Braz. J. Bot (2015) 38(2):333–342
DOI 10.1007/s40415-014-0130-x

Chromosome studies in Spiranthinae and Cranichidinae (Orchidaceae)

Leonardo R. S. Guimarães · Pedro Mercado-Ruaro ·
Andréa M. Corrêa · Gerardo A. Salazar ·
Fábio de Barros

Received: 16 October 2014 / Accepted: 19 December 2014 / Published online: 11 January 2015

Abstract The chromosome numbers of eighteen species of Spiranthinae from Brazil and Mexico and two of Cranichidinae from Ecuador were analysed. Sixteen chromosome records are presented for the first time: *Aulosepalum riodelayense* ($2n = 64$), *A. tenuiflorum* ($2n = 60$), *Cyclopogon luteoalbus* ($2n = 36$), *Dichromanthus aurantiacus* ($2n = 40$), *Eltroplectris calcarata* ($2n = 42$), *Pelexia funckiana* ($2n = 46$), *Sarcoglottis assurgens* ($2n = 46$), *S. richardiana* ($2n = 50$), *S. rosulata* ($2n = 33$), *S. sceptrodes* ($2n = 46$), *S. schaffneri* ($2n = 46$), *S. scintillans* ($2n = 46$), *Stenorrhynchos albidomaculatum* ($2n = 46$), *S.* cf. *speciosum* ($2n = 46$), *Ponthieva andicola* ($2n = 26$) and *P. pilosissima* ($2n = \pm 42$). For *Mesadenella cuspidata*, a chromosomal number variation ($2n = 38/42$) was found. In addition, ideograms of five Brazilian species are presented. Chromosome data support phylogenetic relationships suggested by previous cytological and molecular studies.

Keywords Brazil · Cytotaxonomy · Ideogram · Karyotype · Mexico

Introduction

The family Orchidaceae comprises approximately 25,000 species (Dressler 2005) distributed in 850 genera (Atwood 1986; Pridgeon et al. 1999; Chase et al. 2003). It has a cosmopolitan distribution, with the exception of the regions always covered with snow and extreme deserts, but is most abundant and diverse in humid tropical and subtropical forests. As for the classification, the most recent systems for Orchidaceae are those proposed by Dressler (1993) and Pridgeon et al. (1999, 2001, 2003, 2005, 2009, 2014), the latter based also on molecular data, proposing the division of the family into five subfamilies: Apostasioideae, Vanilloideae, Cypripedioideae, Orchidoideae and Epidendroideae. In the subfamily Orchidoideae, there are six tribes; one of them is the Cranichideae, with six subtribes: Cranichidinae, Galeottiellinae, Goodyerinae, Manniellinae, Pterostylidinae and Spiranthinae (Pridgeon et al. 2001, 2003).

Subtribe Cranichidinae has 16 genera and about 210 species, all of them endemic to the Neotropics (Cribb and Pridgeon 2003; Salazar et al. 2003), and is characterized by the non-resupinate flowers, comparatively short column and brittle pollinia (Dressler 1993). In turn, subtribe Spiranthinae comprises about 40 genera and 470 species almost exclusively restricted to the Neotropics, except for the cosmopolitan genus *Spiranthes* Rich. (Salazar 2003). Its members can be recognized by the tubular flowers, margins of the lip adhered to the sides of the column, forming a deep nectary, and the soft granulose pollinia (Dressler 1993).

There are few cytotaxonomic studies on Spiranthinae, and these are concentrated especially on the genus *Spiranthes* with $x = 15$, 22 and $2n = 30$ to 74 (Vij and Vohra 1974; Sheviak 1982; Tanaka and Kamemoto 1984; Martínez 1985;

L. R. S. Guimarães (✉) · A. M. Corrêa · F. de Barros
Núcleo de Pesquisa Orquidário do Estado, Instituto de Botânica,
Caixa Postal 68041, São Paulo, SP 04045-972, Brazil
e-mail: leo.rsguimaraes@hotmail.com

P. Mercado-Ruaro · G. A. Salazar
Departamento de Botánica, Instituto de Biología, Universidad
Nacional Autónoma de México, Apartado Postal 70-367,
04510 México, D.F., Mexico

Brandham 1999). For the Neotropical species, Martínez (1981, 1985), Felix and Guerra (2005), Daviña et al. (2009) and Grabiele et al. (2010, 2013) have made the most significant contributions. On Cranichidinae, chromosome counts are reported only for *Ponthieva mandonii* Rchb.f. ($2n = 46$; Martínez 1985) and six species of *Prescottia* R.Br. (one with $n = 48$ and the others with $2n = 48$; Felix and Guerra 2005).

The aim of this study was to determine the chromosome numbers and other chromosomal features of eighteen species of Spiranthinae and two of Cranichidinae, as part of our ongoing systematic and evolutionary studies of these orchid groups (Guimarães 2014; Guimarães et al., unpublished data).

Materials and methods

Eighteen species of Spiranthinae from Brazil and Mexico, and two species of Cranichidinae from Ecuador were studied (Table 1). Samples were cultivated in the greenhouses of the “Núcleo de Pesquisa Orquidário do Estado/Instituto de Botânica” (NP-OE/IBt in São Paulo, Brazil) and of “Herbario AMO” (Mexico City, Mexico). Vouchers were deposited at the herbarium of the Institute of Botany (SP), Mexico’s National Herbarium (MEXU) and the herbarium of the Faculty of Superior Studies of the National Autonomous University of Mexico, campus Zaragoza (FEZA).

Mitotic studies were performed in root tips pretreated with 8-hydroxyquinoline 0.2 mM (8-Hq) for 24 h at 4 °C (SP samples) and for 5 h at 18 °C (MEXU and FEZA samples), fixed in absolute ethanol:glacial acetic acid (3:1) for 3–24 h at room temperature (25 °C) and stored in a freezer at −20 °C. To prepare the slides, root tips were hydrolysed in 5 N HCl for 12–30 min at room temperature, frozen in liquid nitrogen to remove the coverslip, stained with 2 % Giemsa for the Brazilian specimens (Guerra 1983), hydrolysed in 1 N HCl for 12 min at 60 °C and stained according to the Feulgen technique for the remaining specimens (Mercado-Ruaro and Delgado-Salinas 1998). The meristems were soaked in a drop of 2 % aceto-orcein and then squashed.

