Taxonomic and Cytogenetic Analysis of Species of the *Anthurium* (Araceae) Genus Native to the Brazilian Atlantic Forest

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ABSTRACT

The taxonomical description of native species is of utmost importance in terms of biodiversity survey and for checking the maintenance conditions of ecosystems. The fact that many species have ornamental features add value to their study because they will consist of important materials for production and trade, helping to reduce the predatory collection and to foster sustainable development. On the other hand, studies on mitotic and meiotic chromosomes – be they through conventional techniques, banding techniques or through techniques of molecular cytogenetics – and also studies on the pollinic morphology through optic or electron microscopy will be of great help for the taxonomical delimitation of genera which, very often, are poorly defined and comprehended.

1. INTRODUCTION

The sustainable economic exploitation of several native species works in the sense of divulging such species and preserving those at a risk of extinction. Native species with ornamental appeal such as the bromeliads (Bromeliaceae family), orchids (Orchidaceae family) and anthuriums (Araceae family) suffer from predatory extraction. These species, together with several types of indoor plants and palm trees, stand out among the most requested ones in the European and American markets of flowers and ornamental plants for being exotic tropical plants (Matsunaga 1995). Besides their contribution to taxonomy, native species that provide ornamental features may be multiplied through seedlings or *in vitro* cultivation; in addition their production and trade may become an income source for the inhabitants of the very region where the sample has been collected, therefore reducing predatory collection and contributing for increase of the family income.

Due to the complexity of their systematic principles and the morphological peculiarities of their foliages and inflorescences, the Araceae family plants have drawn the interest of many botanists. However, their morphology is quite differentiated from plant groups results in an own nomenclature rendering its comprehension difficult (Temponi *et al*. 2005).

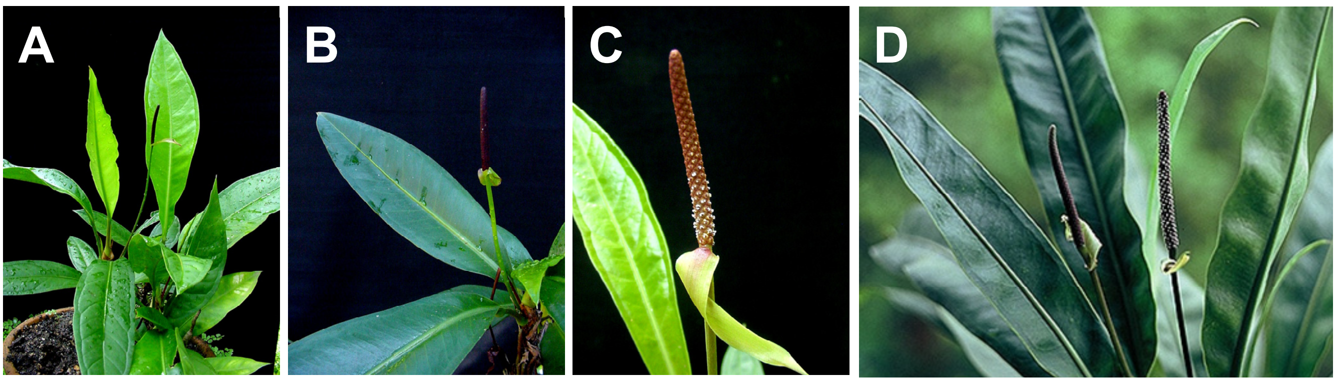

Fig. 1 (**A**) MN1442 (*Anthurium urvilleanum*) habit; (**B**) MN1536 (*Anthurium* sp.) habit; (**C, D**) MN1479 floe (*Anthurium intermedium*) – inflorescence (**C**) and foliar blade, (**D**) Jarenkow wo/no (*Anthurium gaudichaudianum*) inflorescence and foliar blade.

Among the Araceae, a large number of species belonging to the genera *Anthurium, Philodendron*, *Caladium, Monstera*, *Dieffenbachia*, *Zantedeschia*, *Syngonium,* and *Aglaonema* are grown all over the world, both in gardens and indoors, and may be found in the surrounding countryside, outdoors, composing the landscape. The vast genus *Anthurium* is the most widely known genus of this family, with species largely cultivated as ornamental plants, thanks to the beauty of their floral structure (spathe-and-spadix inflorescence) and/or leaves.

The Araceae is a large family of herbaceous monocots with 9 subfamilies (Keating 2002), 110 genera (Gonçalves 2005), and approximately 4,000 species with Lemnaceae family genera included. The majority of the Araceae genera are found in the tropical areas of Tropical and North America, Tropical, Continental and South Africa, Temperate Eurasia, Malaysia, Madagascar and the Seychelles Arquipelago (Coelho and Waechter 2004).

Anthurium Schott is the largest aroid genus, and has an estimated number of 1,100-odd neotropical species (Mayo *et al.* 1996 1997, Bown 2000, Coelho and Waechter 2004). Its distribution area comprises essentially Tropical America, being found from Mexico down to Argentina, with occurrences in the West Indies, too (Mayo 1977). The species of this genus present wide morphologic variation and are found in the wet tropical woods of low and medium elevations, but they also occur in foggy forests, in marshes, on rocky outcrops, on open sandy areas and even in semi-arid regions. As to their characteristics, these species are creeping and soil hemiphytes, epiphytes, lithophytes and, seldom, helophytes or reophytes (Mayo *et al.* 1997). In the most recent revision of the genus by Engler (1905), 486 species are divided into 18 sections. Since the date when this revision was published, a considerably larger number of species has been described (Croat and Sheffer 1983). Nowadays, the genus *Anthurium* is classified as belonging to the Pothoideae subfamily, Potheae tribe, and it is subdivided into 19 sections (Croat and Sheffer 1983, Keating 2002).

