The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2013, **172**, 329–344. With 5 figures

Karyology of the genus *Epidendrum* (Orchidaceae: Laeliinae) with emphasis on subgenus *Amphiglottium* and chromosome number variability in *Epidendrum secundum*

FELIPE NOLLET M. DE ASSIS[1], BRUNO CÉSAR Q. SOUZA[1], ENOQUE MEDEIROS-NETO[1], FÁBIO PINHEIRO[2], ANA EMÍLIA B. SILVA[1] and LEONARDO P. FELIX[1]*

[1]*Departamento de Ciências Biológicas, Universidade Federal da Paraíba, Campus II, Areia, PB, 58.397-000 Rodovia BR 079 – Km 12, Brazil*
[2]*Instituto de Botânica, Núcleo de Pesquisas do Orquidário do Estado, São Paulo, SP 04301-902, Brazil*

Received 21 May 2012; revised 2 December 2012; accepted for publication 17 February 2013

Epidendrum is one of the largest Neotropical genera of Orchidaceae and comprises approximately 1500 species. Only 2.8% of these species have been studied cytologically, demonstrating chromosome numbers ranging from $n = 12$ in *E. fulgens* to $n = 120$ in *E. cinnabarinum*. The present work evaluated the evolution of the karyotypes of *Epidendrum* spp. based on data gathered from the literature and from analyses of the karyotypes of 16 Brazilian species (nine previously unpublished). The appearance of one karyotype with $n = 12$ with one larger chromosome pair in subgenus *Amphiglottium* appears to have occurred at the beginning of the divergence of this lineage, and $x = 12$ probably represents the basic number of this subgenus. *Epidendrum secundum* exhibits wide variation in chromosome numbers, with ten different cytotypes found in 22 Brazilian populations, seven of which were new counts: $2n = 30$, 42, 50, 54, 56, 58 and 84. Most lineages of *Epidendrum* seem to have been secondarily derived from one ancestral stock with $x = 20$, as is seen in the majority of the present-day representatives of the genus. *Botanical Journal of the Linnean Society*, 2013, **172**, 329–344.

ADDITIONAL KEYWORDS: aneuploidy – basic chromosome number – bimodal karyotype – chromosome evolution – cytotypes – disploidy – Epidendroideae – infrageneric categories – karyotype asymmetry – polyploidy.

INTRODUCTION

Orchidaceae comprises approximately 25 000 species divided into five subfamilies, Apostasioideae, Cypripedioideae, Vanilloideae, Orchidoideae and Epidendroideae (Chase *et al*., 2003), the latter having 650 genera and 18 000 species (Cribb & Chase, 2005). *Epidendrum* L. belongs to Epidendroideae and shows extensive morphological diversity and many plesiomorphic characteristics in relation to other Epidendroideae, complicating the delimitation of infrageneric categories (Pinheiro *et al*., 2009).

Epidendrum is one of the largest Neotropical orchid genera, with approximately 1500 species (Chase *et al*., 2003; Hágsater & Soto Arenas, 2005) widely distributed throughout the Neotropics (Hágsater & Soto Arenas, 2005). The genus shows extensive intra- and interspecific morphological variation (Pabst & Dungs, 1975a; Hágsater, 1984; Pinheiro & Barros, 2005, 2007a). It is characterized by having generally cylindrical stems, rarely pseudobulbs, leaves generally distichous, flowers with labellum fused to the

*Corresponding author. E-mail: lpfelix@cca.ufpb.br

base of the column and anthers with two, four, six or eight (generally four) sessile pollinia (Hágsater & Soto Arenas, 2005).

Epidendrum was initially divided into four subgenera: (*Eu-*) *Epidendrum* Lindl., *Spathium* Lindl., *Amphiglottium* Lindl. and *Strobilifera* Lindl. (Lindley, 1852–1859 and, subsequently, Cogniaux, 1898–1902; Pabst & Dungs, 1975a, Brieger, 1976–1977). *Amphiglottium* is subdivided into four sections, *Imbricata* (Salisb.) Brieg., *Bifaria* (Salisb.) Brieg., *Ancipta* (Salisb.) Brieg. and *Amphiglottium* (Salisb.) Brieg., with the latter being subdivided into three subsections, *Integra* Brieg., *Carinata* Brieg. and *Tuberculata* Brieg., of which only *Tuberculata* and *Integra* were recognized as monophyletic. Subsection *Carinata*, in turn, was subdivided into two monophyletic clades by Pinheiro *et al*. (2009): a clade from the Andean region (*E. calanthum* Rchb.f. & Warsz., *E. ibaguense* Kunth and *E. incisum* Rchb.f. & Warsz.) and a clade from the Brazilian Atlantic coast (*E. cinnabarinum* Salzm. ex Lindl., *E. fulgens* H.Focke, *E. denticulatum* Barb.Rodr. and *E. puniceoluteum* F.Pinheiro & F.Barros).

Karyotypic changes in plants have long been recognized as important drivers of species evolution (Grant, 1981; Levin, 2002). Remarkable karyotypic diversity can be seen in the basic numbers, chromosome sizes and the organization of conserved syntenic blocks in different plant families and populations (Levin, 2002; Guerra, 2008), suggesting that chromosome number changes are part of ongoing evolutionary processes and not rare macroevolutionary events (Ramsey & Schemske, 1998). These chromosomal differences can be attributed to a large array of evolutionary processes (Levin, 2002). Adaptive divergence related to the colonization of new habitats (Stebbins, 1971; Grant, 1981), gene duplication (Lynch & Conery, 2000), proliferation of mobile genetic elements (SanMiguel *et al*., 1996) and allo- and autopolyploidy (Ramsey & Schemske, 1998) are widespread mechanisms of karyotypic change that have been studied intensively in recent years (Doyle *et al*., 2008). Investigations of chromosome numbers are therefore an essential first step for plant evolutionary studies, offering a primary framework to test evolutionary hypotheses of diversification from populations to the phylogenies of higher taxa (Stace, 2000; Guerra, 2008).

The chromosome numbers of orchids have been increasingly reported, contributing substantially to the evolutionary studies of these species at different taxonomic levels (Felix & Guerra, 2000, 2010; Koehler *et al*., 2008; Pinheiro *et al*., 2009; Yamagishi-Costa & Forni-Martins, 2009; Chochai *et al*., 2012; Moraes, Leitch & Leitch, 2012; Neubig *et al*., 2012). Different numbers for core Maxillariinae have been observed across different clades (Whitten *et al*., 2007) and centric fusion/fission events generating descendent disploidy have been identified in *Christensonella* Szlach., Mytnik, Górniak & Śmiszek (Koehler *et al*., 2008). Oncidiinae also display extensive variation ($n = 5$–30), with lower numbers being phylogenetically associated with twig epiphytes (Chase *et al*., 2005). The significant chromosome number variation observed in *Epidendrum* (Pinheiro *et al*., 2009) and *Cattleya* Lindl. (Yamagishi-Costa & Forni-Martins, 2009; Antonelli *et al*., 2010) was primarily caused by hybridization and introgression events, evolutionary processes commonly associated with chromosome number reorganization and ploidy evolution (Ramsey & Schemske, 1998; Doyle *et al*., 2008). Gene exchanges between taxa with large differences in chromosome numbers have likewise been observed in *Epidendrum* (Pinheiro *et al*., 2010), challenging the widely held view of 'instant isolation' among species with different chromosome numbers (Coyne & Orr, 2004).

Only 2.8% of *Epidendrum* spp. have been studied cytologically and these demonstrate chromosome number variation between closely related species and among populations within species. The species from the Atlantic clade in subgenus *Amphiglottium* show chromosome numbers ranging from $2n = 24$ in *E. fulgens* (Pinheiro *et al*., 2009) to *c*. $2n = 240$ in *E. cinnabarinum* (Guerra, 2000; Conceição, Oliveira & Barabosa, 2006; Felix & Guerra, 2010). In addition, six different cytotypes are known in *E. secundum* Jacq. ($2n = 28$, 40, 48, 52, 68 and 80), a widespread species from the Neotropical region, and several numeric records have been reported for *E. radicans* Pav. ex Lindl. and *E. xanthinum* Lindl. (Blumenschein, 1960; Pinheiro *et al*., 2009). The most frequent number for the genus is $n = 20$, which has been observed in approximately 70% of the species examined; $n = 20$ is also the most frequent number among other members of subtribe Laeliinae, suggesting that $x = 20$ is the basic number for the genus, and quite possibly for the entire subtribe (Felix & Guerra, 2010). Karyological data for this genus are scarce, which leaves the evolutionary history of the genus unclear; the direction of karyological evolution and many other aspects of its phylogenetic relationships (including its primary basic number) remain unknown.

Epidendrum secundum is one of the most variable and taxonomically less well-understood species (Brieger, 1976–1977), with many synonyms, such as *E. ansiferum* Rchb.f., *E. crassifolium* Lindl., *E. ellipticum* Graham and *E. elongatum* Jacq. (Pinheiro & Barros, 2007a). This taxon is distributed throughout South America and occurs in varied habitats, such as the Andes, the central highlands of Brazil, in rocky field vegetation, along the Atlantic coast and on inselbergs in the Caatinga (Pinheiro & Barros, 2007a), and

demonstrates the ability to colonize new habitats readily. *Epidendrum secundum* belongs to subsection *Tuberculata*, based on the morphology of its lip callus (Pinheiro & Barros, 2007b).