For each species, chromosomes were counted in at least five cells, observed in a Carl Zeiss Jenaval (SP samples) and an Axioscop (MEXU, FEZA samples) optical microscopes and photographed using the Canon PowerShot S3 1S (SP samples) and AxioCam ERc5S (MEXU, FEZA samples) digital cameras.

The best mitotic metaphase spreads were selected from the SP samples for a general chromosome characterization. For five of them, we detailed the karyotypes and prepared the ideograms. Chromosomes were measured using Adobe Photoshop CS 5.1 software, determining the karyotype formula (KF), total chromosome length (TCL, calculated as the sum of all individual sizes of chromosomes), the average length of each chromosome (ALC) and the centromeric index (CI) for each chromosome pair (calculated as the ratio of the short arm and chromosome length). The chromosome types were classified according to the nomenclature of Levan et al. (1964).

Results

Within the subtribe Spiranthinae, *Aulosepalum riodelayense* (Burns-Bal.) Salazar presented $2n = 64$; *A. tenuiflorum* (Greenm.) Garay, $2n = 60$; *Cyclopogon luteoalbus* (A.Rich. & Galeotti) Schltr., $2n = 36$; *Dichromanthus aurantiacus* (Lex.) Salazar & Soto Arenas, $2n = 40$; *Eltroplectris calcarata* (Sw.) Garay & H.R.Sweet, $2n = 42$; *E. triloba* (Lindl.) Pabst, $2n = 46$; *Pelexia funckiana* (A.Rich. & Galeotti) Schltr., $2n = 46$; *Mesadenella cuspidata* (Lindl.) Garay, $2n = 38$ and 42 (variation possibility due to uncertainly or aneusomaty; see Discussion below); *Sarcoglottis assurgens* (Rchb.f.) Schltr., $2n = 46$; *Sarcoglottis* cf. *grandiflora* (Lindl.) Klotzsch, $2n = 46$; *Sarcoglottis richardiana* (Schltr.) Salazar & Soto Arenas, $2n = 50$; *Sarcoglottis rosulata* (Lindl.) P.N.Don, $2n = 33$; *Sarcoglottis sceptrodes* (Rchb.f.) Schltr., $2n = 46$; *Sarcoglottis schaffneri* (Rchb.f.) Ames, $2n = 46$; *Sarcoglottis scintillans* (E.W.Greenw.) Salazar & Soto Arenas, $2n = 46$; *Sauroglossum elatum* Lindl., $2n = 46$; *Stenorrhynchos albidomaculatum* Christenson, $2n = 46$; and *Stenorrhynchos* cf. *speciosum* (Jacq.) Rich., $2n = 46$ (Figs. 1–5, 11–26). All species studied, except *A. riodelayense*, *A. tenuiflorum*, *C. luteoalbus*, *S. richardiana*, *S. scintillans*, *S. albidomaculatum* and *S.* cf. *speciosum*, present a pair of acrocentric or submetacentric chromosomes, which are about twice the size of the remaining chromosomes, which are metacentric. *Mesadenella cuspidata* with $2n = 42$ presents a pair of acrocentric chromosomes about four times as large as the remainder chromosomes (Table 2, Figs. 4, 8). The species *A. riodelayense* (Fig. 11) and *A. tenuiflorum* (Fig. 12) show a bimodal karyotype with six larger chromosomes and the others small.

The chromosome numbers for *E. calcarata* (Table 3, Figs. 1, 6), *A. riodelayense* (Fig. 11), *A. tenuiflorum* (Fig. 12), *C. luteoalbus* (Fig. 13), *D. aurantiacus* (Fig. 14), *P. funckiana* (Fig. 15), *S. assurgens* (Fig. 16), *S. richardiana* (Fig. 18), *S. rosulata* (Fig. 19), *S. schaffneri* (Fig. 20), *S. sceptrodes* (Fig. 21), *S. scintillans* (Fig. 22), *S. albidomaculatum* (Fig. 23) and *S.* cf. *speciosum* (Fig. 24) represent the first mitotic records for those species.

Table 1 List of species analysed of Spiranthinae and Cranichidinae with respective locality, vouchers and chromosome numbers ($2n$), previous counts and references

Taxon	Locality and voucher	$2n$	$2n$—Previous counts
Spiranthinae			
Aulosepalum riodelayense (Burns-Bal.) Salazar	Mexico, Oaxaca, El Vado, Rio del Vado, *Salazar 7553* (MEXU)	64	–
Aulosepalum tenuiflorum (Greenm.) Garay	Mexico, Oaxaca, Ayoquezco de Aldama, *Cruz-Lustre 158* (MEXU)	60	–
Cyclopogon luteoalbus (A. Rich & Galeotti) Schltr	Mexico, Querétaro, Landa de Matamoros, *Cruz-Lustre 970* (MEXU)	36	–
Dichromanthus aurantiacus (La Llave & Lex.) Salazar & Soto Arenas	Mexico, Distrito Federal, Reserva Ecológica del Pedregal de San Ángel, *Cruz-Lustre 155* (MEXU)	40	–
Eltroplectris calcarata (Sw.) Garay & H.R. Sweet	Brazil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi, *Guimarães* et al. *198* (SP)	42	–
Eltroplectris triloba (Lindl.) Pabst	Brazil, Espírito Santo, Linhares, Reserva Biológica Sooretama, *Guimarães* et al. *204* (SP)	46	46—Martínez (1985)
Mesadenella cuspidata (Lindl.) Garay	Brazil, São Paulo, Itararé, *Barros s.n.* (SP)	38/42	46—Martínez (1985), Daviña et al. (2009)
Pelexia funckiana (A. Rich. & Galeotti) Schltr	Mexico, Veracruz, San Andrés Tuxtla, *Salazar 8390* (MEXU)	46	–
Sarcoglottis assurgens (Rchb.f.) Schltr	Mexico, Guerrero, Chilpancingo, Pueblo de Acahuizotla, *Figueroa 302* (MEXU)	46	–
Sarcoglottis cf. *grandiflora* (Lindl.) Klotzsch	Ecuador, *Salazar 8500* (cultivated specimen)	46	46—Martínez (1985), Felix & Guerra (2005), Daviña et al. (2009)
Sarcoglottis richardiana (Schltr.) Salazar & Soto Arenas	Mexico, Chiapas, Ocotalito, Area de Protección de Flora y Fauna Nahá, *Salazar 8227* (MEXU)	50	–
Sarcoglottis rosulata (Lindl.) P.N. Don	Mexico, Oaxaca, Ayoquezco de Aldama, *Cruz-Lustre 142* (MEXU)	33	–
Sarcoglottis sceptrodes (Rchb.f.) Schltr	Mexico, Quintana Roo, Felipe Carrillo Puerto, *Hágsater 12236* (MEXU)	46	–
Sarcoglottis schaffneri (Rchb.f.) Ames	Mexico, Distrito Federal, Reserva Ecológica del Pedregal de San Ángel, *Cruz-Lustre 156* (MEXU)	46	–
Sarcoglottis scintillans (E.W. Greenw.) Salazar & Soto Arenas	Mexico, Oaxaca, *Ramírez 15* (FEZA)	46	–
Sauroglossum elatum Lindl	Brazil, Minas Gerais, Serra do Cipó, *Guimarães* et al. *159* (SP)	46	46—Martínez (1985)
Stenorrhynchos albidomaculatum Christenson	South America, without location, *Guimarães 294* (cultivated specimen)	46	–
Stenorrhynchos cf. *speciosum* (Jacq.) Rich	Mexico, without location, *Guimarães 293* (cultivated specimen)	46	–
Cranichidinae			
Ponthieva andicola Rchb.f	Ecuador, *Salazar 8428* (cultivated specimen)	26	–
Ponthieva pilosissima (Senghas) Dodson	Ecuador, *Salazar 8427* (cultivated specimen)	±42	–

