



## Chromosome numbers and the systematics of tribe Vochysieae (Vochysiaceae)

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### ABSTRACT

With five neotropical genera and ca. 220 species, Vochysieae is the largest tribe of Vochysiaceae, comprising more than 90 % of its species. Preliminary phylogenetic analyses with *matK* sequence data indicated that Vochysieae may be paraphyletic, separated into the clades QRC (*Qualea*, *Ruizterania* and *Callisthene*) and VS (*Vochysia*, *Salvertia*). Whether the genera and the infrageneric taxa are artificial is still controversial. In this study, we analyzed the distribution of chromosome numbers in mitotic or meiotic cells among 20 species belonging to the genera *Callisthene*, *Qualea*, *Salvertia*, and *Vochysia*, that are native and common in Central and Southeast Brazilian forests or savannas. Species of *Callisthene* and *Qualea* possessed  $2n = 22$ , while species of *Salvertia* and *Vochysia* had  $2n = 24$  (or  $n = 12$ ). These chromosome numbers corroborate the recognition of two groups, and also suggest that chromosome evolution based on numerical variation is conservative in these lineages.

**Keywords:** *Callisthene*, Cytotaxonomy, Myrtales, Neotropics, *Qualea*, *Salvertia*, *Vochysia*, Vochysiaceae

## Introduction

Vochysiaceae is a mainly neotropical family composed of nearly 240 species (Shimizu & Yamamoto 2012). They are trees or shrubs with simple, opposite or whorled leaves, with glandular or non-glandular stipules, zygomorphic flowers, pentamerous calyx with shallowly connate lobes in quinuncial arrangement, the fourth lobe spurred or gibbous, corolla frequently reduced to 1-3 petals, only one fertile stamen, tiny staminodes frequently present, usually 3-carpellate and 3-loculate superior ovary with axillary ovules, and loculicidal capsules with winged seeds (Warming 1875; Stafleu 1952; Kawasaki 2007). This family was classified in Polygalales or allied suprafamilial taxa (e.g. Cronquist 1988) until phylogenetic analyses of molecular,

supported by morphological, embryological and wood anatomical data, placed it within Myrtales as sister family of Myrtaceae (Conti *et al.* 1996; 1997; Soltis *et al.* 2000).

Vochysiaceae is currently composed of two tribes (Erismeae and Vochysieae) and eight genera (or seven if *Ruizterania* is included in *Qualea*), three of them (*Callisthene*, *Qualea* and *Vochysia*) with infrageneric divisions (Warming 1875; Stafleu 1948; 1952; 1953; Keay & Stafleu 1953; Marciano-Berti 1969; Kawasaki 1998; 2007; Litt & Chase 1999; Litt & Cheek 2002; Litt & Stevenson 2003a; b). With nearly 220 species (*Callisthene* - 11 spp., *Qualea* - ca. 50 spp., *Ruizterania* - 14 spp., *Salvertia* - 1 sp., and *Vochysia* - ca. 140 spp.), Vochysieae is the largest tribe of the family, comprising more than 90 % of the species. Litt (1999) performed phylogenetic analyses for the family, including morphological and *matK* sequence data, and found that

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Erismeeae is a monophyletic lineage with distinct genera, while Vochysieae is uncertain. Two clades were recovered: one formed by species of *Vochysia* and the monospecific genus *Salvertia* nested among them (clade VS), and other composed of species of *Qualea*, *Ruizterania* and *Callisthene* (clade QRC). There is no resolution to ascertain if these two clades have a sister relationship or one of them is sister to Erismeeae. This study also suggested that the generic and infrageneric divisions of Vochysieae are artificial, thus indicating the reduction to only two genera (*Qualea s.l.* and *Vochysia s.l.*). The QRC and VS clades (and the genera that compose them) have also been distinguished through peculiar combinations of flower (Litt & Stevenson 2003a; b; Carmo-Oliveira & Morretes 2009), fruit and seed morphological features (Stafleu 1952), together with some embryological characteristics (Boesewinkel & Venturelli 1987). Besides, Mayworm & Salatino (2002) found that the distribution pattern of seed fatty acid types corroborates the distinction of the clades QRC and VS, but also that this kind of data may help discriminating the two subgenera of *Qualea* (*Q. subg. Qualea* and *Q. subg. Amphilochia*). So, a systematic reassessment of Vochysieae is needed, especially to verify if subdivisions can be distinguished within the clades QRC and VS. In this scenario, we carried out this study to verify if chromosome data can contribute to the more comprehensive phylogenetic analysis of Vochysiaceae that G Shimizu, D Gonçalves, A Litt, K Yamamoto, and A Simões (unpubl. res.) are currently working on.