We investigated the karyotype evolution of *Epidendrum* spp., based on data from the literature and from the karyotypes of 16 Brazilian species and 22 populations of *E. secundum* reported here, to determine the chromosome evolution in the genus at different levels (from populations to species belonging to different clades) by: (1) providing chromosome counts for species not yet studied; (2) reviewing known chromosome counts for the genus, especially those for subgenus *Amphiglottium*; (3) establishing the most probable basic chromosome number for the genus; and (4) identifying chromosome evolution patterns in *E. secundum*.

MATERIAL AND METHODS

All of the material analysed was collected in northern, north-eastern, central-western, southern and south-eastern Brazil. Table 1 lists the species analysed, their respective chromosome numbers, previously recorded counts, voucher numbers, asymmetry indices and collection localities. Table 2 lists all previous numerical data available in the literature related to *Epidendrum* spp. All live material was cultivated in the Orchidarium at the Centro de Ciências Agrárias of the Universidade Federal da Paraíba and voucher specimens were deposited in the Jayme Coelho de Moraes Herbarium (EAN) and the São Paulo Botanical Institute (IBT).

The species were mostly identified according to Pabst & Dungs (1975a), and their binomials were updated according to the International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do; Index Kewensis, 2005). Chromosomes were measured using Image Tool software (Donald *et al*., 2007). To determine chromosome numbers, five metaphase cells were examined and counted for each population.

Chromosome analyses were undertaken using root tips pretreated with 0.002 M 8-hydroxyquinoline at 4 °C for 24 h. The material was then fixed in absolute ethanol–glacial acetic acid (3 : 1, v/v) for 3–24 h at room temperature (25 °C) and subsequently stored at –20 °C. To prepare the slides, root tips were hydrolysed in 5 M HCl at room temperature, frozen in liquid nitrogen to remove the coverslip, stained with 2% Giemsa (Guerra, 1983) and mounted in Entellan. The best chromosome preparations were photographed using an Olympus D-540 digital camera coupled to an Olympus CX40 microscope. The images were optimized for better contrast and brightness with Adobe Photoshop CS3 Extended Version 10.0.

Three metaphases with well-defined chromosome morphologies were used for chromosome measurements in each species, employing ©UTHSCSA image tool® version 3.0 software. The chromosome arm ratios (length of the long arm divided by the length of the short arm) were calculated to classify chromosomes as metacentric, submetacentric or acrocentric (metacentric, 1–1.4; submetacentric, 1.5–2.9; acrocentric, ≥ 3.0) (Guerra, 1986). To determine the karyotype asymmetry versus symmetry, the index of Romero Zarco (1986) was calculated to estimate the intrachromosomal asymmetry [$A_1 = 1 - (\Sigma b/B)/n$, where b are the average lengths of the short arms of each chromosome pair, B are the average lengths of the long arms of each chromosome pair and n is the number of chromosome pairs] and interchromosomal asymmetry ($A_2 = S/X$, where S is the standard deviation and X is the average chromosome length). In addition, the Stebbins classification was applied to access the karyotype category for each species.

The phylogenetic tree based on the maximum likelihood criterion for *trnL-trnF* (Pinheiro *et al*., 2009) was redrawn with Corel Photo Paint Version X5 in order to facilitate the understanding of chromosome number variation in subgenus *Amphiglottium*.

RESULTS

New counts for *Epidendrum* (total of nine) are presented in bold type in Table 1 for the species: *E. armeniacum* Lindl. (Figs 1A, 5A), *E. proligerum* Barb.Rodr. (Fig. 1B), *E. pseudodifforme* Hoehne & Schltr. (Figs 1C, 5B), *Epidendrum* sp. nov. (aff. *difforme*) (Fig. 1D), *E. ramosum* Jacq. (Fig. 1G), *E. viviparum* Lindl. (Figs 1H, 5E) and *E. tridactylum* Lindl. (Fig. 1I), all with $2n = 40$; *E. orchidiflorum* Salzm. ex Lindl. (Fig. 2B) with $2n = 112$; *Epidendrum* sp. with $2n = 38$ (Fig. 2F); and *E. secundum* (Fig. 3A–J) with $2n = 30, 42, 50, 54, 56, 58$ and 84. All of the species had semi-reticulated interphase nuclei (Figs 1E, G, I, 2D, E, 3E, G), proximal prophase condensation patterns (Figs 1D, 2C) and interspecific variation in number, size and distribution of the chromocentres. In order to discuss the base numbers, Figure 4, based on the phylogenetic tree proposed by Pinheiro *et al*. (2009), shows the numerical chromosome variation for each species belonging to subgenus *Amphiglottium*.

Values obtained for the Romero Zarco asymmetry indices (A_1 and A_2) and the Stebbins categories of classification (St) indicated that the karyotypes were largely symmetrical (Table 1), except in *Epidendrum* sp. nov. (aff. *difforme*) (Fig. 1D), *E. ramosum* (Fig. 1G), *E. viviparum* (Figs 1H, 5E), *E. secundum* (Fig. 3B–J) with $2n = 40$, 42, 50, 54, 56, 58 and 84, *E. fulgens* (Figs 2D, 5F) and *E. denticulatum*

Table 1. Taxa of *Epidendrum* spp. (*sensu* Pabst & Dungs, 1975a,b; Pinheiro *et al.*, 2009) analysed, with provenance, voucher number, chromosome numbers ($2n$), previous counts ($2n$), references, number of individuals per population, chromosome size range (μm), Romero Zarco asymmetry indices (A_1 and A_2) and Stebbins classification (St). New counts are printed in bold type. The references corresponding to the previous counts are indicated by asterisks and listed at the end of the table

Taxonomic groups/species	Provenance	Voucher number	Chromosome number ($2n$)	Previous counts ($2n$)	References*	Number of individuals/ population	Chromosome size range (μm)	A_1	A_2	St
Sect. *Epidendrum* II										
Group Spathaceae										
Armeniacum alliance										
E. armeniacum Lindl.	Taquaritinga do Norte, PE	L.P.Felix, 12091	**40**			01	1.44–2.53	0.13	0.16	1A
Group Racemosae										
Proligerum alliance										
E. proligerum Barb. Rodr.	Taquaritinga do Norte, PE	L.P.Felix, 12092	**40**			04	1.71–2.99	0.21	0.15	1A
Group Subumbellatae										
Difforme alliance										
E. pseudodifforme Jacq.	Taquaritinga do Norte, PE Areia, PB	L.P.Felix, 12094	40	40	TK84, PI09	10	1.50–2.76	0.17	0.15	3A
***Epidendrum* sp. nov.** (aff. *difforme*)	Pacoti, CE	L.P.Felix and M. F. Oliveira, 46	**40**			01	1.92–2.69	0.50	0.20	1B
E. latilabre Lindl.	Taquaritinga do Norte, PE Guaramiranga, CE	L.P.Felix, 12095	40	40	FG10	02	1.53–2.57	0.14	0.14	1A
Nocturnum alliance										
E. nocturnum Jacq.	Taquaritinga do Norte, PE Belém do Pará, PA	L.P.Felix, 9177	80	40, 80	TK84	10	1.39–2.53	0.29	0.14	2A
Group Paniculatae										
Paniculatum alliance										
E. paniculatum Ruiz and Pavon	Alto Paraíso, GO Taquaritinga do Norte, PE	L.P.Felix, 12096	40	40	TK84	02	1.43–2.45	0.20	0.14	1A
Ramosum alliance										
E. ramosum Jacq.	Taquaritinga do Norte, PE Brejo da Madre de Deus, PE	L.P.Felix, 12097	**40**			05	1.48–2.60	0.17	0.22	1B
Sect. *Psilanthemum*										
E. viviparum Lindl.	Cultivated	L.P.Felix, 12098	**40**			02	1.17–2.34	0.20	0.15	1B
Ex genus *Amblostoma*										
E. tridactylum Lindl.	Taquaritinga do Norte, PE	L.P.Felix, S/N	**40**			02	1.46–2.66	0.15	0.40	1A