Few works have been developed with the *Anthurium* genus since the last great treatment by Engler (1905), mainly in the eastern area of Brazil where, in spite of some species having been described (Barroso 1957 1970, Catharino *et al.* 1990, Sakuragui and Mayo 1999, Coelho 2000, Mayo 1978 2000, Gonçalves 2001) and floras performed (Coelho 2000 and this manuscript), further systematic studies on this genus are still needed that, according to Croat (1991), is poorly defined and comprehended.

According to Croat (1998), future work on *Anthurium* must concentrate in two areas: the Andean countries, especially Colombia, Ecuador and Peru as well as in Brazil. An estimated number of 123 species were taxonomically determined in Brazil alone (Mayo *et al*. 1996 1997, Coelho and Waechter 2004). A total of 15 new Brazilian species of this genus were described in the last six to seven years (Sakuragui and Mayo 1999, Coelho and Mayo 2000, Mayo *et al*. 2000, Coelho and Leoni 2004, Coelho 2004).

The section *Urospadix* was described by Engler (1878) in order to gather 23 species, four out of which occur in the southeast of Brazil. This is the largest group defined by Engler, being, however, the most artificial in the genus (Croat and Sheffer 1983). The species are concentrated in the east and southeast of Brazil. The most modern classification of the *Anthurium* sections is that of Croat and Sheffer (1983) which remains until today quite similar to that of Engler (1905). The species of the section *Urospadix* are recognized for having the following characters: short internodes, sylleptic mesophylls which are most of the times decomposed, a foliar leaf blade that is generally oblong-lanceolate, lanceolate, lineal lanceolate and longer than wide, primary lateral veins which are generally numerous and evident, and sub-globous pods (Engler 1878, Croat and Sheffer 1983). The section is considered taxonomically complex with many species that are yet little known and new for science; besides, added to that, there is poor knowledge of the sites where they occur and scarce collection in these areas. Therefore, performed works add a significant contribution to the genus and to the Araceae family as a whole.

The difficulty in framing some species of the *Anthurium* genus taxonomically, mainly regarding those that till then have belonged to the "*harrisii*" complex (*A. urvilleanum* (**Fig. 1A**)*, A. harrisii, A intermedium, A. jilekii, A. simonii*) is, above all, due to its morphologic plasticity. These species are very similar, with subtle morphologic and vegetal differences giving margin to a confusing taxonomy within the group. Thus, considering the scarceness of vegetative and reproductive morphologic data for the taxonomical outline of the genus, more detailed and trustful studies are needed like those in the realm of cytotaxonomy, for example.

Okada and Hotta (1987) and Petersen (1989 1993a) reviewed the bibliography on chromosomes for the species in the *Araceae* family. This family has a pronounced variation in both number and size, as well as in the morphology of the chromosomes. The chromosome numbers range from 2n = 14 (*Ulearum*) to 2n = 168 (*Arisaema*). The size of the aroid chromosomes varies from less than 1 μm to roughly 17 μm, most genera falling in the medium range (3-6 μm). Many species have almost all of their chromosomes metacentric, and some species have acrocentric and subtelocentric chromosomes.

The assumed basic number of chromosomes ranges from x = 5 to x = 27, with most evidences pointing towards a high basic number such as x = 12, probably derived from an ancient event of polyploidization. Taking this hypothesis into account, no modern species of this family constitutes a true primary diploid (Petersen 1989 1993a). Zhonglang *et al*. (2002) have recently described a *Typhonium* species with 2n = 10 (*T. jinpingense*), that supports the basic number x = 5.

With reference to chromosome size and morphology, the general evolutionary pathway seems to have led from small, metacentric chromosomes towards large, acrocentric and telocentric chromosomes (Petersen 1989 1993a). Large chromosomes are rare in the Araceae, and the larger ones are found in the tribes Nephthytideae (Lasioideae), Colocasieae (Colocasioideae), Spathicarpeae, Zomicarpeae, and Ambrosininae (Aroideae), according to Petersen (1989).

There are several taxonomical studies but comparatively few cytogenetical records involving the genera of the Araceae family occurring in Brazil. However, extensive cytogenetical studies of this family have been carried out in North America, Europe and Japan (Ramalho 1995).

The genus with the largest number of species analyzed cytologically is *Anthurium* (193 species) followed by *Arisaema* (95 species), *Cryptocoryne* (62 species), *Philodendron* (38 species), *Amorphophallus* (37 species), and *Colocasia* (33 species), according to reviews by Okada and Hotta (1987) and Petersen (1989 1993a). Mayo *et al.* (1997) considered the *Anthurium* genus to be surprisingly uniform cytologically due to the fact that the vast majority of the species has a diploid number 2n = 30.

The current knowledge on the Atlantic Forest biodiversity, which till recently was virtually non-existent, linked to the continuous effort of performing collections and taxonomical studies on the *Anthurium* species, aiming also at their use for ornamental purposes, is the focus of the study carried out by researchers of the Botanic Garden Research Institute of Rio de Janeiro (JBRJ), Rio de Janeiro, RJ; of the Department of Zoology and Genetics of the Institute of Biology of the Federal University of Pelotas (DZG, IB/UFPel), Pelotas, RS; of the Center for Farming

Research of Mild Climate of the Brazilian Company of Farming Researches (CPACT/Embrapa), Pelotas, RS; of the Palinology Laboratory, National Museum of Rio de Janeiro, RJ and of the Botany Department, University of São Paulo, SP.

These groups aim at contributing for a larger knowledge on the *Anthurium* genus from the Atlantic Forest; solving taxonomical problems particularly of species pertaining to *Flavescentiviridia* and *Obscureviridia* subsections (Urospadix section) and providing elements for both taxonomical studies and works on genetic improvement of anthurium with ornamental potential.

The *Anthurium* Schott species are collected in various forest formations of the Atlantic Forest, kept in cultivation and deposited in the RB/JBRJ Herbarium where the classification of the species is performed. The cytogenetic study is currently being made in the Laboratory of Cellular Biology, DZG, IB/UFPel.