Chromosome numbers and other karyological parameters such as chromosome morphology, genome size, banding and DNA *in situ* hybridization patterns are of great importance, supplying characters for taxonomic studies (Stace 2000). But karyotype information on Vochysiaceae has been limited so far to chromosome numbers of only two species of one genus, *Vochysia lomatophylla* Standl. with  $2n = 22$  (Goldblatt 1979) and *V. venezuelana* Stafleu with  $2n = 24$  (Löve 1987). So, the aim of this article is to provide new chromosome counts for Vochysieae and to verify if and how chromosome numbers vary within and between the clades QRC and VS and among their compounding genera and subgeneric divisions. Also, considering that ploidy level variation related to habitat have been reported for other botanical families (Morawetz 1986; Félix & Guerra 2000), we selected some species of *Callisthene*, *Qualea* and *Vochysia* from forest and cerrado (a Brazilian type of savanna) to analyze this feature. The results are also discussed in the context of the current knowledge about chromosome number in Myrtales.

## Materials and methods

Twenty species belonging to four genera of the tribe Vochysieae (*Callisthene* Mart., *Qualea* Aubl., *Salvertia* A.St.-Hil. and *Vochysia* Aubl.) that are native and common in Central and Southeast Brazil were studied for their

chromosome numbers (Tab. 1). To check for intraspecific variation, individuals of two or three populations from distinct distribution areas were studied for *Qualea cordata* Spreng., *Vochysia cinnamomea* Pohl, *V. tucanorum* Mart. and *Salvertia convallariodora* A.St.-Hil. For the other species, we examined only one population each. Vouchers of the studied specimens are held at the Herbarium of the University of Campinas (UEC). Chromosome numbers were observed in 10 or more metaphasic cells per species. Mitotic or meiotic cells were observed and photographed under Olympus BX51 microscope, using Kodak Imagelink (ISO 25) or Ilford (ISO 50) films. Because flowering and fruiting times of these species are distinct, they were collected for flower buds and/or ripe fruits bearing mature seeds for chromosome studies. Only *S. convallariodora*, *V. cinnamomea* and *V. tucanorum* were studied for both mitotic and meiotic divisions. Mitotic cells were obtained from root tips of germinated seeds, pre-treated with paradichlorobenzene (PDB) saturated solution for 5 hours at 16-18 °C and fixed in 1:3 acetic-alcohol (Carnoy) solution for 24 h, and then stored in freezer (-20 °C), preserved in 70 % ethanol. The root tips were washed in distilled water, hydrolysed in 5 N HCl for 10 min at room temperature and squashed in a drop of 45 % acetic acid. After covering removal, slides were stained in 2 % Giemsa solution (Guerra 1983). For the analysis of microsporogenesis, pollen mother cells were obtained from flower buds fixed in Carnoy's solution and stored in freezer, preserved in 70 % ethanol; young anthers were squashed in 1.2 % aceto-carmine (Medina & Conagin 1964).

## Results

Two distinct chromosome numbers were observed (Tab. 1). The number  $2n = 22$  was found in two species of *Callisthene* (Fig. 1A-B) and four species of *Qualea* (Fig. 1C-D), while  $2n = 24$  (Fig. 1E-H) or  $n = 12$  (Fig. 2) were found in *Salvertia convallariodora* and 13 species of *Vochysia*. So, the basic chromosome numbers of the clades QRC and VS are respectively  $x = 11$  and  $x = 12$ .