Table 1. *Continued*

Taxonomic groups/ species	Provenance	Voucher number	Chromosome number ($2n$)	Previous counts ($2n$)	References*	Number of individuals/ population	Chromosome size range (μm)	A_1	A_2	St
(Subgenus *Amphiglottium*) *Polyanthum* alliance										
E. orchidiflorum Salzm. ex Lindl.	Santa Rita, PB Morro do chapéu, BA	L.P.Felix, 12564	**112**			01	0.58–2.26	0.26	0.34	
E. secundum Jacq.	São Paulo, SP	A.S. Pires (IBt, 2831)	**30**	28, 40, 48, 52, 68 e 80	B60, PI09, FG10	01	1.22–2.44	0.19	0.21	1A
	Santa Bárbara, MG	F. Pinheiro *et al.* (IBt, 17494)	40			01	1.26–3.56	0.18	0.29	1B
	Nova Friburgo, RJ	F. Pinheiro *et al.* (IBt, 17840)	40			01	1.32–3.47	0.18	0.29	1B
	Nova Friburgo, RJ	F. Pinheiro *et al.* (IBt, 17838)	**42**			01	1.08–4.22	0.29	0.30	1B
	Nova Friburgo, RJ	F. Pinheiro *et al.* (IBt, 17839)	**42**			01	1.12–4.15	0.29	0.30	1B
	Roraima	H. Sacher (IBt, 17593)	48			01	1.02–2.13	0.22	0.19	1B
	Cananéia, SP	F. Barros (IBt, 13141)	**50**			01	1.12–3.49	0.22	0.27	1B
	Mariana, MG	P. Brólio, M.B. Silva, G. Neto (IBt, 7052)	**50**			01	1.17–3.38	0.22	0.27	1B
	Ubatuba, SP	E.R. Pansarin (IBt, 17664)	**50**			01	1.15–3.17	0.22	0.27	1B
	Jundiaí, SP	E.R. Pansarin (IBt, 17668)	**50**			01	1.37–3.46	0.22	0.27	1B
	Curitiba, PR	M. Trovo (IBt, 17916)	**50**			01	1.16–3.40	0.22	0.27	1B
	Mogi das Cruzes, SP	M. Trovo (IBt, 18042)	**50**			01	1.20–3.25	0.22	0.27	1B
	Manhuaçú, MG	M. Trovo (IBt, 17990)	**54**			01	1.48–3.45	0.22	0.23	1B
	Santana do Riacho, MG	F. Barros (IBt, 15287)	**56**			01	1.37–3.49	0.23	0.22	2B
	Ubatuba, SP	E.R. Pansarin (IBt, 17662)	**56**			01	1.41–3.41	0.23	0.22	2B
	Santo Antônio do Itambé, MG	J. Leônidas (IBt, 17672)	**56**			01	1.35–3.38	0.23	0.22	2B
	Itatiaia, RJ	M. Trovo (IBt, 17879)	**56**			01	1.37–3.49	0.23	0.22	2B
E. secundum Jacq.	Serra do Rio do Rastro, SC	(IBt, 17924)	**c. 56**			01	1.34–3.46	0.23	0.22	2B
	Carrancas, MG	E.R. Pansarin (IBt, 17665)	**58**			01	1.43–3.85	0.18	0.23	1B
	Camocim de São Félix, PE	L. P. Felix, 12088	**56**			12	1.35–3.41	0.23	0.22	2B
	Fagundes, PB	L. P. Felix, 12090	**56**, 68, **84**			10	1.35–3.41 1.44–3.96 1.38–3.59	0.23 0.25 0.27	0.22 0.18 0.23	2B 1B 1B
	Brejo da Madre de Deus, PE	L. P. Felix, 12089	**84**			02	1.38–3.59	0.27	0.23	1B
Schomburgkii alliance										

Table 1. *Continued*

Taxonomic groups/ species	Provenance	Voucher number	Chromosome number ($2n$)	Previous counts ($2n$)	References*	Number of individuals/ population	Chromosome size range (μm)	A_1	A_2	St
E. cinnabarinum Salzm.	Alcaçuz, RN Serraria, PB Esperança, PB Areia, PB Santa Rita, PB Brejo da Madre de Deus, PE	L.P.Felix, 12106	240	240	G00, CO06	10	0.98–4.51	–	–	–
E. fulgens Brongn.	Panelas, PE São João do Tigre, PB	L.P.Felix, 12515	24	24	TK 84	02	1.21–3.19	0.24	0.23	1B
Denticulatum alliance										
E. denticulatum Barb. Rodr.	Cultivated	Unvouchered	38	40	TK84, PI09	02	1.30–3.08	0.19	0.22	1B
Epidendrum sp.	Ibicoara, BA	Unvouchered	**38**			01	2.18–3.94	0.16	0.20	1B

*B60, Blumenschein (1960); TK84, Tanaka & Kamemoto (1984); G00, Guerra (2000); CO06, Conceição *et al*. (2006); PI09, Pinheiro *et al*. (2009); FG10, Felix & Guerra (2010). Acronyms for Brazilian states: BA, Bahia; CE, Ceará; GO, Goiás; MG, Minas Gerais; PA, Pará; PB, Paraíba; PE, Pernambuco; PR, Paraná; RN, Rio Grande do Norte; RJ, Rio de Janeiro; SC, Santa Catarina; SP, São Paulo; new counts are given in bold type.

(Figs 2E, 5G), which were generally more asymmetrical. Chromosome sizes varied from 0.58 μm in *E. orchidiflorum* to 4.51 μm in *E. cinnabarinum* (Fig. 2C). Chromosome numbers varied from $2n = 24$ in *E. fulgens* to $2n = 240$ in *E. cinnabarinum*.

All of the species analysed from section *Epidendrum* II Pabst & Dungs (Table 1) had a chromosome number of $2n = 40$ (Fig. 1A–G), with symmetrical karyotypes and chromosomes that varied from meta- to submetacentric, except for *E. nocturnum* Jacq. in the Subumbellatae group with $2n = 80$ (Figs 2A, 5C).

In section *Psilanthemum* Pabst & Dungs (Table 1), *E. viviparum* (Figs 1H, 5E) and *E. tridactylum* (Fig. 1I; formerly in the genus *Amblostoma* Scheidw.) both had $2n = 40$. Six of the species studied here, distributed among three alliances in subgenus *Amphiglottium*, demonstrated dramatic inter- and intraspecific chromosome number variation. The chromosomes were difficult to spread, even after removing the epidermis, root cap and root procambium. Descriptions of the karyotypes observed in the populations of subgenus *Amphiglottium* analysed here are provided below.

In the *Polyanthum* Pabst & Dungs alliance, *E. orchidiflorum*, with $2n = 112$, has a karyotype with some chromosomes that were slightly larger than the others (Fig. 2B).

In the *Schomburgkii* Pabst & Dungs alliance, *E. cinnabarinum* stood out in having the highest chromosome number among the samples analysed in this work and in Orchidaceae ($2n = 240$), and by having 14 chromosomes demonstrating late condensation in relation to the others (Fig. 2C, arrows). These late condensing chromosomes also differed by having uniformly decondensed chromatin; the other chromosomes had pericentromeric regions that were more precociously condensed (which is characteristic of the proximal prophase condensation pattern). *Epidendrum fulgens* had the lowest number of chromosomes among the species studied here ($2n = 24$), and had two chromosomes larger than the others (Fig. 2D, arrows).

In the *Denticulatum* Pabst & Dungs alliance, *E. denticulatum* had $2n = 38$, with one pair of metacentric chromosomes that was approximately twice the size of some chromosome pairs (Fig. 2E, arrows, 5G). *Epidendrum* sp. had $2n = 38$ (Fig. 2F), a more asymmetrical karyotype and metacentric to submetacentric chromosomes.

In the *Polyanthum* Pabst & Dungs alliance, *E. secundum* stood out by demonstrating marked numerical chromosome variations, with ten cytotypes with more or less asymmetrical karyotypes being found among 22 populations (Table 1). There were variable numbers of large chromosomes in each cytotype. Chromosome numbers in this species varied from $2n = 30$ to $2n = 84$ among populations from northern, north-eastern, south-eastern and southern Brazil. Only one population of *E. secundum* collected in the northern region (Roraima State) was analysed, and it had individuals with $2n = 48$ (Fig. 3D), with a set of eight large chromosomes and two chromosomes showing distended heteromorphic NORs (Nucleolus Organizer Regions) (Fig. 3D, arrowheads).

Populations of *E. secundum* from north-eastern Brazil showed high intrapopulational chromosome number variations. One population had $2n = 68$ chro-

Table 2. Known chromosome numbers in *Epidendrum* L. (organized *sensu* Dressler, 1993)

Taxa	*n*	2*n*	Sources*
Epidendrum angustatum (T.Hashim.) Dodson (as *Neolehmannia angustata*)		36	GJ94
E. appendiculatum Hashimoto		38	GJ94
E. avicula Lindl. [as *Lanium avicula* (Lindl.) Benth]		40	TK84, GJ94, DA09
E. blepharistes Barker ex Lindl. (as *E. funckii* Rchb. f.)		40	GJ94
E. burtonii Benn. Christ.		80	TK84
E. calanthum Rchb.f. and Warsc.		30	PI09
E. ciliare L.	20	40, 80,160	TK84, G85
E. cinnabarinum Salzm.	108, 124	240	FG10, PW
E. cochlidium Lindl.		28	PI09
E. cooperianum Bateman (as *E. longispathum* Barb. Rodr.)		40	TK84
E. cristatum Ruiz and Pavon (as *E. raniferum* Lindl.)	20	40	TK84
E. cristatum (as *E. tigrinum* Sessé and Moc.)		40	B57
E. denticulatum Barb. Rodr.		40, 38	TK84, PI09, PW
E. difforme Jacq. (as *Neolehmannia difforme*)		40	TK84, PW
E. diffusum Sw.	20	40	TK84
E. ellipticum Grah.		56, 68	TK84, FG10
E. flexuosum G. Mey		28	PI09
E. fulgens Brongn.		24	B57, PI09, PW
E. ibaguense Kunth.		70	PI09
E. lanipes Lindl.		40	G85
E. latilabre Lindl.		40	FG10, PW
E. loefgrenii Cogn.		40	TK84
E. magnoliae Muhl. (as *E. conopseum* R. Br.)	20	40	TK84
E. myrmecophorum Barb. Rodr.		120	PI09
E. nocturnum Jacq.	20	40, 80	TK84, FG10, PW
E. orchidiflorum Salzm. ex Lindl.		ca. 120	PW
E. paniculatum Ruiz and Pav. (as *E. floribundum* Kunth.)		40	TK84, PW
E. patens Sw.		40	TK84
E. proligerum Barb. Rodr.		40	PW
E. propinquum A. Rich. and Galeotti		40	TK84
E. puniceoluteum F. Pinheiro and F. Barros		52	PI09
E. ×purpureum Barb. Rodr.		56; 120	TK84; PI09
E. radicans Pav. ex Lindl.		40, 57, 70, 60, 62, 64	TK84; PI09
E. ramosum Jacq.		40	PW
E. rigidum Jacq.		40	TK84
E. secundum Jacq.		28, 30, 40, 42, 48, 50, 52, 54, 56, 58, 68, 80, 84	FG10, PI09, PW
E. secundum (as *E. brachyphyllum* Lindl.)	30		TK84
E. secundum (as *E. elongatum* Jacq.)		56	TK84
E. secundum (as *E. lindenii* Lindl.)		56	TK84
E. strobiliferum Rchb.f. (as *E. mosenii* Barb. Rodr.)		24	TK84
E. viviparum Lindl.		40	PW
E. xanthinum Lindl.		28, 30, 40, 60, ca. 80	TK84, G88, PI09