A secondary constriction (satellite) was observed on the largest chromosome pair of *M. cuspidata* (Table 2, Figs. 3, 4, 8, 9), *S. assurgens* (Fig. 16) and *S. rosulata* (Fig. 19).

The chromosome number (CN), total chromosome length (TCL) and karyotype formula (KF) of *E. calcarata*, *E. triloba*, *M. cuspidata* and *S. elatum* are given in Table 4.

Regarding the studied representatives of the subtribe Cranichidinae, *Ponthieva andicola* Rchb.f. has $2n = 26$ (Fig. 25) and *Ponthieva pilosissima* (Senghas) Dodson, $2n = \pm 42$ (Fig. 26). Both are first chromosome counts for these species. *Ponthieva pilosissima* presents 10 large chromosomes, 20 median chromosomes and ca. 12 small

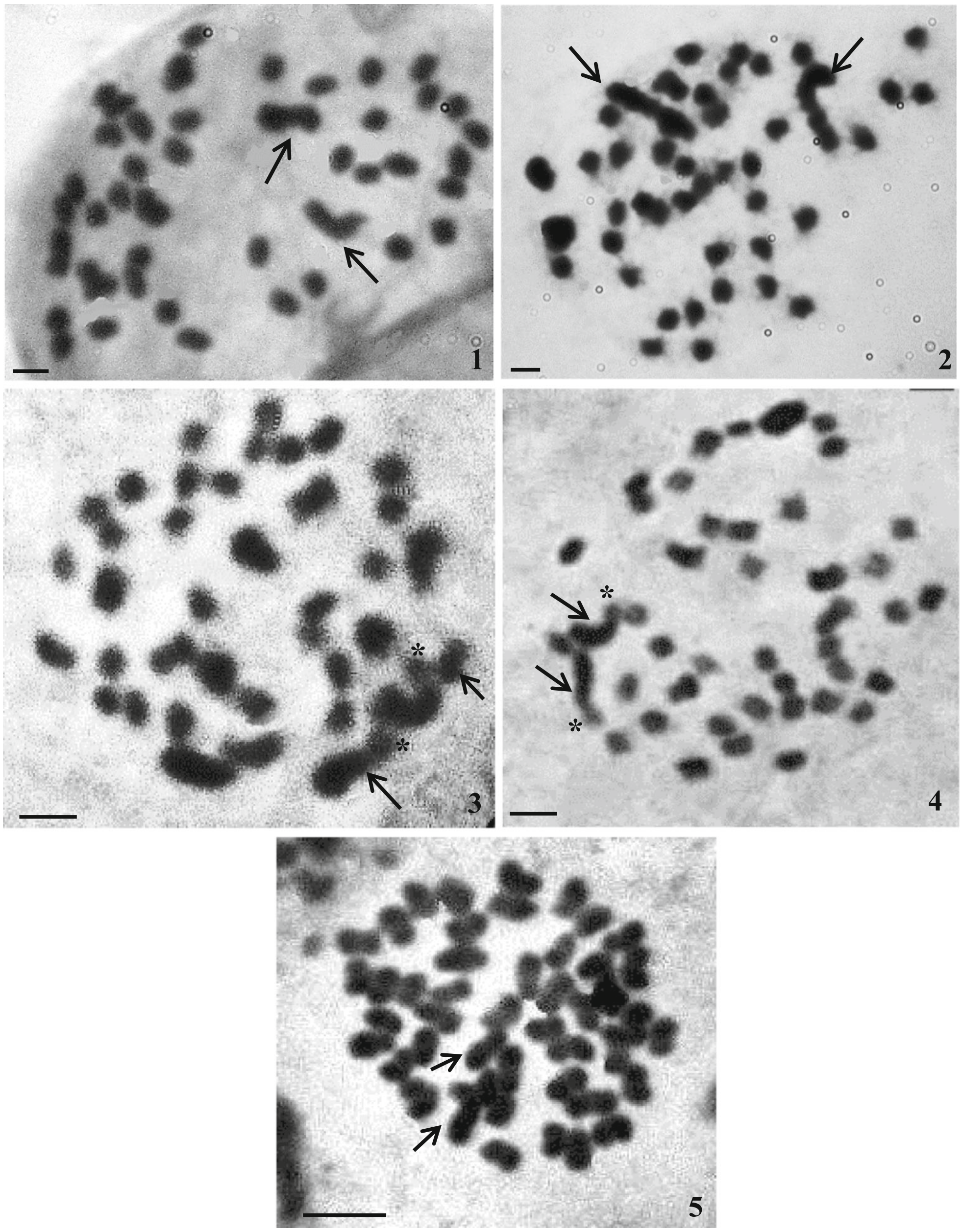

Fig. 1–5 Chromosomes of species of Spiranthinae studied. **1** *Eltroplectris calcarata* ($2n = 42$). **2** *Eltroplectris triloba* ($2n = 46$). **3–4** *Mesadenella cuspidata* ($2n = 38$ and $2n = 42$, respectively). **5** *Sauroglossum elatum* ($2n = 46$). *Arrows* indicate largest chromosomes in bimodal karyotypes; *asterisks* in **3** and **4** indicate the satellites. *Bar* = 1 µm

chromosomes in a trimodal karyotype. This difficulty of counting is due to the distinct degree of contraction of the different types of chromosomes, so that the small chromosomes may be earlier or later condensed and some of them have a secondary constriction. Thus, we were unable to determine the precise chromosome number of this species.

