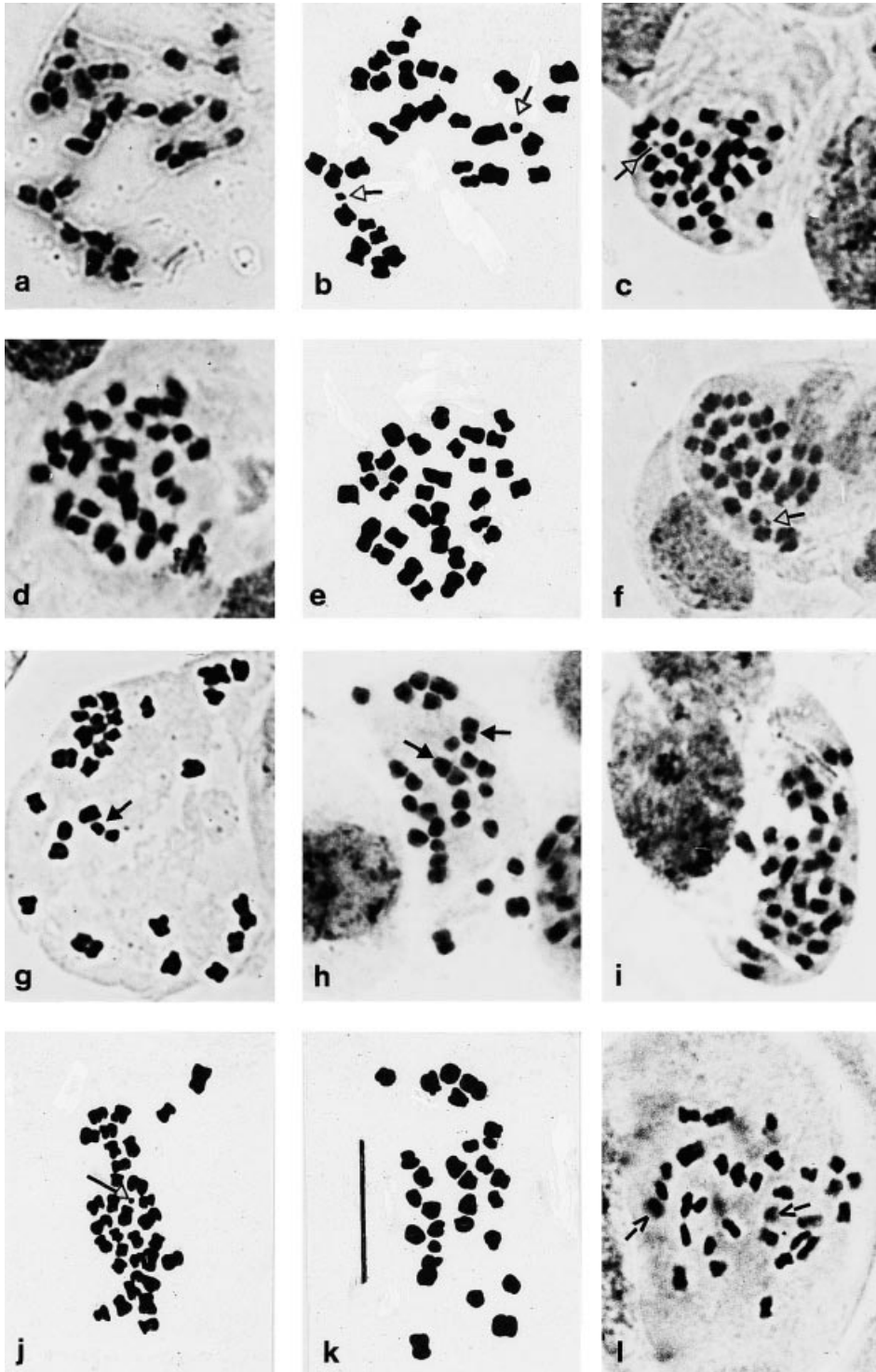
Numbers in brackets, polysomatic counts; *, counts from the present study.

played an important part in species diversification in sect. *Aeschynanthus* (Kiehn & Weber, 1997), but not in others where polyploidy is only of sporadic occurrence.