## Discussion

The results obtained in this study corroborate the separation of the clades QRC and VS. From the two previously reported chromosome numbers for the family,  $2n = 24$  in *V. venezuelana* (*Vochysia* sect. *Vochysiella* subsect. *Calophylloideae*) (Löve 1987) agrees with the pattern of chromosome number we found for VS clade, but the number  $2n = 22$ , reported for *V. lomatophylla* (*Vochysia* sect. *Ciliantha* subsect. *Ferrugineae*) by Goldblatt (1979), does not. There are no figures of the chromosomes in this latter study but it contains a description of a pair of distinctly larger chromosomes bearing satellites of similar size to other chromosomes in the karyotype. As we obtained  $2n = 24$



**Table 1.** Chromosome numbers for 20 Vochysiaceae species. ARB (Andréa Rodrigues Barbosa). GO (Goiás), MG (Minas Gerais), MT (Mato Grosso) and SP (São Paulo). Habitat: Ce (Cerrado), GalF (Gallery forest), MCr (Mesotrophic cerrado), StmF (Subtropical high mountain forest) and TcF (Tropical coastal rain forest).

Species	Specimen, collection site	Habitat	n	2n
<i>Callisthene</i> sect. <i>Callisthene</i>				
<i>Callisthene major</i> Mart.	ARB 213, Uberlândia (MG)	Ce	-	22
<i>Callisthene</i> sect. <i>Cataphyllantha</i>				
<i>Callisthene fasciculata</i> Mart.	ARB 209, Uberlândia (MG)	MCr	-	22
<i>Qualea</i> subg. <i>Qualea</i> sect. <i>Costatifolium</i>				
<i>Qualea grandiflora</i> Mart.	ARB 194, Santana do Riacho (MG)	Ce	-	22
<i>Qualea multiflora</i> Mart.	ARB 215, Uberlândia (MG)	Ce	-	22
<i>Qualea parviflora</i> Mart.	ARB 216, Uberlândia (MG)	Ce	-	22
<i>Qualea</i> subg. <i>Amphilochia</i>				
<i>Qualea cordata</i> Spreng.	ARB 185, Santana do Riacho (MG)	Ce	-	22
	ARB 201, Águas de Lindóia (SP)	Ce	-	22
<i>Salvertia convallariodora</i> A.St.-Hil.	ARB 182, Lagoa Santa (MG)	Ce	12	-
	ARB 183, Lagoa Santa (MG)	Ce	-	24
<i>Vochysia</i> sect. <i>Ciliantha</i> subsect. <i>Discolores</i>				
<i>Vochysia discolor</i> Warm.	ARB 294, Gouveia (MG)	Ce	12	-
<i>Vochysia schwackeana</i> Warm.	ARB 119, São José dos Campos (SP)	StmF	12	-
<i>Vochysia</i> sect. <i>Ciliantha</i> subsect. <i>Ferrugineae</i>				
<i>Vochysia acuminata</i> Bong.	ARB 250, Santana do Riacho (MG)	GalF	12	-
<i>Vochysia ferruginea</i> Mart.	ARB 368, Nova Xavantina (MT)	GalF	12	-
<i>Vochysia selloi</i> Warm.	ARB 229, Santo André (SP)	TcF	12	-
<i>Vochysia</i> sect. <i>Ciliantha</i> subsect. <i>Lutescentes</i>				
<i>Vochysia bifalcata</i> Warm.	ARB 41, Ubatuba (SP)	TcF	-	24
	ARB 15, Rio Claro (SP)	Ce	-	24
<i>Vochysia tucanorum</i> Mart.	ARB 16, Rio Claro (SP)	Ce	-	24
	ARB 113, Lagoa Santa (MG)	Ce	12	-
<i>Vochysia</i> sect. <i>Vochysiella</i> subsect. <i>Calophylloideae</i>				
<i>Vochysia haenkeana</i> Mart.	ARB 347, Caiapônia (GO)	GalF	-	24
<i>Vochysia</i> sect. <i>Vochysiella</i> subsect. <i>Decorticantes</i>				
<i>Vochysia cinnamomea</i> Pohl	ARB 14, Itirapina (SP)	Ce	-	24
	ARB 303, Cristalina (GO)	Ce	12	-
<i>Vochysia elliptica</i> Mart.	ARB 139, Santana do Riacho (MG)	Ce	12	-
<i>Vochysia herbacea</i> Pohl	ARB 338, Goiás Velho (GO)	Ce	12	-
<i>Vochysia pumila</i> Pohl	ARB 336, Pirenópolis (GO)	Ce	-	24
<i>Vochysia rufa</i> Mart.	ARB 208, Uberlândia (MG)	Ce	-	24