*B57, Blumenschein, 1957; TK84, Tanaka & Kamemoto, 1984; G81, Goldblatt, 1981; G85, Goldblatt, 1985; G88, Goldblatt, 1988; GJ94, Goldblatt & Johnson, 1994; PI09, Pinheiro *et al*. (2009); FG10, Felix & Guerra, 2010; PW, present work.

mosomes (Fig. 3I) with asymmetrical karyotypes. Two populations had $2n = 84$ chromosomes (Fig. 3J) with asymmetrical karyotypes and 11 large chromosomes. Cytotypes with $2n = 56$, 68 and 84 chromosomes were found in a population from Fagundes, in Paraíba State.

In south-eastern Brazil, the smallest chromosome number observed in a population of *E. secundum* was

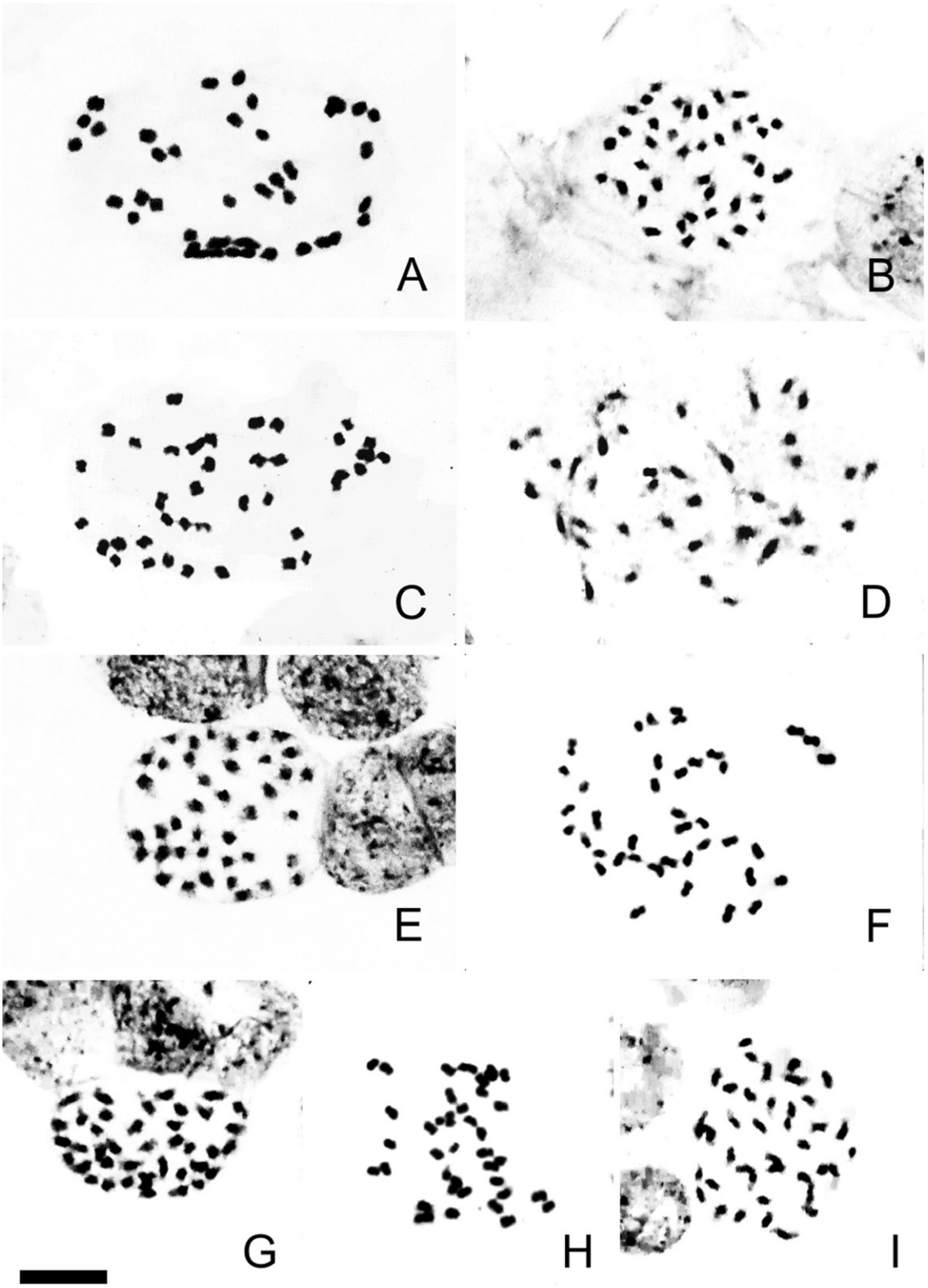

Figure 1. Chromosome complements and interphase nuclei of *Epidendrum* spp.: A, *E. armeniacum*; B, *E. proligerum*; C, *E. pseudodifforme*; D, ***E.* sp. nov.** (aff. *difforme*); E, *E. latilabre*; F, *E. paniculatum*; G, *E. ramosum*; H, *E. viviparum*; I, *E. tridactylum*. Bar in (G) corresponds to 10 μm.