Type B seed group, basic numbers $x=16$ and $x=15$, and dysploidy

Both $x=16$ and $x=15$ occur in all the Type B sections (*X*, *Diplotrichium*, and *Polytrichium*), while *A. parasiticus* ($2n=30, 32$) and *A. longicaulis* ($2n=28, 30$) show

FIG. 2. All somatic metaphases, unless stated otherwise: a, *A. angustifolius*, $2n=30$; b, drawing of (a), satellites prominent; c, *A. arctocalyx*, prometaphase $2n=32$, satellite minute; d, *A. bracteatus*, $2n=32$; e, drawing of (d); f, *A. ceylanicus*, prometaphase, $2n=32$; g, *A. gracilis*, $2n=28$ (Bhutan, Acc. No. 19821972); h, *A. gracilis*, $2n=28$ (Acc. No. 19802575, wild origin unknown); i, *A. hookeri*, prometaphase, $2n=32$; j, *A. magnificus*, $2n=32$, drawing, satellite minute; k, drawing of *A. gracilis* (h); l, *A. vinaceus*, $2n=32$. Open arrows, satellites; solid arrows, overlapping/touching chromosomes; >, out-of-focus chromosomes. Scale bar = $10\mu\text{m}$ in (k) applies to all figures.



dysploid series within a single species. In *A. longiflorus*, polysomatic variations of $2n=21$ and 28 have been encountered in addition to the normal $2n=30$ (Ratter & Prentice, 1964). Dysploid change through chromosome loss is probably responsible for the relationship between $x=16$ and $x=15$ (Ratter, 1975), but see below for further discussion. Polyploids are scarce, recorded so far only in *A. myrmecophilus* and *A. speciosus*, both $2n=64$.

Aeschynanthus gracilis (sect X) and a third basic number, $x=14$

A single previous count of the rare chromosome number of $2n=28$ was recorded by Eberle (1956) for a stock of *A. longicaulis* (as *A. marmoratus* T. Moore), a species where $2n=30$ has been observed in several other accessions (Rogers, 1954; Ratter & Prentice, 1964; Milne, 1975); the only other report of such a number was as a somatic variation within roots of *A. longiflorus*, where $2n=30$ is the prevalent number (Ratter & Prentice, 1964). Understandably, there has been some hesitancy in accepting the presence of a third basic number, $x=14$, in the genus (Kiehn & Weber, 1997). However, it has been confirmed in the present study in *A. gracilis*, a species with a wide geographical range, recorded from China (Yunnan), Thailand, Myanmar, N Vietnam, N India, and Bhutan. This species has a trailing, flexuous habit and thick leaves, uncommon features in sect. X. Its unusual somatic number of $2n=28$ was encountered in five different accessions (Table 1; Appendix). *Aeschynanthus* chromosomes are small, (0.6–1.5 μm ; Kiehn & Weber, 1997) and metaphase spreads with clear morphology rather hard to find; it is nevertheless notable that the karyotype of *A. gracilis* has a large number of acrocentric chromosomes (Fig. 2g,h), compared with other species illustrated in Fig. 2 where metacentrics and submetacentrics predominate. Our study thus clearly demonstrates the existence in *Aeschynanthus* of a third basic number of $x=14$, with possibly a distinctive karyotype.

Aeschynanthus magnificus and *A. vinaceus*

The taxonomic assignment of these two species was at one time uncertain, and they were provisionally placed in sect. *Microtrichium*. Mendum *et al.* (2001) agree with this placement, and in terms of chromosome number, they both have the same somatic number $2n=32$, the predominant number in the Type A seed group.

Ratter (1975) deduced that of the two prevalent basic numbers in *Aeschynanthus*, $x=16$ is ancestral and $x=15$ derived through dysploid reduction. Arguments in favour of this view have also been forwarded by Kiehn & Weber (1997), namely that $x=16$ occurs throughout sect. *Microtrichium*, while *Agalmyla* and *Lysionotus*, also in the tribe *Trichosporeae*, have $x=16$ (Fussell, 1958; Ratter, 1975; Kiehn &

Weber, 1997). On the basis of appendage morphology (Mendum *et al.*, 2001) and molecular data (Denduangboripant & Cronk, 2000), sect. *Microtrichium* is regarded as basal to the genus. There are dysploid series of $2n=30$ and 32 in both Type A seed and Type B seed groups, occurring both inter- and intraspecifically, and this is now extended to $2n=28$ in sect. *X* (Type B seed). Such variation may have been the result of dysploid change in chromosome number which has become established in sects *X*, *Diplotrichium*, and *Polytrichium*, and perhaps sect. *Aeschynanthus*, presumably representing many independent dysploid evolutionary lines (Ratter, 1975), and taken even further to $x=14$ in *A. gracilis* of sect. *X*.