or  $n = 12$  in two or more populations in all of the 13 studied species of *Vochysia*, we believe that  $2n = 22$  in *V. lomatophylla* is a miscount and that those satellites are a distinct pair of chromosomes that were partly joined to others due to technical artefact. A new count is needed to confirm the number of chromosomes in this species, but the consistency of our results indicates that chromosome number is stable within the clades QRC and VS, and then chromosome evolution regarding to numerical variation is conservative in these two lineages of Vochysiaceae.

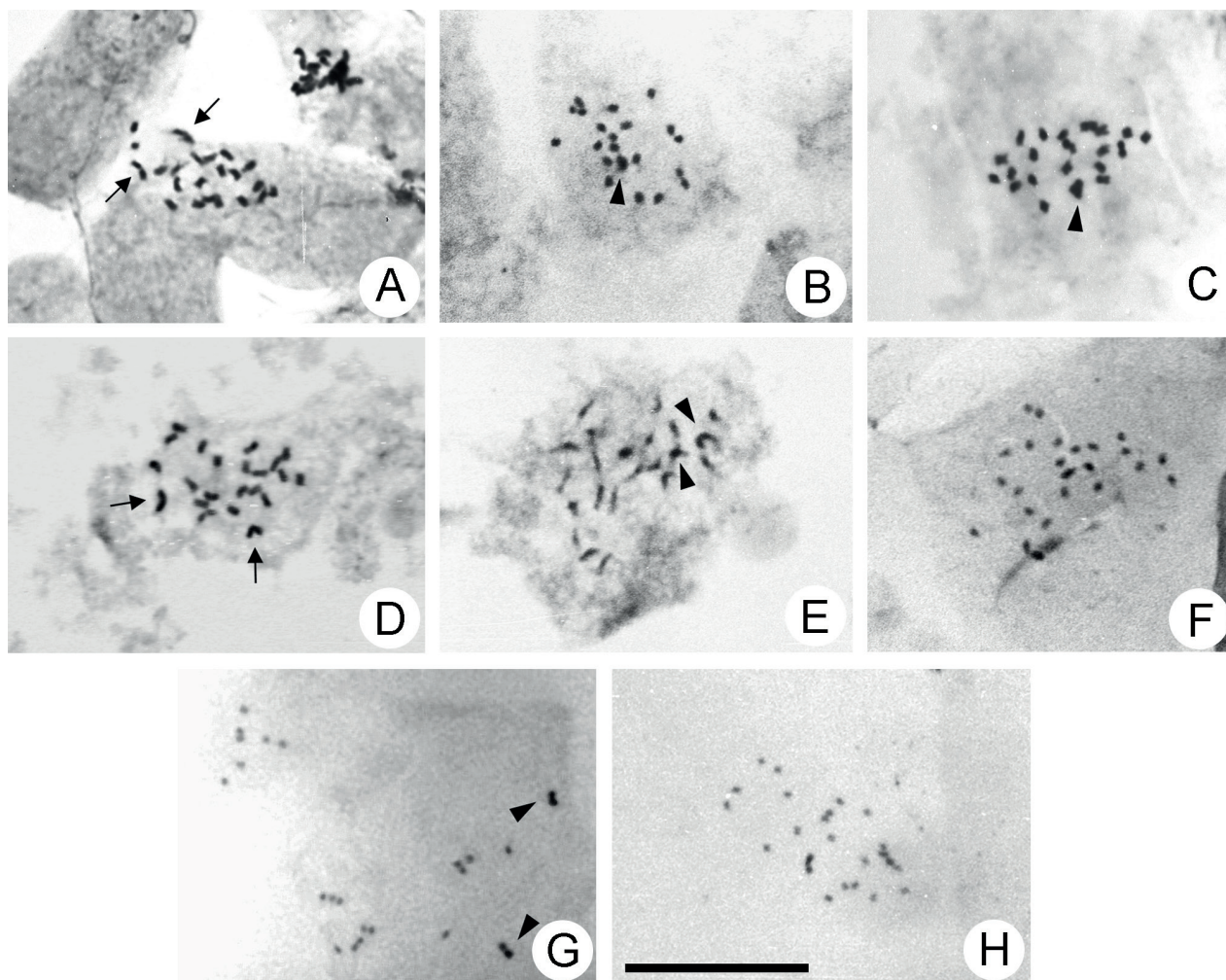
All species of *Qualea* and *Salvertia* studied were collected in cerrado. On the other hand, *Callisthene* and *Vochysia* samples were obtained from different habitats (cerrado and forest physiognomies, see Tab. 1). As both genera presented constant chromosome numbers, speciation related to the occupation of those different habitats may have occurred without change in chromosome number. These results suggest that the difference from  $x = 11$  to 12, or vice versa,