The material is being collected in trips to several municipalities comprising the states of Espírito Santo, Rio de Janeiro, Minas Gerais and São Paulo, in the several forest formations of the Atlantic Forest and, so far, 25 trips have been made. The material is being collected according to the techniques presented by Croat (1985) and, later on, deposited in the herbarium. At present, 170 specimens are under cultivation in greenhouses at the JBRJ. So far, 1,340 exsiccate, representing nine states of Brazil (Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Minas Gerais, Rio de Janeiro, Espírito Santo, Bahia e Sergipe) have been analyzed and deposited in several Brazilian and foreign herbaria.

In most of the collections, latitude, longitude and altitude registers of the specimens are obtained by utilizing the "Global Position System (GPS)" – Garmin 12.

In order to photograph the habit and vegetative and inflorescence details, a Sony-Mavica FD92 digital camera with the highest resolution has been utilized.

We have been using a conventional technique in our cytogenetic analyses, as described by Guerra and Souza (2002) and Singh (2002). We have taken this to be the paradigmatic technique for analysis, and then all the other techniques, such as NOR, C/Giemsa, C/DAPI, and C/CMA$_3$ banding techniques, and the molecular cytogenetic techniques FISH and GISH, will be applied in well prepared slides and will be compared to those first results obtained through the conventional technique.

Young roots are collected from plants maintained in glass jars containing water or in pots with organic soil. To ensure adequate metaphases, the roots are pre-treated with 8-hydroxyquinoline (8-HQ), fixed in 3:1 v/v (ethyl alcohol: acetic acid) and stored in a freezer up to the moment they are used. For slide preparation, the root tips are hydrolyzed in 5N HCl and this hydrolysis is followed by an incubation of the apical meristem in an enzyme solution (cellulase and pectinase). Later the meristem is squashed in a drop of 45% acetic acid. The slides are made permanent by immersion in liquid N_2, air-dried and stained with Giemsa solution.

The chromosomes of the metaphase cells that show better spreading and contraction are counted and analyzed for number and morphology characterization. The metaphase plates are photographed with colour film (ASA 100) at 1,000 X in an optic microscope Olympus BX 51.

In general, the chromosomes of the species of the *Anthurium* genus measure about 3 to 6 μm, a position among the mean chromosome sizes found in the genera of the Araceae family. For description purposes of the anthurium karyotypes, we have considered as large chromosomes those with approximately 6 μm of total length and as small ones those with more or less 3 μm. Those with an intermediary length were considered as medium chromosomes. Another karyotypical description refers to the difference between the size of the larger and smaller chromosomes. When this difference is large, the karyotype is considered bimodal or asymmetric; when the variation is gradual and not much difference in size exists among the chromosomes of the karyotype, it is considered symmetric. The chromosome morphology is described according to Guerra (1986) into metacentric (m), submetacentric (sm), acrocentric (a) and telocentric (tc) chromosomes when the ratio between the chromosome arms (long arm/short arm) varies from 1,00 to 1,49; from 1,50 to 2,99; from 3,00 to ∞; and ∞, respectively.

1.1. *Anthurium urvilleanum* Schott

The *Anthurium urvilleanum* Schott species is native from the Atlantic Forest biome, spreading from Santa Catarina up to Rio de Janeiro with soil, rupicolous and, more rarely, hemiepiphyte habitats. It features folial leaf blade with sharp base to long wedge-shaped and pods which are vinaceous in the apex to greenish or hyaline to the base or, rarely, totally vinaceous. According to Coelho *et al.* (2004), the species has a population in the south of the state of Rio de Janeiro and another in the coastal lowlands of this state (Municipality of Silva Jardim) while their vegetative morphological and reproductive characteristics were not consistent enough in order to separate the species of these two groups.

1.1.1. M. Nadruz 1541 Collection

Collected in the municipality of Parati**,** RJ, it has a diploid chromosome number of 2n = 2x = 30 (**Fig. 2A**). Two pairs of large metacentric chromosomes occur while the remaining ones are smaller. These smaller chromosomes are relatively uniform in size, appearing as metacentric (1 pair), submetacentric (10 pairs) and acrocentric (2 pairs) while one of the pairs of acrocentric chromosomes has satellites, thus forming a symmetric karyotype. The karyotype formula is 6 m + 20 sm + 4 a. This species presents an interesting feature in some cells which is the occurrence of heteromorphism of the nucleolar organizer region (NOR) located right before the satellite of the pair of satellited chromosomes, that is, one of them has a relatively bigger NOR than that of its homologous pair.

1.1.2. M. Nadruz 1543 Collection

Collected in the municipality of Parati, RJ**,** it has cells with 30, 31 and 32 chromosomes. This numerical difference found among the cells of one sole subject is probably due to the absence or presence of one or two accessory chromosomes, the B-chromosomes. Therefore, this species has 2n = 2x = 30 + 0-2 B as diploid number of chromosomes. Its karyotype is symmetric comprising 2 pairs of large metacentric chromosomes, 1 pair of medium metacentric chromosomes, 5 pairs of medium submetacentric chromosomes, 7 pairs of medium acrocentric chromosomes two out of them have satellites. In the cells with 31 and 32 chromosomes, 1 or 2 small acrocentric chromosomes are respectively found. As these small acrocentric chromosomes are much smaller than the smaller ones of the group of 30 chromosomes, it is claimed that these might be accessory chromosomes of the B-type chromosomes. Confirmation of the nature of these quite small chromosomes will only be able to be cleared up by a study of the meiosis or by a banding technique. The basic karyotypical formula of this species is 6 m + 10 sm + 14 a.