CONCLUSIONS

Chromosome reorganization leading to numerical change appears to have taken two different directions within the genus *Aeschynanthus*. Polyploidy seems to have played an important role in diversification, especially in the Type A seed group of species, and particularly in sect. *Aeschynanthus*. Numerical change is achieved mainly through dysploidy, with only limited and sporadic occurrence of polyploidy, in the Type B seed group. On the basis of seed morphology and molecular data, the Type B species appear to be more derived. Counts obtained during this study, together with previous records, suggest the derivation of $2n=28$ and $2n=30$ through dysploid reduction from an ancestral $2n=32$, a number that predominates in species with Type A seed.

Available cytological data for the genus are still limited, and further studies, including karyotype analysis, should bring greater cytotaxonomic understanding.

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REFERENCES

- BENTHAM, G. (1876). Gesneriaceae. In: BENTHAM, G. & HOOKER, J. D. *Genera Plantarum*, Vol. 2, pp. 990–1025. London: Reeve & Co. and Williams & Norgate.
- BORGMANN (1964). Anteil der Polyploiden in der Flora der Bismarck-gebirges von Ostneuguinea. *Zeitschr. Bot.* 52: 118–172.
- BURTT, B. L. & WOODS, P. J. B. (1975). Studies in the Gesneriaceae of the Old World XXXIX. Towards a revision of *Aeschynanthus*. *Notes Roy. Bot. Gard. Edinburgh* 33: 471–489.

- CLARKE, C. B. (1883). Cyrtandreae. In: DE CANDOLLE, A. *Monographia phanerogamarum*, Vol. 5 (1), pp. 1–303. Paris: Sumptibus G. Masson.
- DENDUANGBORIPANT, J. & CRONK, Q. C. B. (2000). High intra-individual variation in ITS sequences in *Aeschynanthus*: implications for phylogenetics. *Proc. R. Soc. Lond. B.* 267: 1407–1415.
- DYER, A. F. (1963). The use of lacto-propionic orcein in rapid squash methods for chromosome preparations. *Stain Tech.* 38: 85–90.
- EBERLE, P. (1956). Cytologische Untersuchungen an Gesneriaceen. I. Mitteilung. Die Struktur der Pachytänchromosomen, sowie eine Reihe neu bestimmter Chromosomenzahlen. *Chromosoma* 8: 285–316.
- FOX, D. P. (1969). Some characteristics of the cold hydrolysis technique for staining plant tissues by the Feulgen reaction. *J. Histo. Cytochem.* 17: 226.
- FUSSELL, C. P. (1958). Chromosome Numbers in the Gesneriaceae. *Baileya* 6: 117–125.
- GOLDBLATT, P. (ed.) (1981). Index to plant chromosome numbers, 1975–1978. St Louis, MI: Missouri Botanical Garden.
- HELLMAYR, E. (1989). Chromosomenzählungen an Blütenpflanzen der Malaiischen Halbinsel. – 5. In: Österreichisches Botanikertreffen in Innsbruck, 25–28 Mai 1989. Innsbruck: Universität.
- JONG, K. (1997). *Laboratory Manual of Plant Cytological Techniques*. Edinburgh: Royal Botanic Garden Edinburgh.
- JONG, K. & MÖLLER, M. (2000). New chromosome counts in *Streptocarpus* (Gesneriaceae) from Madagascar and Comoro Islands, and their taxonomic significance. *Plant Syst. & Evol.* 224: 173–182.
- KIEHN, M. & WEBER, A. (1997). Chromosome numbers of Malayan and other paleotropical Gesneriaceae. II. Tribes Trichosporeae, Cyrtandreae and Epithemateae. *Beitr. Biol. Pflanzen* 70: 445–470.
- LEE, R. E. (1962). Chromosome numbers in the Gesneriaceae. *Baileya* 10: 33–45.
- MALLA, S. B., BHATTARAI, S., GORKHALI, M., SAIJU, H. & KAYASTHA, N. (1978). IOPB chromosome number reports LXII. *Taxon* 27: 519–535.
- MENDUM, M., LASSNIG, P., WEBER, A. & CHRISTIE, F. (2001). Testa and seed appendage morphology in *Aeschynanthus* (Gesneriaceae): phytogeographical patterns and taxonomic implications. *Bot. J. Linn. Soc.* 35 (2).
- MILNE, C. (1975). Chromosome numbers in the Gesneriaceae: V. *Notes Roy. Bot. Gard. Edinburgh* 33: 523–525.
- RATTER, J. A. (1963). Some chromosome numbers in the Gesneriaceae. *Notes Roy. Bot. Gard. Edinburgh* 24: 221–229.
- RATTER, J. A. (1975). A survey of chromosome numbers in the Gesneriaceae of the Old World. *Notes Roy. Bot. Gard. Edinburgh* 33: 527–543.
- RATTER, J. A. & MILNE, C. (1970). Chromosome numbers in the Gesneriaceae: IV. *Notes Roy. Bot. Gard. Edinburgh* 30: 183–187.
- RATTER, J. A. & PRENTICE, H. T. (1964). Chromosome numbers in the Gesneriaceae: II. *Notes Roy. Bot. Gard. Edinburgh* 25: 303–307.
- RATTER, J. A. & PRENTICE, H. T. (1967). Chromosome numbers in the Gesneriaceae: III. *Notes Roy. Bot. Gard. Edinburgh* 27: 205–209.
- ROGERS, O. M. (1954). Some chromosome counts in the Gesneriaceae. *Baileya* 2: 14–18.
- SCHLECHTER, R. (1923). Gesneriaceae papuanae. *Bot. Jahrb.* 58: 263–283.
- WANG, W. T. (1984). *Aeschynanthus*. *Bull. Bot. Lab. N. E. Forestry Institute* 4 (1): 26–30.

