First karyological report in Larnax and Deprea (Solanaceae)

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First karyological report in *Larnax* and *Deprea* (Solanaceae)

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Short title: First karyological report in *Larnax* and *Deprea*

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The study of chromosomes can help to understand the relationships among related species or clusters of species. *Deprea* and *Larnax* have been controversial about their position and classification within nightshade plants family; besides, their chromosomes have still not been studied. We described for the first time several chromosomes features and discussed them with leaf, flower and fruit traits, proposing possible changes in the current classification and evolutive considerations.

Abstract

Somatic chromosomes of 12 samples belonging to seven *Larnax* Miers species and three *Deprea* Raf. species are studied. Chromosome number and karyotype analysis of both genera are reported for the first time. All taxa have 2n = 24. The most frequent haploid karyotype formula (8/12 samples) is 9 metacentric (m) + 3 submetacentric (sm) chromosomes, whereas *L. glabra* (Standl.) N.W. Sawyer and *Larnax* sp. display 10 m + 2 sm. Karyotypes of *L. nieva* S. Leiva & N.W. Sawyer and *D. cuyacensis* (N.W. Sawyer

& S. Leiva) S. Leiva & Lezama are remarkable for the highest number of sm chromosome pairs, with 7 m + 5 sm and 5 m + 7 sm, respectively, presenting the highest intrachromosomal asymmetry index (A_1), whereas *Larnax* sp. and *L. glabra* show the lowest A_1. Most samples (9/12) examined have only one pair of chromosomes with nucleolar organizer regions (NORs), whereas *L. glabra, Larnax* sp., and *D. cuyacensis* possess two pairs of NORs. Systematic considerations about the monophyly of *Larnax* and *Deprea* are provided. The different karyotype parameters obtained, together with morphological characters, are discussed to single out the species.

Key words: AgNOR - cytogenetics - Feulgen - monophyly - South America

Introduction

Larnax Miers and *Deprea* Raf. are two neotropical genera placed in the tribe Physalideae, with 36 species (Deanna *et al.* 2014) and 10 species (Cueva and Treviño 2012; Barboza *et al.* 2013), respectively. From a phylogenetic perspective, *Larnax* and *Deprea* are currently positioned as sisters to Withaninae and Iochrominae clades, but remain isolated in a small unnamed subclade (Olmstead *et al.* 2008; Särkinen *et al.* 2013). Both genera occur in South America, from Costa Rica to Bolivia (Sawyer 2005; Leiva González S., Deanna R., Barboza, G.E., unpubl. data), inhabiting cloud pre-montane and montane forests. Furthermore, many species are of pharmacological interest as a source of withanolides, which can have leishmanicidal activity (e.g., *L. glabra* (Standl.) N.W. Sawyer; Cardona *et al.* 2005) or potential cancer chemopreventive activity (*L. subtriflora* (Ruiz & Pav.) Miers [*sub nom. Deprea subtriflora*]; Su *et al.* 2003; Misico *et al.* 2011).

Historically, the taxonomy of *Larnax* and *Deprea* has been confusing, since several authors (Dunal 1852; Hemsley 1882; Zahlbruckner 1892; D´Arcy 1973, 1993) adopted different criteria to transfer species between these genera or to other genera (*Physalis*, *Withania* Pauquy, or *Athenaea* Sendtn.). Furthermore, differentiation between *Larnax* and *Deprea* has been unclear and has varied according to different taxonomic opinions. The most recent morphological cladistic work (Sawyer 2005) attempted to demonstrate the monophyly of *Deprea* and *Larnax* using corolla, stamen, and pollen characters, although ambiguous results were obtained using DNA sequence data in cladistic analysis. Moreover, the most recent phylogenetic analysis including both genera showed them as polyphyletic (Särkinen *et al.* 2013).