Discussion

Some of the counts obtained here differ from those found in literature. Martínez (1985) and Daviña et al. (2009) reported a $2n = 46$ cytotype for a specimen of *Mesadenella cuspidata* collected in Argentina, while in this study we found a chromosomal number variation of $2n = 38/42$ for a single specimen collected in Brazil. This difference in chromosomal number could be due to the difficulty of counting, to the possibility that these specimens could actually represent different species of *Mesadenella*, or merely to intra-polymorphism in this feature. Another possible explanation is that Martínez (1985) and Daviña et al. (2009) interpreted the secondary constrictions (satellites) of some chromosomes as distinct chromosomes, as was the case with the legume *Oxyrhynchus* (Palomino and Mercado 1983). Analysis of additional specimens of this

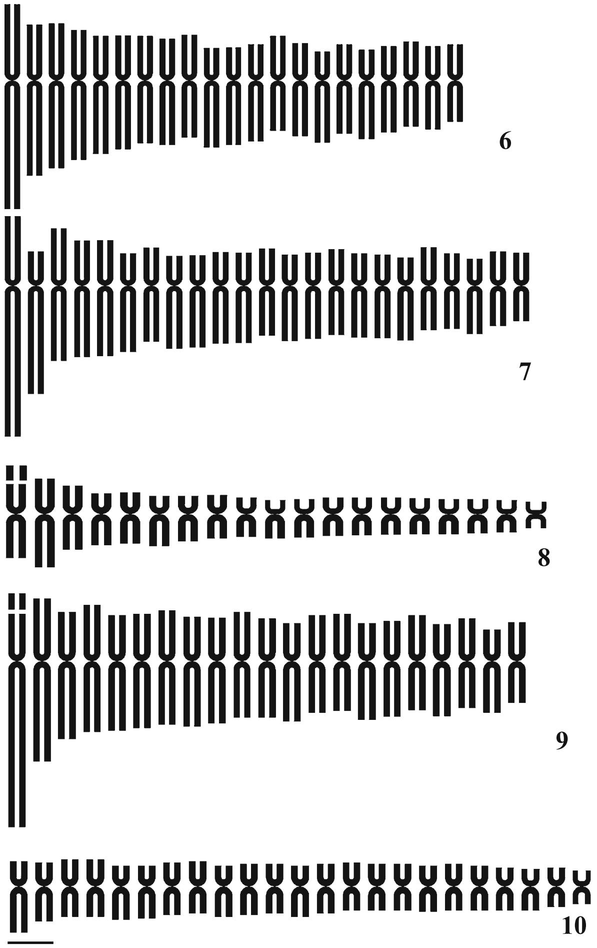

Fig. 6–10 Ideograms of some species of Spiranthinae studied. **6** *Eltroplectris calcarata* ($2n = 42$). **7** *Eltroplectris triloba* ($2n = 46$). **8–9** *Mesadenella cuspidata* ($2n = 38$ and $2n = 42$, respectively, with one pair with satellites). **10** *Sauroglossum elatum* ($2n = 46$). *Bar* = 1 μm

species, preferably from different geographic origins, would help to clarify the issue.

The variation of the chromosome number in *M. cuspidata* in the present study may be due to the difficulty of counting or to the occurrence of aneusomaty. Davide et al. (2007) defined polysomaty and aneusomaty as the intra-individual variations in chromosome number, related to the occurrence of polyploidy and aneuploidy, respectively. In the case of aneusomaty, it would represent the first observation of this condition in Spiranthinae, although it has been documented in orchids of the rupicolous epidendroid genus *Hoffmannseggella* of Laeliinae (Yamagishi-Costa and Forni-Martins 2009, 2013). Few cases of aneusomaty in plants are described in literature, but this phenomenon is common in families such as Orobanchaceae (Greilhuber and Weber 1975), Boraginaceae (Bigazzi and Selvi 2003) and Fabaceae (Rodrigues et al. 2009).

Martínez (1985) also noted that the species of Spiranthinae with $2n = 46$ often show a bimodal karyotype, with one pair of large chromosomes and the remainder ones much smaller. The present study confirms this bimodal karyotype (with some exceptions), as has been found in other species of the subtribe by Felix and Guerra (2005). This karyotype bimodality supports the inclusion of the genera *Eltroplectris* and *Mesadenella* in the *Stenorrhynchos* clade sensu Salazar et al. (2003). On the other hand, *Sarcoglottis assurgens*, *S.* cf. *grandiflora*, *S. sceptrodes*, *S. schaffneri* and *S. scintillans* share the same chromosome number ($2n = 46$) of other species of *Sarcoglottis* and *Pelexia* previously analysed (Martínez 1985; Dematteis and Daviña 1999; Felix and Guerra 2005; Daviña et al. 2009), which reinforces the proposal that *Sarcoglottis* really belongs in the *Pelexia* clade sensu Salazar et al. (2003). The meaning of the discordant chromosome numbers of *Sarcoglottis richardiana* ($2n = 50$) and *S. rosulata* ($2n = 33$) remains unclear and must be carefully studied by analysis of additional specimens of both species.

According to Martínez (1985), the basic number $x = 23$ for the Spiranthinae, as well as the bimodal chromosome condition, seems to be the distinguishing characteristic of the subtribe, which Felix and Guerra (2005) suggest as a significant feature to separate it from subtribe Goodyerinae, which belongs to a different lineage within the tribe Cranichideae (Salazar et al. 2003). The origin for this high basic number, compared to Goodyerinae (with $x = 10$, 14?, Brandham 1999; Felix and Guerra 2005), is not clear but could be due to polyploidy, although the single pair of large chromosomes does not support this hypothesis, unless the polyploids have chromosomes derived from different species, *i.e.* the origin would be fully allopolyploid as supposed by Martínez (1985).