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APPENDIX

Aeschynanthus species investigated

Taxon	Accession	Collection details
<i>A. angustifolius</i> (Bl.) Steud.	19881452	Argent 88/66; Sumatra, Berastagi, Utera Province, S of Medan; 1200m; epiphyte.
<i>A. arctocalyx</i> Mendum & Madulid	19922776	Argent GAM 21; Philippines, Palawan, Mt Mantalingajan.
<i>A. boschianus</i> de Vriese	19570134	Winchcombe; wild origin unknown.
<i>A. bracteatus</i> Wall. ex DC.	19970165	Cherry 123; Vietnam, 1800m. (ex RBG Sydney).
<i>A. buxifolius</i> Hemsley	19970178	Goodwin & Cherry 384; Vietnam, Lào Cai Province, 4km from Ban Khoang village, on roadside bank, 2000m (ex RBG Sydney).
<i>A. ceylanicus</i> Gardner	19850904	Ponsonby 201; Sri Lanka, Central Province, Hakgala; epiphyte in montane forest (ex RBG Kew).
<i>A. gracilis</i> Parish ex C. B. Clarke	19802575	ex Marine Selby Botanic Garden, USA; wild origin unknown.
	19802720	ex Smithsonian Inst., USA (78.505); wild origin unknown.
	19821969	Grierson & Long 3607; Bhutan, Sarbhang District, Singi Khola, 390m; subtropical terai forest on river bank.
	19821970	Grierson & Long 3945; Bhutan, Gaylegphug District, Karai Khola above Aie bridge, 510m; subtropical forest.
	19821972	Grierson & Long 4125; Bhutan, Gaylegphug District, Rang Khola, 980m; warm broad-leaved forest on steep river bank.
<i>A. hookeri</i> C. B. Clarke	19892128	McBeath KEKE 45; Nepal, Basantpur, N of Chitre, 2360m; forested ridge.
<i>A. lineatus</i> Craib	19970163	Wallace, Chambers & Curry 423; China, Yunnan, near Tengchang Yun, Monkey bridge, 1650m; gorge in rich forest.
<i>A. longicaulis</i> Wall. ex R.Br.	19621423	Woods 1739; Peninsular Malaysia, Perlis, Biakang forestry reserve.
<i>A. magnificus</i> Stapf	19812958	Aberdeen University ABD26; Sabah, Mt Kinabalu, Trus Madi, 1600m; sandstone.
<i>A. vinaceus</i> P. Woods	19672118	Burt & Martin 5026; Sarawak, hill W of Melinau Falls.