must have been a remarkable evolutionary event at the origin of the clades QRC and VS. On the other hand, the constancy of chromosome number in these clades makes this kind of evidence useless for the analysis of the generic and infrageneric divisions within the clades.

Evolutionary lineages may, or may not, be associated with variation in chromosome number. For example, Lombello & Forni-Martins (2003) found that the two subfamilies of Malpighiaceae can be differentiated by their predominant basic chromosome numbers ( $x = 5$  in Malpighioideae and  $x = 6$  in Byrsonimoideae) combined with fruit type and habitat. Differently, Mansanares *et al.* (2002; 2007a; b) found that different genera (*Lychnophora*, *Lychnophoriopsis* and *Paralychnophora*) of subtribe Lychnophorinae (Asteraceae) share various chromosome numbers ( $2n = 34, 36$  and  $38$ ), indicating that in this case, this feature has no discriminative value at generic level. So, stability in chromosome number may be a characteristic of lineages at different taxonomic



Chromosome numbers and the systematics  
of tribe Vochysieae (Vochysiaceae)



**Figure 1.** Mitotic metaphases of Vochysiaceae species. **A-D.** QRC clade,  $2n = 22$  chromosomes. **A.** *Callisthene fasciculata*. **B.** *C. major*. **C.** *Qualea cordata*. **D.** *Q. parviflora*. **E-H.** VS clade,  $2n = 24$  chromosomes. **E.** *Salvertia convallariodora*. **F.** *Vochysia haenkeana*. **G.** *V. rufa*. **H.** *V. tucanorum*. Scale bar = 10  $\mu\text{m}$ . Arrowheads point to two overlapped chromosomes, arrows point to one larger chromosome.