1.1.3. M. Nadruz 1394 Collection

Collected in the municipality of Parati, RJ, it has cells with 60, 61 or 62 chromosomes. This numerical difference among the cells of a sole sample is probably due to the absence or presence of 1 or 2 accessory chromosomes of the B-type, respectively. Thus, it can be said that this species has a diploid chromosome number of 2n = 4x = 60 + 0-2 B. The karyotype is symmetric comprising 4 pairs of large metacentric chromosomes, 4 pairs of medium metacentric chromosomes, 11 pairs of medium submetacentric chromosomes and 11 pairs of medium acrocentric chromosomes. The basic karyotype formula is 16 m + 22 sm + 22 a. The presence of a satellite is noticed in two pairs of acrocentric chromosomes that present a NOR that is quite elongated. One of the pairs of large metacentric chromosomes has a secondary constriction in its long arm.

1.1.4. M. Nadruz 1442 Collection

Collected in the municipality of Silva Jardim, RJ, it has 2n = 4x = 60. Out of these 60 chromosomes, 4 pairs are large metacentric chromosomes while two of them are pairs of satellite medium acrocentric ones. The remaining chromosomes are very similar in size with morphology difficult to distinguish but among them medium chromosomes of metacentric, submetacentric and acrocentric morphology are found. This genotype is being studied again, inclusive with banding techniques for better morphological definition of these chromosomes.

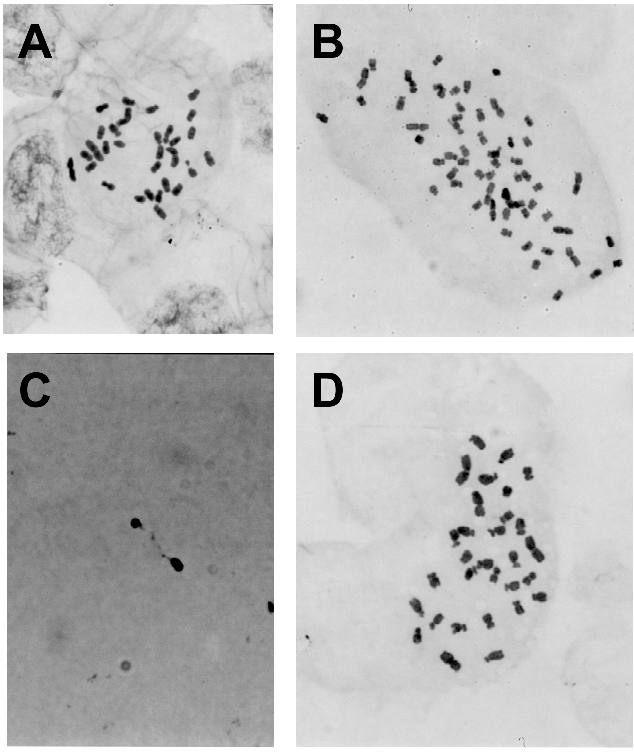

Fig. 2 (**A**) MN1541, 2n = 2x = 30 chromosomes; (**B**) MN1374, 2n = 4x = 60 chromosomes; (**C**) satellite chromosome with long and loosely constriction, (**D**) *Anthurium gaudichaudianum* 2n = 2x = 30.

The M. Nadruz 1541 and 1543 specimens have been classified as of the same species taking into consideration the similar vegetative and reproductive morphological characters with occurrence in the same area and vegetal formation. The authors consider this species as *A. urvilleanum*, not only for the characteristics found in the main work by Schott (1860) but also for the location type in the state of Santa Catarina, confirming the distribution of the species from the south up to the southeast of Brazil limited in the north by the metropolitan area of the state of Rio de Janeiro. However, in spite of the basic diploid chromosome number being equal (2n = 2x = 30), it has been found that the karyotype formulas are not identical and that the M. Nadruz 1543 collection has two additional chromosomes, which are probably B accessory chromosomes.

Regarding the M. Nadruz 1394 and 1442 specimens, both with 2n = 4x = 60, Coelho *et al*. (2004) consider them similar to the M. Nadruz 1541 and 1543 specimens (*A. urvilleanum*). However, in view of the presented cytogenetic results, one proposes a more accurate re-evaluation of the vegetative and reproductive morphological characteristics, and of the cytogenetics characterization in order to outline the taxonomical position of these subjects more precisely.

1.2. *Anthurim harrisii* (Graham) G.Don

The *Anthurium harrisii* species is native to the Atlantic Forest biome, endemic from the state of Rio de Janeiro and grows within salty marsh vegetation and on rocky coasts close to the sea. It features a base that is generally obtuse to nearly heart-shaped in the folial leaf blade and the presence of carinas on the petiole adaxial face and of greenish pods (Coelho *et al*. 2004).

1.2.1. Araújo A wo/no. Collection

Collected in the municipality of Búzios, RJ, it has 2n = 2x = 30 chromosomes. Two metacentric chromosome pairs are relatively large, 9 pairs are medium submetacentric chromosomes, 3 pairs are medium acrocentric chromosomes one out of which has satellites and 1 pair is of small acrocentric chromosomes. Their karyotype is symmetric and features karyotypic formula: 4 m + 18 sm + 8 a.

1.2.2. M. Nadruz 1426 Collection

Collected in the municipality of Carapebus, RJ, it has the somatic chromosome number 2n = 4x = 60. It comprises 4 pairs of relatively large metacentric chromosomes, 5 pairs of medium metacentric chromosomes, 16 pairs of medium submetacentric chromosomes and 5 pairs of medium acrocentric chromosomes. Two pairs of acrocentric chromosomes have satellites with relatively long NOR. It is a symmetric karyotype whose karyotypical formula is 18 m + 32 sm + 10 a.

1.2.3. Marquete 3034 Collection

Collected in the municipality of Rio das Ostras, RJ, it has 30 chromosomes (2n = 2x = 30) comprising two pairs of large metacentric chromosomes, one pair of small metacentric chromosomes, one pair of small acrocentric chromosomes with satellite, having the remaining 11 pairs of chromosomes with submetacentric or acrocentric morphology.