The evolutionary significance of a single pair (or three pairs in *Aulosepalum* ssp.) of larger chromosomes in a bimodal karyotype also remains obscure, especially in cases where this condition is present in species with different chromosome numbers, e.g. *Eltroplectris* sp. with $2n = 26$ (Martínez 1985; Daviña et al. 2009) and *Sacoila* sp. with $2n = 46$ (Cocucci 1956; Martínez 1985; Felix and Guerra 2005; Daviña et al. 2009; Grabiele et al. 2010). Furthermore, this kind of karyotype appears to be quite rare in other groups of Orchidaceae. Felix and Guerra (2000) analysed 44 species of cymbidioid orchids and found bimodality in only two of them. Apparently, a bimodal karyotype is a specialized genomic architecture that could be selected for its functionality (Stebbins 1971; Kenton et al. 1990).

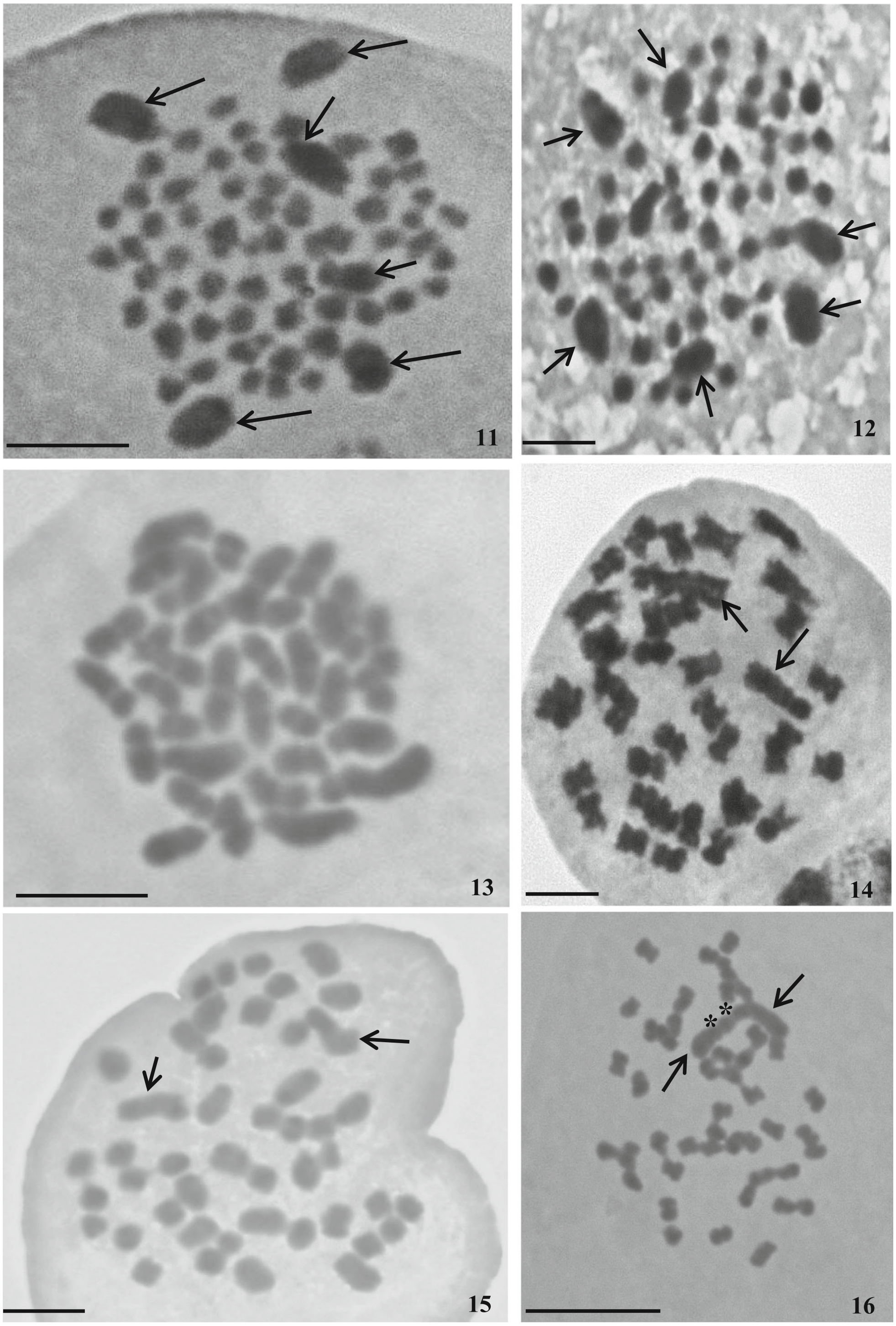

Fig. 11–16 Chromosomes of species of Spiranthinae studied. **11** *Aulosepalum riodelayense* ($2n = 64$). **12** *Aulosepalum tenuiflorum* ($2n = 60$). **13** *Cyclopogon luteoalbus* ($2n = 36$). **14** *Dichromanthus aurantiacus* ($2n = 40$). **15** *Pelexia funckiana* ($2n = 46$). **16** *Sarcoglottis assurgens* ($2n = 46$). *Arrows* in **11–12, 14–16** indicate largest chromosomes in bimodal karyotypes; *asterisks* in **16** indicate the satellites. *Bar* = 5 µm (**11–15**); 10 µm (**16**)

Ponthieva pilosissima, with $2n = \pm 42$, is a typical case of trimodal karyotype. This condition was observed only in Hyacinthaceae (Forrest and Jong 2004), Ruscaceae (Yamashita and Tamura 2004; Meng et al. 2005) and Orchidaceae (this study). All these families belong to the order Asparagales, and this type of karyotype could be a