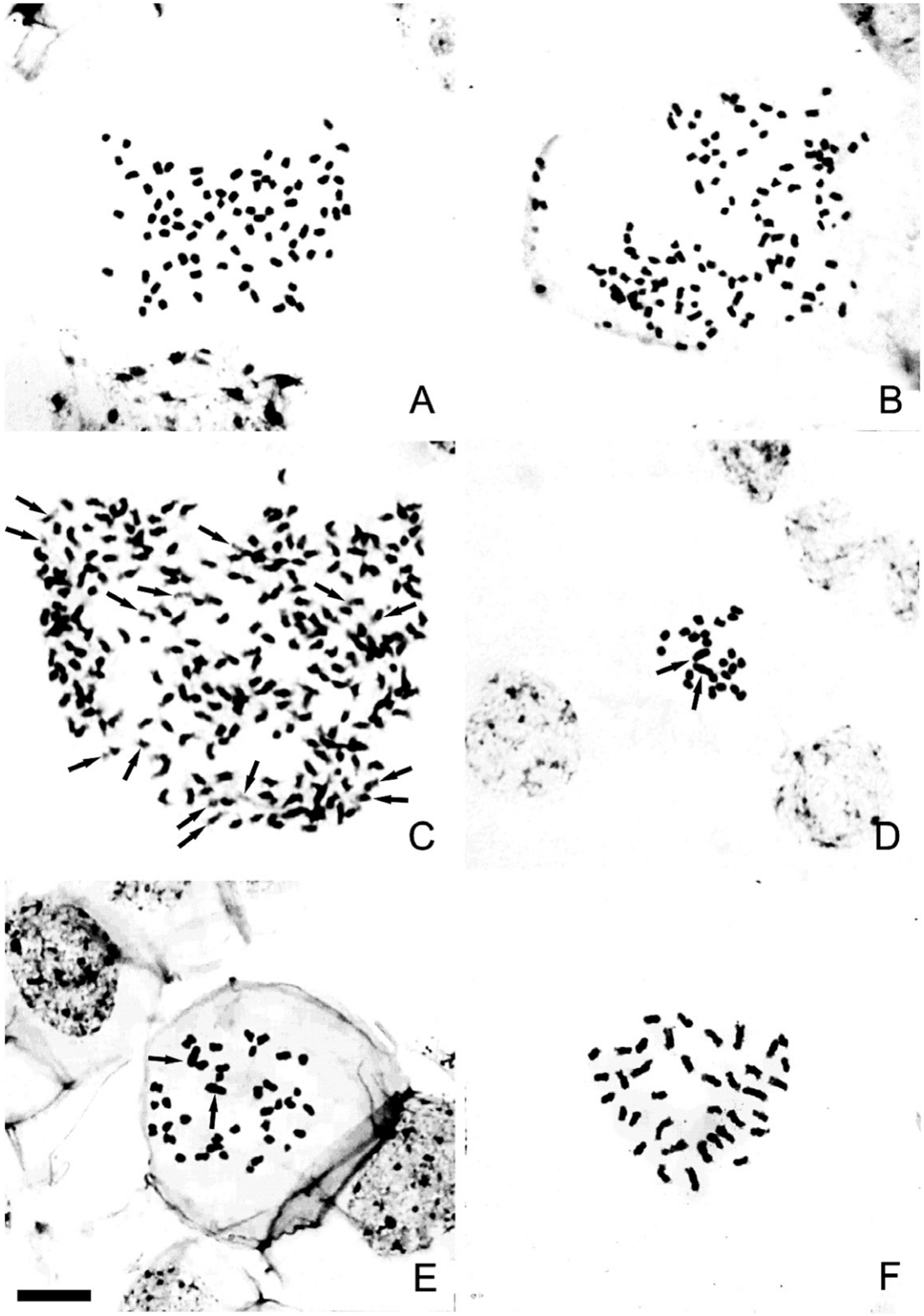

Figure 2. Chromosome complements and interphase nuclei of *Epidendrum* spp.: A, *E. nocturnum* ($2n = 80$); B, *E. orchidiflorum* ($2n = 112$); C, *E. cinnabarinum* ($2n = 240$); D, *E. fulgens* ($2n = 24$); E, F, *E. denticulatum* (E) and *Epidendrum* sp. (F), both with $2n = 38$. The arrows in (C) indicate late condensation chromosomes. The arrows in (D) and (E) indicate the largest chromosomes of the set. Bar in (E) corresponds to 10 μm.

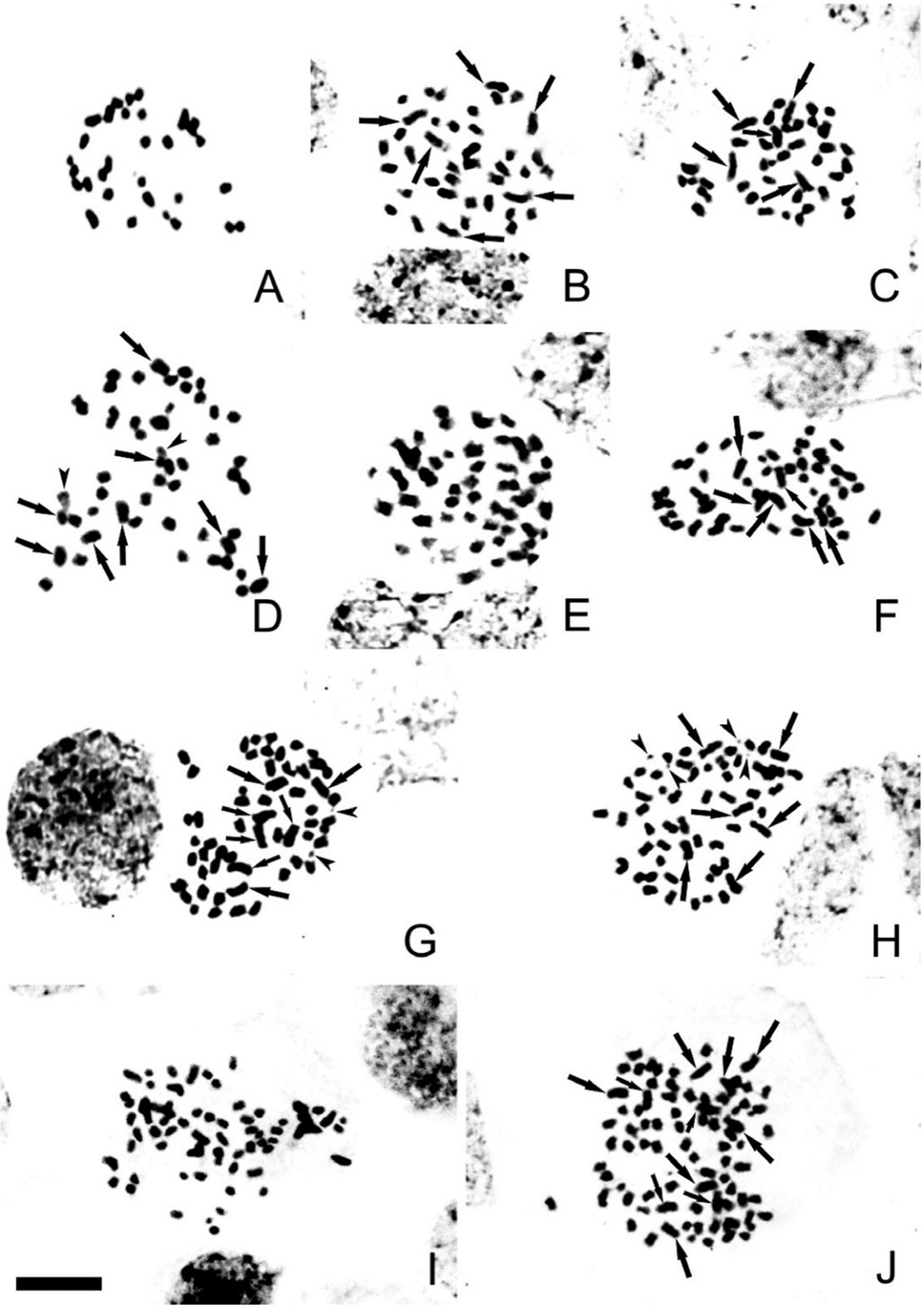

Figure 3. Chromosome complements and interphase nuclei of the *Epidendrum secundum* complex and their provenance: A, São Paulo ($2n = 30$); B, Santa Bárbara ($2n = 40$); C, Nova Friburgo ($2n = 42$); D, Roraima ($2n = 48$); E, Ubatuba ($2n = 50$); F, Manhuaçú ($2n = 54$); G, Santana do Riacho ($2n = 56$); H, Carrancas ($2n = 58$); I, Fagundes ($2n = 68$); J, Brejo da Madre de Deus ($2n = 84$). Arrows indicate the largest chromosomes of the set. Arrowheads indicate satellites. Bar in (I) corresponds to 10 μm.

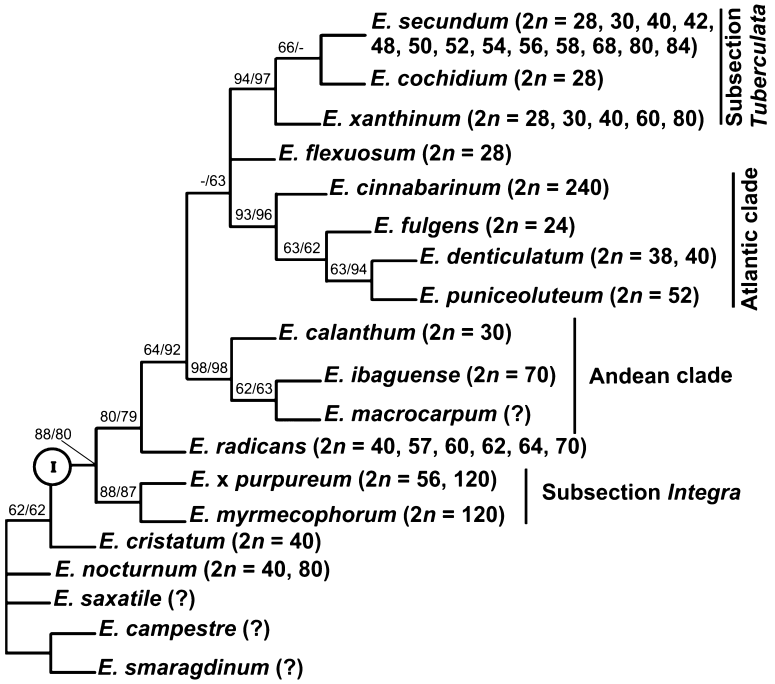

Figure 4. Phylogenetic tree based on maximum likelihood criterion for *trnL-trnF* of subgenus *Amphiglottium* (Pinheiro *et al*., 2009), with the numerical chromosome variation indicated for each species. Maximum likelihood/maximum parsimony bootstrap support values above 50% are indicated above the tree branches.

$2n = 30$ (Fig. 3A); two populations had $2n = 40$ (Figs 3B, 5H), two had $2n = 42$ (Figs 3C, 5I) and five populations had $2n = 50$ (Fig. 3E) with a pair of large chromosomes. One population from Minas Gerais State had $2n = 54$ (Fig. 3F) with six large chromosomes. Five populations from southern Brazil had $2n = 56$ chromosomes with seven large chromosomes (Fig. 3G, arrows) and two satellites (Fig. 3G, arrowheads). One population from Carrancas, Minas Gerais, had $2n = 58$ (Fig. 3H), with six large chromosomes (Fig. 3H, arrows) and four satellites (Fig. 3H, arrowheads).

Only two populations of *E. secundum* were analysed from the southern region of Brazil: one with $2n = 50$ from Curitiba (Paraná State) with an asymmetrical karyotype and a set of large chromosomes, and one with *c*. $2n = 56$ from Serra do Rio do Rastro (Santa Catarina State).