The Araújo A wo/no. and M. Nadruz 1426 species have been determined as *A. harrisii* by Coelho *et al*. (2004). However, they were collected at different localities and as they have different chromosome numbers (2n = 2x = 30 and 2n = 4x = 60, respectively), even with the same basic characteristics of the G.Don species, one draws the conclusion that they are probably considered distinct species or species under process of speciation or species pertaining to a euploid series, like *A. gracile* and *A. scandens*, new studies being needed in order to determine each of their correct taxonomic position.

Marquete 3034 and Araújo A wo/no. have the same chromosome number (2n = 2x = 30) but not the same chromosome feature. They occur in the same habitat and in close locations featuring the same morphological characteristics of G.Don's description. However, in order to draw a conclusion if they belong to the *A. harrisii species*, deeper cytogenetic studies will be needed.

Marchant (1974) also analyzed the *A. harrisii* species (Grahn) G. Don whose exsiccate is deposited under number 6876.1 at the Herbarium of the Royal Botanic Garden of Kew, London. This author found the diploid number 2n = 2x = 30 + 5 fragments, but looking for the photo presented in the paper, these fragments to be satellites or very small B chromosomes, more rarely. However, in 1977, Bhattacharya made a different description for this species: 28 chromosomes plus two B super numerical chromosomes. The result found by this author sums up 30 chromosomes, so probably the two smaller chromosomes have been confused for B chromosomes.

1.3. *Anthurium intermedium* Kunth

Anthurium intermedium is one of the species that Coelho *et al*. (2004) consider as possessing the wider geographic range among the species of the *Flavescentiviridia* subsection, occurring from the farthest southern area of Bahia, in the central regions, from the north coast and south of Espírito Santo, south, woods zone and metropolitan area of Belo Horizonte in Minas Gerais, north and central Paraíba, mountains, metropolitan area, south and one single register in the coastal lowlands of Rio de Janeiro and Paraíba valley, south coast and macro metropolitan area of São Paulo. It has, in general, soil habits and, occasionally, saxicole, rupicolous and hemiphyte habits, being generally esciophyle, more rarely, semi-esciophyle and semihelyophile in the forests of lowlands, submontana and montana and, more rarely, of altitude up to approximately 1,600 m.

1.3.1. M. Nadruz 1479 collection (Fig. 1C)

Collected in the municipality of Caparaó, MG, it has 2n = 2x = 30 chromosomes. They comprise two pairs of relatively large metacentric chromosomes, three pairs of medium metacentric chromosomes, eight pairs of medium submetacentric chromosomes, two pairs of small acrocentric chromosomes one out of which has satellites. It is a symmetric karyotype whose karyotype formula is equal to 10 m + 16 sm + 4 a.

1.3.2. M. Nadruz 1374 collection

Collected in the municipality of Rio de Janeiro, RJ, it presents 2n = 4x = 60 in a symmetric karyotype (**Fig. 2B**). This collection comprises four pairs of large metacentric chromosomes, five pairs of medium metacentric chromosomes, 12 pairs of medium submetacentric chromosomes and nine pairs of acrocentric chromosomes two pairs out of which have satellites (**Fig. 2C**). The karyotype formula is 18 m + 24 sm + 18 a.

1.3.3. Silva wo/no. collection

Collected in the municipality of Magé, RJ, it has 2n = 4x = 60 + 1 B. This specimen presents four pairs of large metacentric chromosomes, five pairs of medium metacentric chromosomes, 11 pairs of medium submetacentric chromosomes and 10 pairs of medium acrocentric chromosomes plus one extra submetacentric chromosome. Two pairs of acrocentric chromosomes have satellites, one of them with a long satellite and a long NOR area. It has a symmetric karyotype with basic karyotype formula 18 m + 22 sm + 20 a.

1.3.4. M. Nadruz 1451 collection

Collected in Itatiaia, RJ, it has 2n = 4x = 60 chromosomes. It features a symmetric karyotype with the presence of one pair of large metacentric chromosomes, three pairs of relatively large metacentric chromosomes and the remaining chromosomes are medium-sized with metacentric, submetacentric and acrocentric morphology. In some cells, there is the presence of chromatinic mass linked to an acrocentric chromosome resembling a very big satellite.

The M. Nadruz 1374 and Silva wo/no. specimens have a close geographic distribution, occurring in the same habitat. Adding up the similarities in the chromosome number to the characteristics presented in the original description by Kunth, these species may be considered as *A. intermedium*.

According to the chromosome differences demonstrated above and the distinct geographic positions of the M. Nadruz 1479 and 1451 subjects, one may suggest that they are distinct species of *A. intermedium*. Consequently, there is the probability that these species be considered as new species. However, this fact will only be confirmed by a deeper cytogenetic and taxonomical study on more subjects from these populations.

Gaiser (1927) had already counted the haploid number of chromosomes of one subject, at the time identified erroneously as *A. harrisii* var *beyrichianum* (Kunth) Engler (at present, a synonym for *A. intermedium*, as per Coelho and Mayo 2004), having found a haploid number n = 15 what coincides with a diploid somatic number of 30 chromosomes of the species M. Nadruz 1479. This author do not described neither the presence of extra-numerary chromosomes, nor the presence of long and loosely NOR.

1.4. *Anthurium parasiticum* (Vell.) Stellfeld

Anthurium parasiticum is very frequent in the Atlantic Forest in most of the vegetation types. It is recognized mainly for the cataphylls and prophylls, generally entire on the apex and slightly decomposed and straw-colored towards the stem base, for the folial leaf blade generally elliptical-lanceolate to lanceolate with the obtuse to fragmented base and for the roundish petiole axially below and grooved with obtuse rims adaxially. It is often confused with *A. intermedium* Kunth, being however different because this one presents cataphylls and prophylls generally deciduous towards the stem base, lanceolate folial leaf blade with sharp to long wedge-shaped base, roundish to carinated petiole axially below and plain with carinated rims adaxially. One re-organization is presented in article I, in Coelho and Mayo (2004), regarding the taxons of the "*olfersianum*" complex, formed by five varieties as per Engler'treatment (1905).