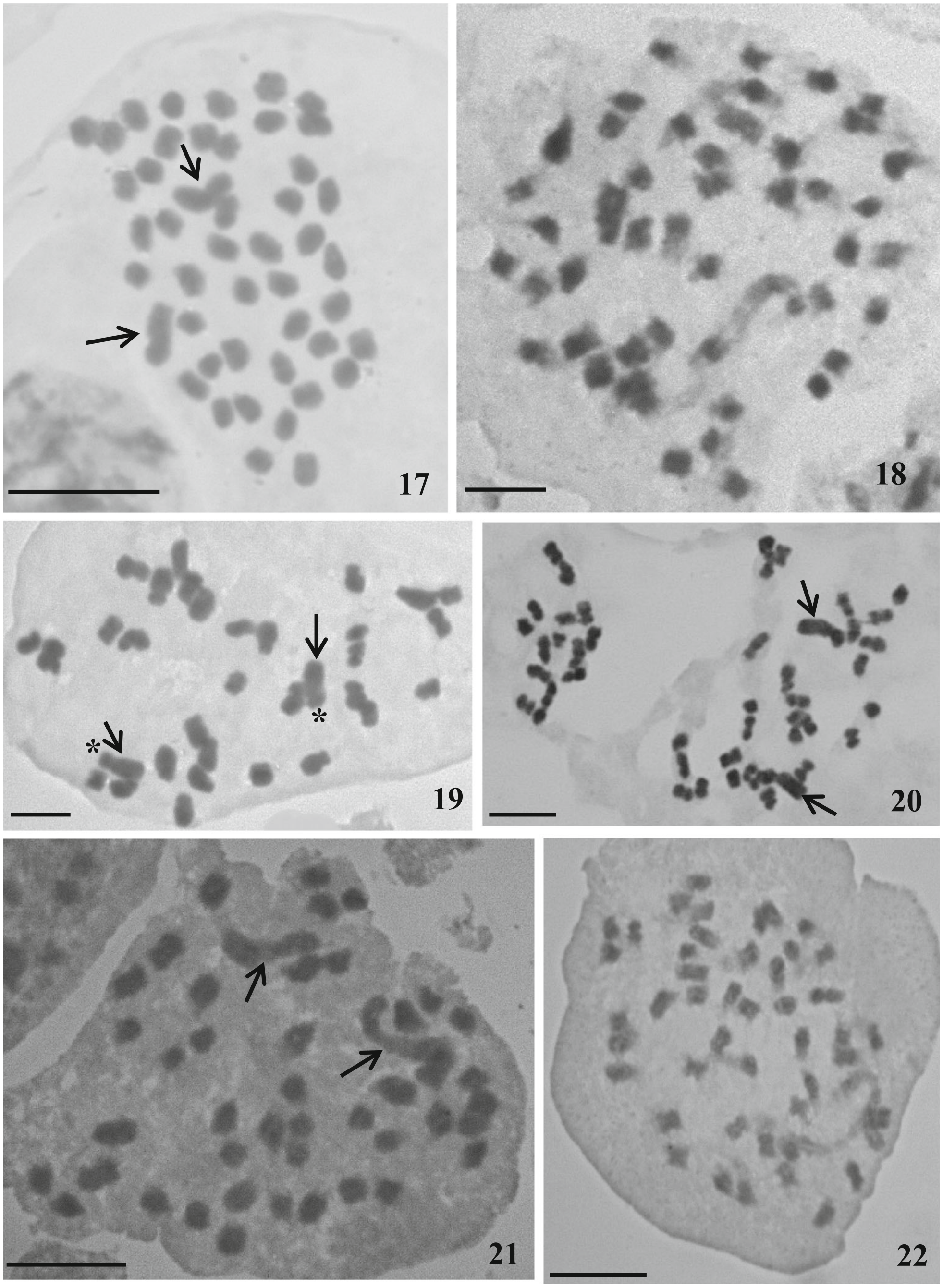

Fig. 17–22 Chromosomes of species of Spiranthinae studied. **17** *Sarcoglottis* cf. *grandiflora* (2*n* = 46). **18** *Sarcoglottis richardiana* (2*n* = 50). **19** *Sarcoglottis rosulata* (2*n* = 33). **20** *Sarcoglottis schaffneri* (2*n* = 46). **21** *Sarcoglottis sceptrodes* (2*n* = 46). **22** *Sarcoglottis scintillans* (2*n* = 46). *Arrows* in **17**, **19**–**21** indicate largest chromosomes in bimodal karyotypes; *asterisks* in **19** indicate the satellites. *Bar* = 5 μm (**18**–**20**); 10 μm (**17**, **21**–**22**)

homoplasy (parallel/convergent character) among those groups.

This study confirms some previous observations (Martínez 1985; Daviña et al. 2009) on chromosome numbers in Spiranthinae, but also contributes new chromosome counts, expanding the cytogenetic knowledge of the subtribe, and also of the family Orchidaceae. Further analyses are needed to clarify the different chromosome numbers found for *M.*

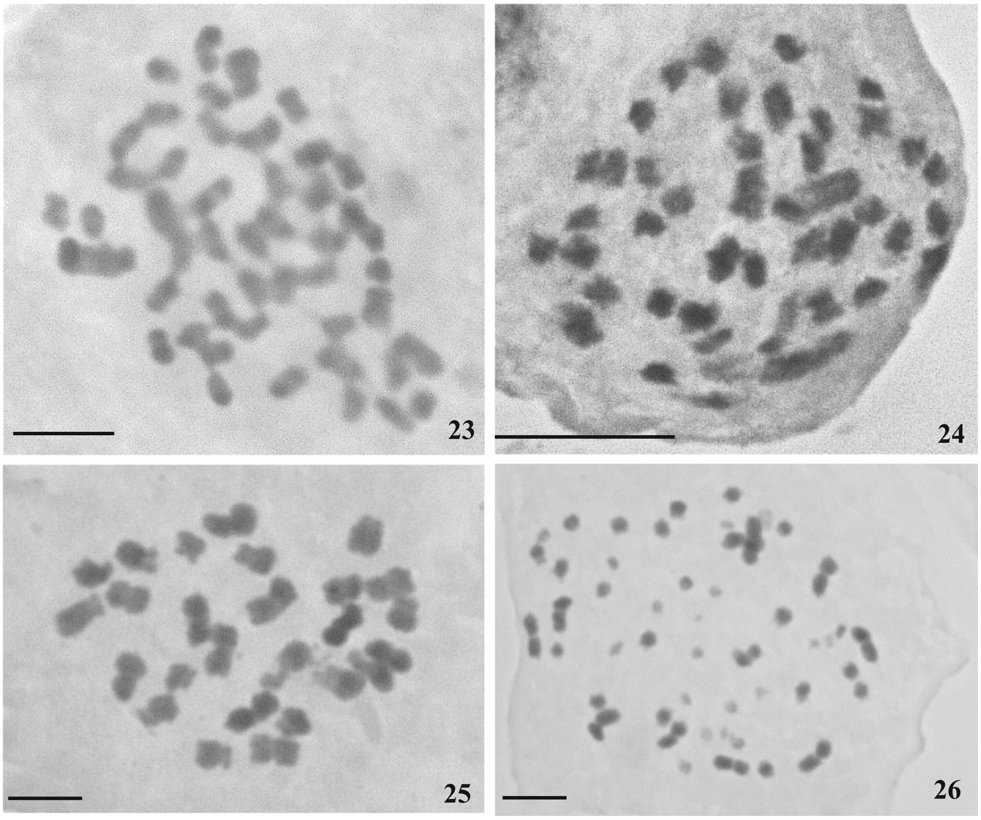

Fig. 23–26 Chromosomes of species of Spiranthinae and Cranichidinae studied. **23** *Stenorrhynchos albidomaculatum* (2*n* = 46). **24** *Stenorrhynchos* cf. *speciosum* (2*n* = 46). **25** *Ponthieva andicola* (2*n* = 26). **26** *Ponthieva pilosissima* (2*n* = ±42). *Bar* = 5 μm (**23, 25**–**26**); 10 μm (**24**)