DISCUSSION

Polyploidy is considered to be the most important type of chromosomal alteration in angiosperm evolution (Stebbins, 1971; Soltis *et al*., 2003; Guerra, 2008). Total genome sizes can vary up to 60-fold in Epidendroideae (from 0.3 to 19.8 pg) (Leitch *et al*., 2009) and polyploidy appears to have had an important role in the evolution of the DNA content of the genus, as a ten-fold variation in ploidy of this genus has been observed. The numerical chromosomal variation extremes observed in *Epidendrum* occur in species growing in terrestrial or rupicolous habitats (*E. fulgens* with $n = 12$ and *E. cinnabarinum* with $n = 120$, respectively) within this predominantly epiphytic genus (Pabst & Dungs, 1975b).

As a whole, Orchidaceae appears to have maintained a positive correlation between DNA content and terrestrial habitat (Leitch *et al*., 2009), but polyploidy in *Oncidium* Sw. is related to terrestrial or rupicolous habitats (Felix & Guerra, 2000). Rupicolous species of *Laelia* Lindl. (as delimited by Pabst & Dungs, 1975a) also appear to have higher chromosome numbers than epiphytes (Blumenschein, 1957; Yamagishi-Costa & Forni-Martins, 2009). Although the highest ploidy levels were seen in terrestrial representatives of *Epidendrum* (such as *E. orchidiflorum* and *E. cinnabarinum*), the smallest known chromosome number for the genus was observed in *E. fulgens* ($2n = 24$), a species with terrestrial or rupicolous habitats. In addition, chromosome numbers in *E. ciliare* L. (which is principally epiphytic) range from $n = 20$ to $n = 80$ (Kamemoto, 1950; Blumenschein, 1960), suggesting that polyploidy (and possibly the quantity of DNA) in *Epidendrum* is influenced, but not restricted, by the type of habitat occupied by these plants.

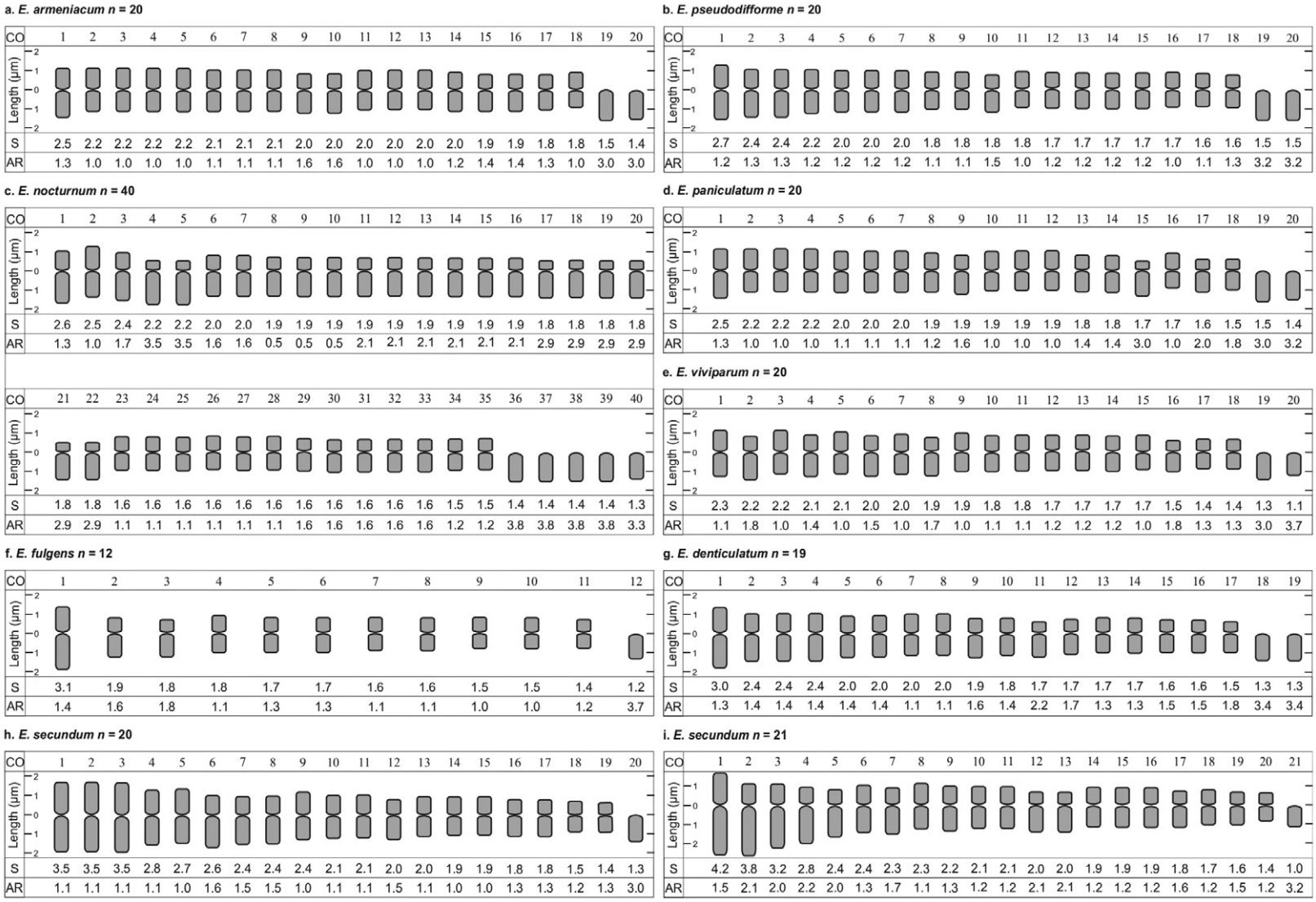

Figure 5. Idiograms of *Epidendrum* spp. showing chromosome size (S) and arm ratio (AR). Chromosomes are ordered by decreasing size (CO).

Epidendrum is quite variable in chromosome number, and polyploidy and disploidy events have both affected its evolution (Hágsater & Soto Arenas, 2005; Pinheiro *et al*., 2009). Karyological variations were observed, especially in subgenus *Amphiglottium*; data from the literature and new counts for nine species in the present work show numerical variation from $2n = 24$ to $2n = 240$. Previous counts of $2n = 240$ for *E. cinnabarinum* (Guerra, 2000; Conceição *et al*., 2006; Felix & Guerra, 2010), $2n = 40$ for *E. paniculatum* Ruiz & Pav. (Tanaka & Kamemoto, 1984) and $2n = 80$ for *E. nocturnum* (Blumenschein, 1960) were confirmed. Other counts of $2n = 40$ (Blumenschein, 1960) and $2n = 56$ (Tanaka & Kamemoto, 1984) for *E. denticulatum* were not confirmed in the present sample. *Epidendrum denticulatum* occurs principally in 'restinga' (coastal) and 'cerrado' (savanna) vegetation areas, and its morphological variations make it difficult to identify, often being confused with *E. secundum* (Pinheiro & Barros, 2007a). It is possible that the divergent karyological records published for this species are related to problems in its circumscription.

In addition to the unpublished data for *E. secundum*, analyses of chromosome number variability (with seven new counts presented here) suggest the occurrence of reproductively isolated chromosomal lineages in this taxon. A similar pattern was observed in at least one other species of subgenus *Amphiglottium* (*E. xanthinum*, with $2n = 28$, 30, 40, 60) (Pinheiro *et al*., 2009). This type of intraspecific chromosome number variability has been reported previously for *E. secundum* (Blumenschein, 1960; Pinheiro *et al*., 2009).

Epidendrum secundum showed high intrapopulational variation in chromosome number. Pinheiro *et al*. (2009) identified six different cytotypes with $2n = 28$, 40, 48, 52, 68 and 80, whereas ten cytotypes were found in the present investigation (Table 1). Furthermore, the chromosomes vary greatly in size, resulting in accentuated asymmetry and bimodality of the karyotypes. Asymmetrical karyotypes have traditionally been considered as derivatives of ancestral cytotypes that were more symmetrical (Stebbins, 1971; Arditti, 1992). *Epidendrum secundum* shows a broad geographical distribution, occurring in sympatry with many other species across its distributional range. It is possible that hybridization and introgression have played important roles in chromosome number variation in this species, as observed in other closely related species (Pinheiro *et al*., 2010).

In addition to *E. secundum*, high ploidy was observed in *E. nocturnum* (of the Subumbellatae group) with $2n = 80$, *E. orchidiflorum* with $2n = 112$, *E. cinnabarinum* with $2n = 240$ (of the subgenus *Amphiglottium*) and *E. ciliare* with $2n = 80$ and 160 (Kamemoto, 1950; Blumenschein, 1960) (of section *Aulizeum sensu* Pabst & Dungs, 1975a). Polyploidy also occurs in the genera *Prosthechea* Knowles & Westc. and *Cattleya* Lindl. (in subtribe Laeliinae) (Felix & Guerra, 2010), suggesting that polyploidy has occurred several times in diverse members of this subtribe.

Epidendrum nocturnum, a highly polymorphic species distributed throughout the tropical Americas, is composed of four formally recognized groups (Brieger & Bicalho, 1977) that may occur sympatrically (although in well-defined microhabitats) without any evidence of intergradation (Carnevali & Romero, 1996). There are records of populations of this species with $2n = 40$, 74, 80 and 85 (Blumenschein, 1960; Tanaka & Kamemoto, 1984). This range of variation is thought to reflect ancient combinations of genomes adjusted to apomixis (Brown, 1951; Szlachetko & Veyret, 1996). Many species previously included in the delimitation of *E. nocturnum* are actually distinct taxa, such as *E. tridens* Poepp. & Endl., *E. longicolle* Lindl. and *E. mininocturnum* Dodson (Carnevali & Romero, 1996).