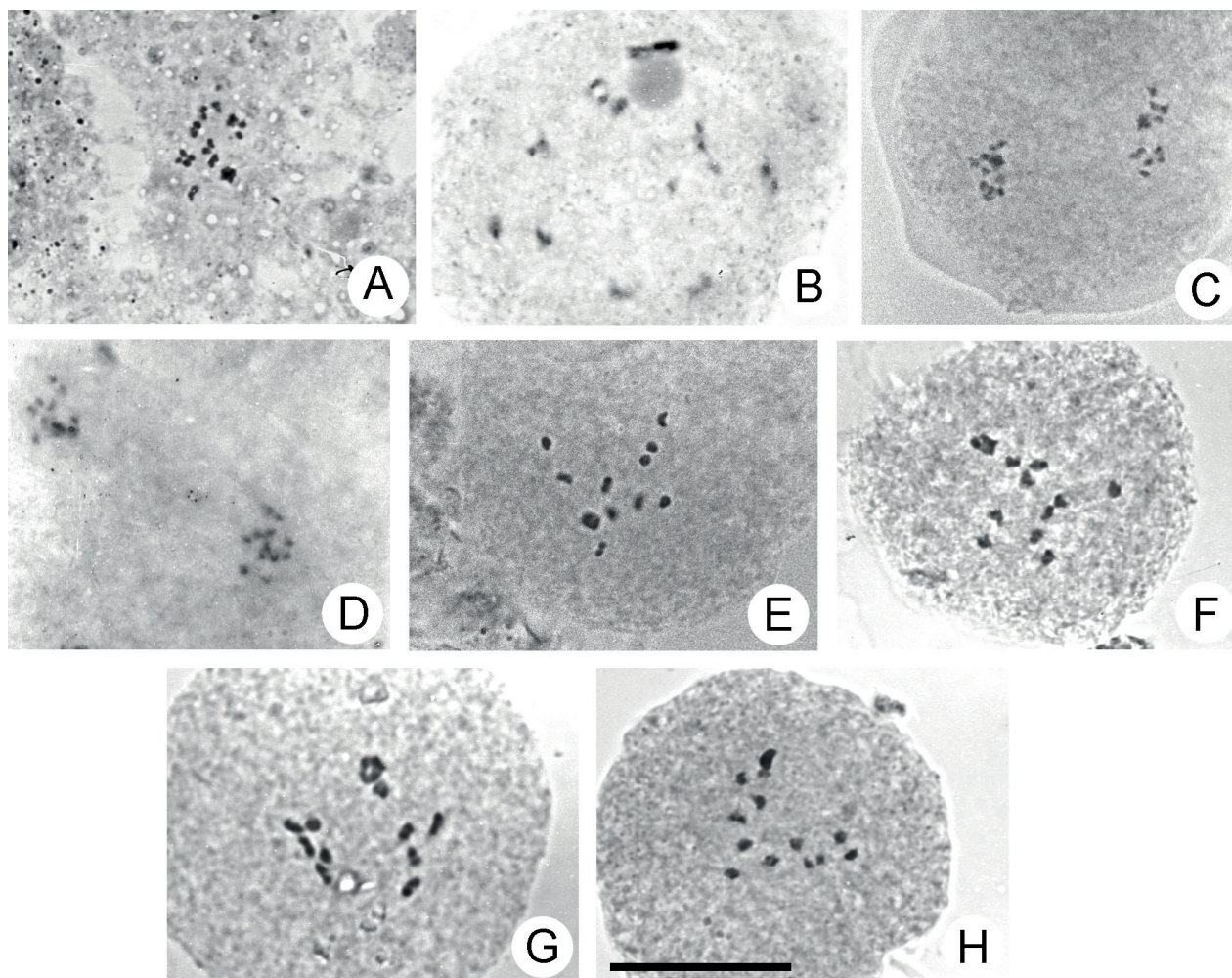
ranks. In Myrtales, it may be found in few lineages within this order, as in the clades Onagraceae/Lythraceae and Myrtaceae/Vochysiaceae, as follows.

Myrtales encompasses nine families and about 13000 species (Stevens 2001 onwards). Combretaceae is sister to the rest of this order (Berger *et al.* 2016), which comprises three main branches: the Onagraceae/Lythraceae clade, the Myrtaceae/Vochysiaceae clade, and a lineage with the clades Melastomataceae and CAP (Crypteroniaceae, Alzateaceae and Penaeaceae) (APG IV 2016). Chromosome numbers have been determined for species of most of the families of Myrtales (Rice *et al.* 2015) and are variable among and inside them: mainly  $n = 12$  and  $13$  for Combretaceae (Renard *et al.* 1983; Ohri 1996);  $n = 5, 6, 7, 8, 9, 10, 11, 14, 15$  to  $28, 32$  for Onagraceae and Lythraceae (e.g. Raven & Tai 1979; Tobe *et al.* 1986; Graham & Cavalcanti 2001);  $n = 11$  and  $12$  for Vochysiaceae (Goldblatt 1979; Löve 1987; this study);  $n = 12$  for Myrtaceae-Psiloxyloideae (Wilson *et al.* 2005); mainly  $n = 11$  for Myrtaceae-Myrtoideae (Rye 1979; Costa *et al.* 2006a; b);  $n = 7$  to  $40$ , mainly  $n=17$  and

$18$  for Melastomataceae-Melastomoideae (Solt & Wurdack 1980; Almeda & Chuang 1992);  $n = 7$  for Melastomataceae-Olisbeoideae (= Memecylaceae);  $n = 14$  for Alzateaceae (Almeda 1997); and  $n = 10$  to  $12$  for Penaeaceae (Stephens 1909; Dahlgren 1968; 1971) which also has two different records,  $n = 10$  (Goldblatt 1976) and  $n = 12$  (Takhtajan 1997), for *Olinia* (Oliniaceae, now included in Penaeaceae).