1.4.1. Araújo B wo/no

Collected in the municipality of Macaé, RJ, it has 2n = 4x = 60, 61e 62 chromosomes.This anthurium features four pairs of large metacentric chromosomes, five pairs of medium metacentric chromosomes, 10 pairs of medium submetacentric chromosomes and 11 pairs of medium acrocentric chromosomes two pairs out of which have satellites plus one metacentric or submetacentric chromosome of similar size to the smaller chromosomes of the karyotype that can be a B chromosome. It has a symmetric karyotype with basic formula 18 m + 20 sm + 22 a.

1.5. *Anthurium* sp.

This species occurs in native formations in the woods of the lowlands of the state of Espírito Santo. It has a soil habit and grows in sites that are relatively shady.

1.5.1. M. Nadruz 1536 Collection

Collected in the municipality of Linhares, ES, it has 2n = 4x = 60, 61 and 62 chromosomes. It presents a symmetric karyotype with medium chromosomes of difficult morphological characterization but it can be noticed among them the occurrence of metacentric, submetacentric and acrocentric chromosomes. One of the pairs of acrocentric chromosomes has satellites. It also presents four pairs of relatively large metacentric chromosomes.

In spite of the difficulty of interpreting the morphological characterization of the chromosomes, the M. Nadruz 1536 (**Fig. 1B**) species has a karyotype with 60 chromosomes plus B-chromosomes like *A. intermedium* (vide Silva wo/no.), however it differs in the vegetative morphology and in the geographic distribution and can be considered a new species.

1.6. *Anthurium gaudichaudianum* Kunth

It is an epyphytic species of shady and wet locations present in the states of Paraná, Santa Catarina, Rio Grande do Sul and São Paulo occurring in woods of low and high hillsides and in rupestrian fields. It can be recognized for the cartaceae folial leaf blades, presence of long entire cataphylls and stipe (Coelho *et al*. 2006).

1.6.1. Jarenkow wo/no. Collection

Collected in the municipality of Torres, RS, it presents 2n = 2x = 30 (**Fig. 2D**). This species has 2 pairs of larger metacentric chromosomes, 1 pair of smaller metacentric chromosomes, 3 pairs of acrocentric chromosomes, and 9 submetacentric chromosomes pairs, who are almost of the same size. Satellites have been visualized in one pair of acrocentric chromosomes. The karyotype formula is 6 m + 18 sm + 6 a.

It is evident that the techniques of chromosome banding and of molecular cytogenetics can respond better to the questions of interspecific taxonomical differentiation. There is a great distribution of both diploid and polyploid species among plants that can present a large or very subtle phenotype variation among the different ploidy. In the case of minimum variations, if the occurrence of cryptic species is noticed, such findings hinder the recognition of the different polyploids at a taxonomical level. In most of these cases, conventional cytogenetics can lead to good answers, mainly when polyploids of different levels are involved, as is the case of polyploid complexes.

Conventional cytogenetics brings information such as number, size and chromosome morphology, presence of chromosomes with satellites and length of the NOR area, making it possible to formulate the karyotype of a certain species. However, the different degrees of condensation where the chromosomes are found in the metaphasic plates make it difficult to obtain satisfactory results. It is important to remember, as Stace (2000) points out, that the conventional technique does not succeed in differentiating a great part of the genomes due to the fact that in certain genomes there is the occurrence of some if not of all chromosomes with much similarity both in size as in morphology. It is also worth remembering that good metaphase plates that present well spread chromosomes and chromosomes in an adequate condensation degree are not always easy to obtain in some plant families.

The difficulty in obtaining good metaphase plates from some genera of the Araceae family poses a problem to chromosome counting, as highlighted by Petersen (1989 1993b) in a study of the genus *Scindapsus*. Moreover, in 1993, Marutani *et al.* studying *Anthurium andraeanum* cv. 'Kaumana,' pointed out that the stickiness of the chromosomes raised difficulties in the meiotic configuration analysis. Ramalho (1995) emphasized the difficulty in obtaining a good spread of the chromosomes of Araceae species, *Monstera adansonii*, *Aglaonema commutatum,* and *Anthurium scandens*. The chromosomes are sticky and tend to cluster. We obtain better results for *Anthurium* using the enzyme solution. A good metaphase plate is an essential condition for obtaining good results not only at the banding and molecular techniques but also at the conventional technique.

Within the Araceae family, acrocentric and telocentric chromosomes seem to be confined to the genera with medium-sized or large chromosomes, which are usually considered advanced, while the small and metacentric chromosomes (appr. 1-3 μm) are characteristic (typical) of species that are classified in basal positions in the phylogeny (Petersen, 1993a). The *Anthurium* species may be classified in an intermediary position based on their medium to small chromosomes and by the presence of chromosomes with acrocentric morphology.

The diploid complement of chromosomes found in some specimens of *Anthurium* are in accordance with the findings for most of the species in this extensive genus. The diploid number 2n = 2x = 30 was also found in other species of *Anthurium* that had their karyotypes described or reviewed after Sheffer and Croat's 1983 review (see **Table 1**). Furthermore, the size of the chromosomes is similar to that of other species of the genus, like *A. warocqueanum*, whose metacentric and submetacentric large chromosomes are nearly 6.0 μm, and the small and medium chromosomes range from 2.8 to 4.0 μm (Kaneko and Kamemoto 1979) and, according to **Table 1**, the size of the anthuriums' chromosomes range from 1.6 μm to 6.71 μm.

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There are satellites in one or two pairs of *Anthurium* chromosomes, in diploid and tetraploid species respectively, but they are not visualized in some metaphase plates – particularly those with extremely condensed chromosomes. In cells with a lesser condensation of the chromosomes, the satellites may resemble supernumerary B-chromosomes, due to its loose attachment to the chromosome caused by a long and loosely NOR.

Table 1 Species of *Anthurium* whose chromosome number and/or karyotype was described or reviewed after Sheffer and Croat (1983), assuming x = 15.