Subgenus *Amphiglottium* shows a surprising variation in chromosome number, comparable with Orchidaceae as a whole. *Habenaria* Willd. (Orchidoideae) and *Oncidium* (subtribe Oncidiinae) have chromosome numbers ranging from $n = 14$ to $n = 80$ and $n = 7$ to $n = 84$, respectively (Felix & Guerra, 1998, 2000, 2005). *Habenaria* is clearly polyphyletic (Bateman *et al*., 2003), but Oncidiinae is monophyletic (Chase *et al*., 2003). *Epidendrum*, as traditionally delimited as polyphyletic, and genera such as *Orleanesia* Barb.Rodr., *Amblostoma* Scheidw. and *Lanium* (Lindl.) Benth., should be included in *Epidendrum* (Hágsater & Soto Arenas, 2005). Subgenus *Amphiglottium* formed one monophyletic clade with moderate (Van den Berg *et al*., 2000) to strong (Pinheiro *et al*., 2009) phylogenetic support, making the subgenus especially interesting as a model for numerical chromosome evolution in Orchidaceae. Reproductive isolation is commonly affected by differences in chromosome numbers and asymmetries among chromosomes (Cozzolino, D'Emerico & Widmer, 2004). These karyotype differences build instant isolation barriers between species with different ploidy levels (Coyne & Orr, 2004). However, there are growing numbers of studies showing that different chromosome numbers and ploidy levels between species can occur at small scales without being barriers to gene flow among them (Jersáková *et al*., 2010; Trávníček *et al*., 2011). Indeed, the different chromosome numbers observed in subgenus *Amphiglottium* do not affect reproductive compatibilities between its species, as gene flow across ploidy levels was observed (Pinheiro *et al*., 2009).

In addition to polyploidy, aneuploidy is a major source of the discrepant chromosome numbers differing from the euploid series. These alterations would be maintained through vegetative reproduction, because *E. secundum* and other species of subgenus *Amphiglottium* reproduce quite easily by budding at their floral stem nodes. These variable chromosome numbers may also be related to the wide polymorphic and morphological continuum observed in this subgenus, one of the most variable in the genus (Hágsater & Soto Arenas, 2005; Pinheiro & Barros, 2007b; Pinheiro *et al*., 2009).

The identification of the basic number was described by Guerra (2000) as the haploid number of a taxon that most parsimoniously explains the chromosome number variation in that taxon and in other closely related taxa. Based on this concept, Felix & Guerra (2000, 2005) proposed $x = 7$ as the basic chromosome number of the cymbidioid clade, of the majority of terrestrial orchids and of Orchidaceae. *Epidendrum*, however, seems to have followed a distinct evolutionary pattern. As can be seen in Figure 4, *E. cristatum* Ruiz & Pav., sister group of subgenus *Amphiglottium*, has $2n = 40$, suggesting that current representatives of *Epidendrum* are palaeopolyploids with $x = 20$. In addition, approximately 70% of the species in this genus for which karyological records are available have $n = 20$, followed by $n = 28$ and 40 (Felix & Guerra, 2010). Similar numbers, especially $n = 20$, occur widely in all of the genera of subtribe Laeliinae (numerical records exist for 7.4%), suggesting that $x = 20$ is really the basic number for the genus. However, smaller haploid numbers were observed in *E. fulgens* ($n = 12$) and *E. denticulatum* ($n = 19$), some of the more derived species in subgenus *Amphiglottium*. As $n = 20$ is the most frequent number in the subtribe, and at least one species of the related subtribe Pleurothallidinae has $n = 10$ (Felix & Guerra, 2010), it would be reasonable to consider $x_1 = 10$ as the basic primary number for the genus, with $n = 12$ resulting from ascendant disploidy, and $n = 19$ probably resulting from descendant disploidy from an ancestor with $x_2 = 20$. However, the notable presence of one large chromosome pair in *E. fulgens* (and in *E. denticulatum*) also occurs in several species of subgenus *Amphiglottium*, including all of the cytotypes of *E. secundum* that have been analysed, confirming the monophyly of *Amphiglottium*, as suggested by Pinheiro *et al*. (2009), and it is also a good indicator of the ploidy of the species composing this subgenus. Any other hypothesis to explain the origin of this chromosome pair would be less parsimonious, because it requires the occurrence of other chromosome alteration events (deletions and fusions, at least) in this and in the other chromosome pairs in each cytotype. However, the number of large chromosomes did in fact vary among the species, although they did not correspond to the ploidy expected for an $x = 10$ ancestral stock. These data suggest that some species of subgenus *Amphiglottium*, especially *E. secundum*, have been derived from disploidy/aneuploidy events that have led to the formation of secondary basic numbers. Although chromosome counts are relatively rare in *Epidendrum* (only 42 have been reported to date, representing just 2.8% of all *Epidendrum* spp.), 70% showed $n = 20$, 30 or 40. This same variation has also been observed in other representatives of subtribe Laeliinae, which clearly have $x = 20$ as their secondary basic number (Felix & Guerra, 2010). Angiosperms as a whole are considered to be ancient polyploids that underwent diploidization through massive genetic change or through reductions in chromosome numbers (Adams & Wendel, 2005) and satellite DNA amplification and deletion (Sharma & Raina, 2005). It is quite probable that these processes are still active in the karyological evolution of *Epidendrum*, and that they are especially notable in subgenus *Amphiglottium*.

From the strict viewpoint of the numerical variations in chromosome number for *Epidendrum*, the basic primary number that would most parsimoniously explain their relationship with other species of Laeliinae would be $x = 10$, even though this number has not been observed among the modern species of this genus. In subgenus *Amphiglottium*, a karyotype with $n = 12$ and with a pair of large chromosomes seems to have occurred at the start of the divergence of this lineage, and $x = 12$ probably represents a basic number restricted to this subgenus. The other lineages of *Epidendrum* seem to have been derived secondarily from an ancestral stock with $x = 20$, which is seen in the majority of the current representatives of this genus.

ACKNOWLEDGEMENTS

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and research grants, and the anonymous reviewers for their valuable comments and suggestions to improve the manuscript.

REFERENCES

Adams KL, Wendel JF. 2005. Polyploidy and genome evolution in plants. *Current Opinion in Plant Biology* **8:** 135–141.

Antonelli A, Verola CF, Parisod C, Gustafsson ALS. 2010. Climate cooling promoted the expansion and radiation of a threatened group of South American orchids (Epidendroideae: Laeliinae). *Biological Journal of the Linnean Society* **100:** 597–607.

Arditti J. 1992. *Fundamentals of orchid biology*. New York: John Wiley and Sons.

Bateman RM, Hollingsworth PM, Preston J, YI-Bo L, Pridgeon AM, Chase MW. 2003. Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* **142:** 1–40.

Blumenschein A. 1957. Estudos citológicos na família Orchidaceae. PhD Thesis, Universidade de São Paulo.

Blumenschein A. 1960. Número de cromossomas de algumas espécies de orquídeas. *Publicação Cientifica do Instituto de Genética USP* **1:** 45–50.

Brieger FG. 1976–1977. Gattungsreihe Epidendra. In: Brieger FG, Maatsch R, Senghas K, eds. *Schlechter die Orchideen*. Berlin: Paul Parey, 509–549.

Brieger FG, Bicalho AD. 1977. Observações taxonômicas em *Epidendrum nocturnum* Jacq. *Relatório Científico* **11:** 23–28.

Brown WV. 1951. Apomixis in *Zephyranthes texana*. *American Journal of Botany* **38:** 697–702.

Carnevali G, Romero GA. 1996. Orchidaceae Dunstervillorum VII: the *Epidendrum nocturnum* alliance in the Venezuelan Guayana and the Guianas. *Lindleyana* **11:** 239–249.

Chase MW, Cameron KM, Barrett RL, Freudenstein JV. 2003. DNA data and Orchidaceae systematics: a new phylogenetic classification. In: Dixon KW, Kell SP, Barrett RL, Cribb PJ, eds. *Orchid conservation*. Kota Kinabalu: Natural History Publications (Borneo), 69–89.

Chase MW, Hanson L, Albert VA, Whitten WM, Williams NH. 2005. Life history evolution and genome size in subtribe Oncidiinae (Orchidaceae). *Annals of Botany* **95:** 191–199.

Chochai A, Leitch IJ, Ingrouille MJ, Fay MF. 2012. Molecular phylogenetics of *Paphiopedilum* (Cypripedioideae; Orchidaceae) based on nuclear ribosomal ITS and plastid sequences. *Botanical Journal of the Linnean Society* **170:** 176–196.

Cogniaux A. 1898–1902. *Epidendrum*. In: Martius CFP, Eichler AG, Urban I, eds. *Flora brasiliensis*. Munich: Oldenbourg, 30–186.

Conceição LP, Oliveira ALPC, Barabosa LV. 2006. Characterization of the species *Epidendrum cinnabarinum* Salzm. (Epidendroideae: Orchidaceae) occurring in Dunas do Abaaeté – Salvador, Ba, Brasil. *Cytologia* **71:** 125–129.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Cozzolino S, D'Emerico S, Widmer A. 2004. Evidence for reproductive isolate selection in Mediterranean orchids: karyotype differences compensate for the lack of pollinator specificity. *Proceedings of the Royal Society of London B: Biological Science* **271:** 259–262.