Table 2 Chromosome measurements of *Mesadenella cuspidata*

Pair	*Mesadenella cuspidata* (2*n* = 38)				*Mesadenella cuspidata* (2*n* = 42)			
	ATL (μm)	CI	Type	SC	ATL (μm)	CI	Type	SC
1	0.49	40.82	m	+	1.33	22.26	a	+
2	0.60	39.50	sm	–	1.02	38.42	sm	–
3	0.43	44.71	m	–	0.79	38.61	sm	–
4	0.33	36.36	sm	–	0.79	44.30	m	–
5	0.34	39.71	sm	–	0.72	39.86	sm	–
6	0.34	42.65	m	–	0.71	41.55	m	–
7	0.30	40.00	sm	–	0.68	40.44	m	–
8	0.29	43.86	m	–	0.71	44.37	m	–
9	0.26	37.25	sm	–	0.66	41.22	m	–
10	0.26	39.22	sm	–	0.61	38.52	sm	–
11	0.26	42.31	m	–	0.60	39.17	sm	–
12	0.26	43.14	m	–	0.66	46.56	m	–
13	0.25	44.00	m	–	0.62	43.09	m	–
14	0.25	44.90	m	–	0.60	42.02	m	–
15	0.24	44.68	m	–	0.57	40.35	m	–
16	0.23	43.48	m	–	0.61	47.11	m	–
17	0.23	44.44	m	–	0.52	37.86	sm	–
18	0.22	44.19	m	–	0.61	48.76	m	–
19	0.18	45.71	m	–	0.59	48.31	m	–
20	–	–	–	–	0.56	47.75	m	–
21	–	–	–	–	0.50	48.00	m	–

ATL Average of total chromosome length, *CI* centromeric index, Type of chromosome: *m* metacentric, *sm* submetacentric, *a* acrocentric and *SC* secondary constriction: + presence and − absence

Table 3 Chromosome measures of *Eltroplectris calcarata*, *E. triloba* and *Sauroglossum elatum*

Pair	*Eltroplectris calcarata*			*Eltroplectris triloba*			*Sauroglossum elatum*		
	ATL (μm)	CI	Type	ATL (μm)	CI	Type	ATL (μm)	CI	Type
1	1.71	37.13	sm	1.81	31.58	sm	0.45	34.83	sm
2	1.26	36.90	sm	1.17	24.03	a	0.37	39.73	sm
3	1.21	39.26	sm	1.09	43.32	m	0.33	37.88	sm
4	1.09	38.71	sm	0.96	38.74	sm	0.33	38.46	sm
5	0.99	37.56	sm	0.95	39.47	sm	0.36	47.22	m
6	0.95	39.47	sm	0.81	32.72	sm	0.36	47.22	m
7	0.83	32.53	sm	0.76	32.24	sm	0.32	40.63	m
8	0.89	38.98	sm	0.76	33.11	sm	0.31	40.32	m
9	0.82	33.74	sm	0.75	36.67	sm	0.33	44.62	m
10	0.90	41.34	m	0.74	36.49	sm	0.32	44.44	m
11	0.76	31.58	sm	0.77	40.26	m	0.33	47.69	m
12	0.81	37.04	sm	0.71	35.92	sm	0.31	45.16	m
13	0.75	34.23	sm	0.68	33.82	sm	0.31	45.90	m
14	0.86	44.44	m	0.71	38.30	sm	0.29	43.86	m
15	0.78	40.00	sm	0.69	37.23	sm	0.30	50.00	m
16	0.75	40.27	m	0.69	38.41	sm	0.29	48.28	m
17	0.72	39.58	sm	0.72	42.66	m	0.29	48.28	m
18	0.79	46.84	m	0.71	42.55	m	0.29	47.37	m
19	0.70	41.01	m	0.62	35.77	sm	0.28	45.45	m
20	0.71	45.77	m	0.68	46.32	m	0.27	44.44	m
21	0.65	46.51	m	0.62	42.74	m	0.25	42.00	m
22	–	–	–	0.61	45.90	m	0.25	48.00	m
23	–	–	–	0.56	48.21	m	0.21	47.62	m

ATL Average of total chromosome length, *CI* centromeric index, Type of chromosome: *m* metacentric m, *sm* submetacentric and *a* acrocentric

Table 4 Karyological features of the species analysed

Taxon	CN	TCL	KF
Eltroplectris calcarata (Sw.) Garay & H.R.Sweet	42	18.88	7m + 14sm
Eltroplectris triloba (Lindl.) Pabst	46	18.52	8m + 14sm + 1a
Mesadenella cuspidata (Lindl.) Garay	38	5.71	13m + 6sm
	42	14.41	14m + 6m + 1a
Sauroglossum elatum Lindl.	46	7.10	19m + 4sm

CN Chromosome number, *TCL* total chromosome length, *KF* karyotypic formula, *m* metacentric, *sm* submetacentric and *a* acrocentric

cuspidata and to understand the trimodal karyotype reported for *P. pilosissima*.

Acknowledgments The authors are grateful to Fernanda Lo Schiavo and Leandro C.S. Ventura (NP-OE/IBt) for assistance in the laboratory, and Eric Hágsater (AMO Herbarium) and Gabriela Cruz-Lustre (UNAM) for providing plant material for this study. This study was supported by a scholarship of the São Paulo Research Foundation (FAPESP) [Grant Numbers 2010/16353-1, 2012/19778-9] to LRSG and by a productivity grant of the Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [grant number 304506/2013-3] to FB.