Species	2n	Karyotype	Chromosomes			Author/Year
			Satellites	B	Size	
A. warocqueanum (*Aw*)	30	4 m/sm + 26 sm	-	3	2,8 – 6,0 µm	Marutani and Kamemoto 1983
(selfed offspring of *Aw*)	30	4 m/sm + 26 sm	-	0-6	4 m/sm L + 26 sm/a M	
A. gracile	40	-	1 pair	-	-	Guerra 1986b
A. scherzerianum	14, 28	-	-	-	1,6 – 3,8 µm	Subramanian and Munian 1988
	44	18 m + 16 sm + 10 a	-	-		
A. amnicola	30	4 m + 8 a + 18 m/a	1 pair	-	4 m L; 6 a FL; 20 m/a S	Marutani *et al.* 1988
A. beyrichianum	30	-	-	-	-	Okada and Hotta 1987
A. chelseiense	30	-	-	-	-	
A. concinnatum	30	-	-	-	-	
A. condobrium	30	-	-	-	-	
A. ferrierense	30	-	-	-	-	
A. flavo-viride	30	-	-	-	-	
A. froebelli	30	-	-	-	-	
A. gloriosum	30	-	-	-	-	
A.grusonii	30	-	-	-	-	
A. motafrontenense	30	-	-	-	-	
A. roseum	30	-	-	-	-	
A. venosum	30	-	-	-	-	
A. calamus	40	-	-	-	-	
A. acussatum	30	-	-	-	-	Petersen 1989
A. clidemioides	30	-	-	-	2 – 4 µm	
A. gymnopus	30	-	-	-	3 – 4 µm	
A. affine	30	symmetric	1 pair; large	-	uniform	Carvalheira *et al.* 1991
A ranchoanum	44	-	-	-	-	Sheffer 1991
A. arenicola	aneuploid at tetraploid level			-		
A. andraeanum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	Marutani *et al.* 1993
A. caperatum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. formosum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. kamemotoanum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. lindeneanum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. roseospadix	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. subsignatum	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. cf. sanctifidense	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
Anthurium sp.	30	4 m/sm + 2 a + 24 ?	1 pair	-	4 sm/m L; 2 a FL; 24 S	
A. gargaranum	30	4 m/sm + 2 a + 24 ?	1 pair	1	4 sm/m L; 2 a FL; 24 S	
A. ochranthum	30	6 m/sm + 24 ?	1 pair	2	4 sm/m L; 2 m FL; 24 S	
A. nymphaeifolium	30	2 m + 2 a + 26 ?	1 pair	-	2 m L; 2 a L; 26 S	
A affine	30	30 sm/m	1 pair	-	Small variation	Ramalho 1995
A. cf. parasiticum	30	30 m/sm/a	-	-	-	
A. petrophylum	30	30 m/sm	1 pair	-	-	
A. gracile	40	40 m/sm/a	-	-	uniform	
A. scandens ssp scandens	48	48 m/sm	1 pair	-	-	
A. pentaphyllum var. *pentaphyllum*	60	60 m/sm	3 pairs FL	-	uniform	
A. affine	30	sm: the greater number	-	1-2	2,51 – 6,71 µm	Oliveira *et al.* 1995
A. longipes	30	sm: the greater number	-	-	2,51 – 6,71 µm	
A. pentaphyllum var. *pentaphyllum*	30	sm: the greater number	-	-	2,51 – 6,71 µm	
A. bellum	90	sm: the greater number	-	-	2,51 – 6,71 µm	
A. pentaphyllum var. *pentaphyllum*	30, 60	-	-	-	-	Guerra and Mayo 1996
Anthurium sp.	30	-	-	-	-	Silva and Guerra 1997
A. pentaphyllum var. *pentaphyllum*	30, 60	-	-	-	-	
A. pageanum	30	-	-	-	-	Croat and Sheffer 1998
A. pittiori	30	-	-	-	-	
A. terryae	30	-	-	-	-	
A. utleyi	30	-	-	-	-	
A. signatum	30, 34	-	-	-	-	
A. gracile	20, 40, 60	-	-	-	-	
A. solitarium	60	-	-	yes	-	
A. affine	30	16 m + 14 sm + 2 ?	1 pair	1-4	-	Cotias-de-Oliveira 1999
A. pentaphyllum var. pentaphyllum	30	-	1 pair	-	-	
	60	-	2 pairs	-	-	
A. bellum	90	-	2 pairs	-	-	

m = metacentric; sm = submetacentric; a = acrocentric; L = large; FL = fairly large; M = medium; S = small

Different chromosome numbers for the same species or even in the same plant, like the counting of 2n = 30 and 2n = 32 reported for *A. scherzerianum* and *A. andraeanum* (see reviews of Sheffer and Croat 1983, Okada and Hotta 1987, Petersen 1989), may be due to the presence of either large and loosely attached satellites or small B chromosomes. Sheffer and Kamemoto (1976) and Sheffer and Croat (1983) emphasize that these satellites could be mistaken for small chromosomes. B-chromosomes occur in 12% of the Araceae species, as reported by Sheffer and Croat (1983). Satellites and/or B-chromosomes are also reported in articles published after their extensive review (see **Table 1**).

Two pairs of chromosomes (metacentric or submetacentric), larger than the rest of the karyotype, can be verified in some *Anthurium* species, such as *A. andraeanum*, *A. crystallinum*, and *A. warocqueanum* (Kaneko and Kamemoto 1978 1979, Ali 1979, Marutani and Kamemoto 1983).

According to **Table 1**, still other *Anthurium* species present these 4 larger-sized chromosomes: *A. amnicola*, as studied by Marutani *et al*. (1988), and 11 other species analyzed by Marutani *et al*. (1993). Exceptions are *A. ochranthum,* whose one fairly large chromosome is metacentric – instead of acrocentric like the rest, and *A. nymphaeifolium*, that presents one acrocentric pair of large chromosomes. Marutani *et al*. (1993) concluded that the karyotypes of these 2 species originated from rearrangements of the typical/standard karyotype they found in the other 10 species they studied: 4 large metacentric or submetacentric chromosomes, 2 fairly large acrocentric chromosomes, and 24 smaller chromosomes, who include the 2 satellite chromosomes. According to these authors, a karyotype obtained through conventional techniques does not differentiate between sections of the genus, but could draw a distinction between some species within the sections, and the same is true for the different ploidies that are found in some species.