Cribb PJ, Chase MW. 2005. Distribution. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN, eds. *Genera Orchidacearum*. Oxford: Oxford University Press, 3–8.

Donald C, Brent DS, McDavid WD, Greer DB. 2007. UTHSCSA ImageTool (IT) – Version 3.0. Available at: http://ddsdx.uthscsa.edu/dig/download.html (accessed 24 July 2007).

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF. 2008. Evolutionary genetics of genome merger and doubling in plants. *Annual Review of Genetics* **42:** 443–461.

Dressler RL. 1993. *Phylogeny and classification of the orchid family*. Cambridge: Cambridge University Press.

Felix LP, Guerra M. 1998. Cytogenetic studies of species of *Habenaria* (Orchidoideae: Orchidaceae) occurring in the northeast of Brazil. *Lindleyana* **13:** 224–230.

Felix LP, Guerra M. 2000. Cytogenetics and cytotaxonomy of some Brazilian species of cymbidioid orchids. *Genetic and Molecular Biology* **23:** 957–978.

Felix LP, Guerra M. 2005. Basic chromosome numbers of terrestrial orchids. *Plant Systematics and Evolution* **254:** 131–148.

Felix LP, Guerra M. 2010. Variation in chromosome number and the basic number of subfamily Epidendroideae (Orchidaceae). *Botanical Journal of the Linnean Society* **163:** 234–278.

Goldblatt P. 1981. Index to plant chromosome numbers 1975–1978. *Monographs in Systematic Botany from the Missouri Botanical Garden* **5.**

Goldblatt P. 1985. Index to plant chromosome numbers 1982–1983. *Monographs in Systematic Botany from the Missouri Botanical Garden* **13.**

Goldblatt P. 1988. Index to plant chromosome numbers 1984–1985. *Monographs in Systematic Botany from the Missouri Botanical Garden* **23.**

Goldblatt P, Johnson DE. 1994. Index to plant chromosome numbers 1990–1991. *Monographs in Systematic Botany from the Missouri Botanical Garden* **40.**

Grant V. 1981. *Plant speciation*. New York: Columbia University Press.

Guerra M. 1983. Variação no conteúdo de DNA nuclear de *Pisum sativum* L. *Ciência e Cultura* (SBPC) **35:** 1661–1663.

Guerra M. 1986. Reviewing the chromosome nomenclature of Levan *et al*. *Brazilian Journal of Genetics* **9:** 21–40.

Guerra M. 2000. Chromosome number variation and evolution in monocots. In: Wilson KL, Morrison DA, eds. *Monocots II: systematics and evolution*. Melbourne: CSIRO, 127–136.

Guerra M. 2008. Chromosome numbers in plant cytotaxonomy: concepts and implications. *Cytogenetic and Genome Research* **120:** 339–350.

Hágsater E. 1984. Towards an understanding of the genus *Epidendrum*. In: Tan KW, ed. *Proceedings of the Eleventh World Orchid Conference*. Miami, FL: 11th World Orchid Conference, 195–201.

Hágsater E, Soto Arenas MA. 2005. *Epidendrum* L. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN, eds. *Genera Orchidacearum, Vol. 4*. Oxford: Oxford University Press, 236–251.

Index Kewensis. 2005. The International Plan Names Index. Available at: http://www.ipni.org/ipni/plantnamesearchpage.do (accessed 28 September 2011).

Jersáková J, Castro S, Sonk N, Milchreit K, Schödelbauerová I, Tolasch T, Dötterl S. 2010. Absence of pollinator-mediated premating barriers in mixed-ploidy

populations of *Gymnadenia conopsea* s.l. (Orchidaceae). *Evolutionary Ecology* **24:** 1199–1218.

Kamemoto H. 1950. Polyploidy in cattleyas. *American Orchid Society Bulletin* **19:** 366–373.

Koehler S, Cabral JS, Whitten M, Williams NH, Singer RB, Neubig KM, Guerra M, Souza AP, Amaral MCE. 2008. Molecular phylogeny of the neotropical genus *Christensonella* (Orchidaceae, Maxillariinae): species delimitation and insights into chromosome evolution. *Annals of Botany* **102:** 491–507.

Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW, Fay MF. 2009. Genome size diversity in orchids: consequences and evolution. *Annals of Botany* **104:** 469–481.

Levin DA. 2002. *The role of chromosomal change in plant evolution*. New York: Oxford University Press.

Lindley J. 1852–1859. *Epidendrum*. In: Lindley J, ed. *Folia Orchidacea*. London: Matthews, 1–97.

Lynch M, Conery JS. 2000. The evolutionary fate and consequences of duplicate genes. *Science* **290:** 1151–1155.

Moraes AP, Leitch IJ, Leitch AR. 2012. Chromosome studies in Orchidaceae: karyotype divergence in Neotropical genera in subtribe Maxillariinae. *Botanical Journal of the Linnean Society* **170:** 29–39.

Neubig KM, Whitten WM, Williams NH, Blanco MA, Endara L, Burleigh JG, Silvera K, Cushman JC, Chase MW. 2012. Generic recircumscriptions of Oncidiinae (Orchidaceae: Cymbidieae) based on maximum likelihood analysis of combined DNA datasets. *Botanical Journal of the Linnean Society* **168:** 117–146.

Pabst GFJ, Dungs F. 1975a. *Orchidaceae brasiliensis*. Band 1. Hildesheim: Brücke Verlag Kurt Schmersow.

Pabst GFJ, Dungs F. 1975b. *Orchidaceae brasiliensis*. Band 2. Hildesheim: Brücke Verlag Kurt Schmersow.

Pinheiro F, Barros F. 2005. Avaliação das relações intra e interespecíficas no complexo *Epidendrum secundum* e espécies afins (Orchidaceae) através de AFLP. Dissertation, Universidade de São Paulo.

Pinheiro F, Barros F. 2007a. Morphometric analysis of *Epidendrum secundum* (Orchidaceae) in southeastern Brazil. *Nordic Journal of Botany* **25:** 129–136.

Pinheiro F, Barros F. 2007b. *Epidendrum secundum* Jacq. e *E. denticulatum* Barb. Rodr. (Orchidaceae): caracteres úteis para a sua delimitação. *Hoehnea* **34:** 563–570.

Pinheiro F, Barros F, Palma-Silva C, Meyer D, Fay MF, Suzuki RM, Lexer C, Cozzolino S. 2010. Hybridization and introgression across different ploidy levels in the Neotropical orchids *Epidendrum fulgens* and *E. puniceoluteum* (Orchidaceae). *Molecular Ecology* **19:** 3981–3994.

Pinheiro F, Koehler S, Corrêa AM, Salatino MLF, Salatino A, Barros F. 2009. Phylogenetic relationships and infrageneric classification of *Epidendrum* subgenus *Amphiglottium* (Laeliinae, Orchidaceae). *Plant Systematics and Evolution* **283:** 165–177.

Ramsey J, Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29:** 467–501.

Romero Zarco C. 1986. A new method for estimating karyotype asymmetry. *Taxon* **35:** 526–530.

SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL. 1996. Nested retrotransposons in the intergenic regions of the maize genome. *Science* **274:** 765–768.

Sharma S, Raina SN. 2005. Organization and evolution of highly repeated satellite DNA sequences in plant chromosomes. *Cytogenetic and Genome Research* **109:** 15–26.

Soltis DE, Senters AE, Zanis MJ, Kim S, Thompson JD, Soltis PS, Ronse Decraene LP, Endress PK, Farris JD. 2003. Gunnerales are sister to other core eudicots, and exhibit floral features of early-diverging eudicots. *American Journal of Botany* **90:** 461–470.

Stace CA. 2000. Cytology and cytogenetics as a fundamental resource for the 20th and 21st centuries. *Taxon* **49:** 451–477.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Szlachetko DL, Veyret Y. 1996. Deux nouvelles espèces de Spiranthinae (Orchidaceae) de Guyane française. *Orchidées Culture et Protection* **25:** 20–21.

Tanaka R, Kamemoto H. 1984. Chromosomes in orchids: counting and numbers. In: Arditti J, ed. *Orchid biology: reviews and perspectives III*. Ithaca, NY: Cornell University Press, 234–283.

Trávníček P, Kubátová B, Čurn V, Rauchová J, Krajníková E, Jersáková J, Suda J. 2011. Remarkable coexistence of multiple cytotypes of the *Gymnadenia conopsea* aggregate (the fragrant orchid): evidence from flow cytometry. *Annals of Botany* **107:** 77–87.

Van den Berg C, Higgins WE, Dressler RL, Whitten WM, Arenas A, Culham A, Chase MW. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Lindleyana* **15:** 96–114.

Whitten WM, Williams NH, Blanco MA, Endara L, Neubig K, Koehler S, Carnevali G, Singer R. 2007. Molecular phylogenetics of *Maxillaria* and related genera (Orchidaceae: Cymbidieae) based upon combined molecular data sets. *American Journal of Botany* **94:** 1860–1889.

Yamagishi-Costa H, Forni-Martins ER. 2009. Hybridization and polyploidy: cytogenetic indications of *Hoffmannsenggella* (Orchidaceae) species evolution. *International Journal of Botany* **5:** 93–99.