References

Atwood JT (1986) The size of the Orchidaceae and the systematic distribution of epiphytic orchids. Selbyana 9:171–186

Bigazzi M, Selvi F (2003) Chromosome variation in Anatolian species of *Nonea* Medik. (Boraginaceae), with special reference to endemics and *N. persica*. Caryologia 56:509–519

Brandham P (1999) Cytogenetics. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) Genera Orchidacearum, vol 1, General Introduction, Apostasioideae, Cypripedioideae. Oxford University Press, Oxford, pp 67–80

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

Cocucci AE (1956) Cromosomas gaméticos de *Stenorrhynchos australis* Lindl. y *Tradescantia radiata* Clarke. Trab Mus Bot (Cordoba) 2:781–783

Cribb PJ, Pridgeon AM (2003) Subtribe Cranichidinae. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) Genera Orchidacearum, vol 3, Orchidoideae (part 2) Vanilloideae. Oxford University Press, Oxford, pp 23–24

Davide LC, Techio VH, Nunes JD, Pereira AV (2007) Variação cromossômica numérica em *Pennisetum*. Ci Agrotecnol 31:398–405

Daviña JR, Grabiele M, Cerutti JC, Hojsgaard DH, Almada RD, Insaurralde IS, Honfi AI (2009) Chromosomes studies in Orchidaceae from Argentina. Genet Mol Biol 32:811–821

Dematteis M, Daviña JR (1999) Chromosome studies on some orchids from South America. Selbyana 20:235–238

Dressler RL (1993) Phylogeny and classification of the orchid family. Dioscorides Press, Portland

Dressler RL (2005) How many orchid species? Selbyana 26:155–158

Felix LP, Guerra M (2000) Cytogenetics and cytotaxonomy of some Brazilian species of Cymbidioid orchids. Genet Mol Biol 23:957–978

Felix LP, Guerra M (2005) Basic chromosome numbers of terrestrial orchids. Plant Syst Evol 254:131–148

Forrest LL, Jong K (2004) Karyotype asymmetry in *Galtonia* and *Pseudogaltonia* (Hyacinthaceae). Edinburgh J Bot 60:569–579

Grabiele M, Honfi AI, Cerutti JC, Fernandez V, Franco D, Daviña JR (2010) Cytogenetic analysis in the terrestrial orchid *Sacoila argentina* (Griseb.) Garay from Paraguay. Bot Stud 51:337–342

Grabiele M, Cerutti JC, Hojsgaard DH, Almada RD, Daviña JR, Honfi AI (2013) Comparative cytogenetics in *Cyclopogon* (Orchidaceae). Biologia (Bratislava) 68:48–54

Greilhuber J, Weber A (1975) Aneusomaty in *Orobanche gracilis*. Plant Syst Evol 124:67–77

Guerra M (1983) O uso de Giemsa na citogenética vegetal: comparação entre a coloração simples e o bandeamento. Cienc Cult 35:190–193

Guimarães LRS (2014) Filogenia e citotaxonomia do clado *Stenorrhynchos* (Spiranthinae, Cranichideae, Orchidoideae, Orchidaceae). PhD Thesis, Institute of Botany

Kenton A, Dickie JB, Langton DH, Bennett MD (1990) Nuclear DNA amount and karyotype symmetry in *Cypella* and *Hesperoxiphion* (Tigridieae; Iridaceae). Evol Trends Pl 4:59–69

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position of chromosomes. Hereditas 52:201–220

Martínez AJ (1981) Notas citotaxonomicas sobre el género *Cyclopogon* Presl. Parodiana 1:139–148

Martínez AJ (1985) The chromosomes of orchids VIII. Spiranthinae and Cranichidinae. Kew Bull 40:139–147

Meng Y, Nie ZL, Yang YP, Gu ZJ (2005) Karyomorphology of *Maianthemum* sensu lato (Polygonatae, Ruscaceae). J Plant Res 118:155–162

Mercado-Ruaro P, Delgado-Salinas A (1998) Karyotypic studies on species of *Phaseolus* (Fabaceae: phaseolinae). Am J Bot 85:1–9

Palomino G, Mercado P (1983) Cytological studies in the genus *Oxyrhynchus* (Leguminosae). In: Brandham PE, Bennett MD (eds) Kew Chromosome Conference II. George Allen and Unwin, London

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (1999) Genera Orchidacearum, vol 1: general introduction, Apostasioideae, Cypripedioideae. Oxford University Press, Oxford

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (2001) Genera Orchidacearum, vol 2: Orchidoideae (part 1). Oxford University Press, Oxford

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (2003) Genera Orchidacearum, vol 3: Orchidoideae (part 2), Vanilloideae. Oxford University Press, Oxford

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (2005) Genera Orchidacearum, vol 4: Epidendroideae (part one). Oxford University Press, Oxford

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (2009) Genera Orchidacearum, vol 5: Epidendroideae (part two). Oxford University Press, Oxford

Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) (2014) Genera Orchidacearum, vol 6: Epidendroideae (part three). Oxford University Press, Oxford

Rodrigues RS, Corrêa AM, Forni-Martins E, Tozzi AMGA (2009) Números cromossômicos em espécies de *Acosmium* Schott e *Leptolobium* Vogel (Leguminosae, Papilionoideae). Acta Bot Bras 23:902–906

Salazar GA (2003) Subtribe Spiranthinae. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (eds) Genera Orchidacearum, vol 3, Orchidoideae (part 2) Vanilloideae. Oxford University Press, Oxford, pp 164–278

Salazar GA, Chase MW, Soto Arenas MA, Ingrouille M (2003) Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences. Am J Bot 90:777–795

Sheviak CJ (1982) Biosystematic study of the *Spiranthes cernua* complex. Bull NY State Mus 48:1–73

Stebbins GL (1971) Chromosomal evolution in higher plants. Edward Arnold, London

Tanaka R, Kamemoto H (1984) Chromosomes in orchids: counting and numbers. In: Arditti J (ed) Orchid biology: reviews and perspectives III. Cornell University Press, Ithaca, pp 323–410

Vij SP, Vohra N (1974) Cytomorphological studies in the genus *Spiranthes* Rich. Cytologia 39:139–143

Yamagishi-Costa J, Forni-Martins ER (2009) Hybridization and polyploidy: cytogenetic indications for *Hoffmannseggella* (Orchidaceae) species evolution. Int J Bot 5:93–99

Yamagishi-Costa J, Forni-Martins ER (2013) Chromosomal diversity in two synchronopatric species of *Hoffmannseggella* (Orchidaceae). Nordic J Bot 31:505–509

Yamashita J, Tamura MN (2004) Phylogenetic analyses and chromosome evolution in Convallarieae (Ruscaceae sensu lato), with some taxonomic treatments. J Plant Res 117:363–370