In the genus *Anthurium*, there are four euploid series based on the basic numbers x = 10, 12, 14, and 15. Since it was the most common to occur, x = 15 was regarded to be ancestral for the genus by Gaiser (1927) and Marchant (1973). However, Sheffer and Kamemoto (1976), Sheffer and Croat (1983), Grayum (1990), Petersen (1993a) postulated x = 12 as ancestral, supported by cytological and non-cytological evidences that put the species of the section *Tetraspermium* in a phylogenetic basal position within the genus.

Sheffer and Croat (1983) determined the chromosome number of 86 species of *Anthurium,* describing the chromosome number of 51 species for the first time. According to their analysis and review, the chromosome numbers found in a total of 137 species of the *Anthurium* genera ranged from 2n = 24 to 2n = 90, with 2n = 30 being the most common number. In this total number of species (137) we do not include 4 subspecies, 4 varieties and 3 hybrids. The results presented in that review stated that the genus *Anthurium* has 74.4% of its species with 2n = 2x = 30; 9.5% with 2n = 2x = 30 and 2n = 2x = ≠30 in the same species (probably most of the numbers that differ from 30 are miscountings due to the presence of B chromosomes); 4.4% with 2n = 4x = 60; 4.4% with 2n = 2x = 30 and 2n = 4x = 60 in the same species; 0.7% with 2n = 6x = 90 (*A. supianum*); and 6.6% with various chromosome numbers (varying from 2n = 24 to 2n = 34, plus a count of 2n = ca.124 for *A. lucidum* carried out by Marchant in 1973, though not yet confirmed by other researchers). According to the data presented, *A. gracile* with 2n = 30, 40, 60; *A. scandens* with 2n = 24, 28, 48; and *A. imperiale, A. wallisii, A. microspadix, A. digitatum, A. pentaphyllum,* and *A. hookeri* with 2n = 30, 60 form euploid series.

Other researchers have carried out very extensive reviews that confirm 2n = 2x = 30 to be the most common chromosome number, having studied new and roughly the same species listed by Sheffer and Croat, in 1983. Okada and Hotta (1987) published a list with the Aracea's chromosome numbers and reported another 13 *Anthurium* species with 2n = 2x = 30, and only 1 species (*A. calamus*) with a different number (see **Table 1**). Petersen (1989) described the chromosome number of 2 species for the first time, and included another one that had not been listed, neither by Sheffer and Croat (1983), nor by Okada and Hotta (1987), all of them with 2n = 2x = 30 (**Table 1**). The 2n = 30 number was also found in 20 other species of *Anthurium* (**Table 1**) described after 1983 by Carvalheira *et al*. (1991), Marutani *et al.* (1993), Ramalho (1995), Guerra and Mayo (1996), Croat and Sheffer (1998), Cotias-de-Oliveira (1999).

The size and morphology of the *Anthurium* chromosomes is similar in various species of the genus, like *A. warocqueanum* (Kaneko and Kamemoto 1979), with 2n = 2x = 30, whose metacentric and submetacentric large chromosomes have nearly 6.0 μm, and the small and medium chromosomes range from 2.8 to 4.0 μm. The size of the anthurium's chromosomes range from 1.6 μm to 6.71 μm and the morphology are metacentric, submetacentric and acrocentric.

The karyotypes of the native species of anthurium from the Atlantic Forest, Brazil, in general, follow the trend found in great part of the species of the *Anthurium* genus already described cytogenetically. Thus, a symmetric karyotype is seen with the number 2n = 2x = 30 or 2n = 4x = 60; medium-sized chromosomes - between approx. 6 and 3 μm; presence of 2 and 4 pairs of metacentric chromosomes that are larger than the other ones - 2 pairs in the cells with 30 chromosomes and 4 pairs in the cells with 60 chromosomes; occurrence of small differences in size among the remaining chromosomes that have metacentric, submetacentric and/or acrocentric morphology; presence of satellites in 1 pair of medium or small chromosome in the 2n=30 and in 2 pairs in the 2n = 60, with elongated NOR; presence of B chromosomes and/or quite large satellites.

The doubts that have arisen as to the taxonomical classification of the Brazilian native species and to the karyotype of these species will be cleared up, as said previously, by the study of a larger number of subjects of the populations of anthurium where the collections have been made. Further cytogenetic studies will also be needed mainly those that will involve the several banding and molecular cytogenetic techniques.

2. CONCLUSION

The karyotypes of the native species of anthurium from the Atlantic Forest, Brazil, in general, follow the trend found in great part of the species of the *Anthurium* genus already described cytogenetically. Thus, a symmetric karyotype is seen with the number 2n = 2x = 30 or 2n = 4x = 60; medium-sized chromosomes - between appr. 6 and 3 μm; presence of 2 and 4 pairs of metacentric chromosomes that are larger than the other ones - 2 pairs in the cells with 30 chromosomes and 4 pairs in the cells with 60 chromosomes; occurrence of small differences in size among the remaining chromosomes that have metacentric, submetacentric and/or acrocentric morphology; presence of satellites in 1 pair of medium or small chromosome in the 2n=30 and in 2 pairs in the 2n = 60, with elongated NOR; presence of B chromosomes and/or quite large satellites.

The doubts that have arisen as to the taxonomical classification of the Brazilian native species and to the karyotype of these species will be cleared up, as said previously, by the study of a larger number of subjects of the populations of anthurium where the collections have been made. Further cytogenetic studies will also be needed mainly those that will involve the several banding and molecular cytogenetic techniques.

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