Considering that variation in chromosome number in Myrtaceae is not frequent (see Rye 1979; Costa & Forn-Martins 2006a; b; 2007; Costa *et al.* 2008), stability in chromosome number seems to be characteristic of the clade Myrtaceae/Vochysiaceae. In this clade, it is particularly noticeable that the basic chromosome numbers  $x = 11$  and  $x = 12$  separate the two subfamilies of Myrtaceae (Myrtoideae and Psiloxyloideae), as well as the clades QRC and VS in Vochysieae. Another feature shared by the members of this clade is the small size of the chromosomes. Costa & Forn-Martins (2007) have found very small chromosomes ( $< 2.0 \mu\text{m}$ ) in Myrtaceae, and we can see in Fig. 1 that Vochysiaceae also present small chromosomes (ca.  $1.0 \mu\text{m}$ ). This may be





**Figure 2.** Meiotic cells of Vochysiaceae species from VS clade, with  $n = 12$  bivalents. **A.** *Salvertia convallariodora* (metaphase I). **B.** *Vochysia elliptica* (diakinesis). **C.** *V. herbacea* (metaphase II). **D.** *V. discolor* (anaphase II). **E.** *V. schwackeana* (metaphase I). **F.** *V. acuminata* (metaphase I). **G.** *V. ferruginea* (metaphase I). **H.** *V. selloi* (metaphase I). Scale bar = 10  $\mu\text{m}$ .

a common feature in Myrtales since it has also been found in other families of this order, like Lythraceae (0.5-4.0  $\mu\text{m}$ ) (Tjio 1948; Graham 2007) and Melastomataceae (0.5-3.0  $\mu\text{m}$ ) (Solt & Wurdack 1980; Almeda & Chuang 1992).

Evolution based on variation in chromosome number seems to have occurred in different ways in the two larger families of Myrtales, Melastomataceae (ca. 170 genera and 5400 species) (Goldenberg *et al.* 2015) and Myrtaceae (ca. 142 genera and 5500 species) (Wilson 2011). The basic chromosome number in Melastomataceae varies considerably, especially in subfamily Melastomoideae, which most frequent haploid numbers have been reported to be  $n = 9$ , 12 or 17 (Solt & Wurdack 1980), while  $n = 11$  is very frequent and widespread among different groups of Myrtaceae. Sytsma *et al.* (2004) found that the rates of sequence evolution were accelerated and seemingly related to morphological and ecological diversification and changes in chromosome number in Melastomataceae, a family that comprises woody, herbaceous and even epiphytic species, from forests or savannas but sometimes from wet areas

such as lakes or stream shores as well (Renner 1993). Otherwise, rates of sequence evolution were found to be slow in Myrtaceae (Sytsma *et al.* 2004), a family comprised only of trees or shrubby species from forests and savannas.

In Myrtaceae, the sister family of Vochysiaceae, polyploidy and disploidy are common chromosome evolutionary events, the first one being mostly limited to the fleshy-fruited Myrtoideae (McVaugh 1968; Costa & Forni-Martins 2006a; b) and the latter one being more common in some dry-fruited genera (Rye 1979) like *Eucalyptus* (Matsumoto *et al.* 2000). Vochysiaceae, or at least the two clades comprised in its larger tribe Vochysieae, seems to follow a similar pattern to Myrtaceae, comprising only trees or shrubs in forests or savannas and with stability in chromosome number but, so far, with no records of polyploidy. The pattern of occurrence of two basic chromosome numbers ( $x = 11$  or 12) in Vochysieae suggests that one event of disploidy or aneuploidy occurred early in the evolution of this group. Although the sister relationships between Erismeae, QRC and VS clades are still uncertain (Litt 1999), in a scenario



## Chromosome numbers and the systematics of tribe Vochysieae (Vochysiaceae)

where Vochysieae is monophyletic (Sytsma *et al.* 2004), the change in the basic chromosome number must have contributed to the subsequent separation of the clades VS and QRC.

Further studies to verify the chromosome number in more species of Vochysiaceae are desirable, especially including Amazonian and Andean species of Vochysieae and American and African members of Erismeae. Knowing the basic chromosome number of Erismeae will add more evidences to infer the relationships among the members of the family, as well as help to unravel some aspects of the chromosome evolution in the Myrtaceae/Vochysiaceae clade.

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