In the Physalideae tribe most of the available cytological information is restricted to reports of chromosome number or meiotic studies. This tribe has x = 12 (Badr *et al.* 1997; Rego *et al.* 2009; Barboza *et al.* 2010; Chiarini *et al.* 2010), except for *Quincula* Raf., with x = 11 (Menzel 1950). Many tribe members have a meiotic chromosome number of n = 12 (e.g. Moscone 1992; Bohs 2000; Sousa-Peña 2001), whereas *Withania*, *Nothocestrum* A. Gray, *Tubocapsicum* (Wettst.) Makino, and some species of *Chaemaesaracha* Dammer and *Physalis* have n = 24 or n = 36 (Menzel 1950, 1951; Averett 1973; Carr 1985). The karyotypes are variable among the genera related to *Larnax* and *Deprea*, and are generally composed of metacentric (m) and submetacentric (sm) chromosomes of small size (1 to 4 µm; Badr *et al.* 1997; Rego *et al.* 2009; Barboza *et al.* 2010; Chiarini *et al.* 2010); whereas, chromosomes range from 0.8 to 14 µm within Solanaceae (Bohs 1994, Badr *et al.* 1997; Chiarini *et al.* 2010; Moyetta *et al.* 2013).

Although many cytogenetic works have been conducted in genera belonging to the Physalideae tribe, *Larnax* and *Deprea* have still not been karyologically studied. As part of a broad taxonomic, molecular, and karyosystematic study, karyotype measurements have been performed in 10 *Larnax* and *Deprea* species. The aims of this work are to: (1) determine the chromosome number for both genera, (2) provide a cytological characterization of their species, and (3) contribute to understand the evolutionary and systematic relationships of the genera examined.

Materials and Methods

The provenance of the plant material analyzed is presented in Table 1. The respective voucher specimens were identified by G. E. Barboza, S. Leiva González and R. Deanna, and are deposited at the herbarium of the Botanical Museum of Córdoba, Argentina (CORD).

Mitotic chromosomes were examined in root tip squashes obtained from germinated seeds. When it was difficult to obtain germinated seeds, 200-500 ppm giberellic acid (GA_3) was applied to break dormancy (Ellis *et al.* 1985). Root tips were pretreated either with *para*-dichlorobenzene-saturated solution at room temperature in darkness for 2 h or with 2 mM 8- hydroxyquinoline at room temperature for 2 h and at 6ºC for 3 h, then fixed in 3:1 ethanol: acetic acid mixture, and maintained at 4ºC for the first 24 h and at 20ºC thereafter. Different pre-treatment methods showed same results in species where both techniques were applied.

Feulgen staining. To prepare the slides, root tips were hydrolyzed with 5N HCl at room temperature for 45 min, stained with Schiff reagent for 2 h (Jong 1997) and squashed in a drop of 2% acetic carmine. Slides were made permanent by freezing with liquid CO_2 (Bowen 1956), removing the coverslip.

Karyotype measurements. A total of 97 individuals from 12 samples were analyzed (Table 1). Between 7 and 104 cells per species were examined under a Leica DMLB microscope and photographed with a Leica DC 250 digital camera. Two to 16 metaphase plates from 2-12 individuals of each species were used to take measurements for each chromosome pair: s (short arm length), l (long arm length), and c (total chromosome length). The arm ratio (r = l/s) was calculated to classify the chromosomes following Levan *et al.* (1964): m - metacentric (r = 1.00-1.69), sm - submetacentric (r = 1.70-2.99), st - subtelocentric (r = 3.00-6.99), and t - telocentric (r = 7.00 and up). Satellites were classified according to Battaglia's (1955) terminology. In addition, the following measurements were calculated: haploid karyotype length (HKL), based on the mean chromosome length for each species, average chromosome length (C), average arm ratio (r), and ratio between the longest and the shortest chromosome of the complement (R) (Table 2). Idiograms were based on the mean values for each species. Chromosomes were arranged first into groups according to increasing arm ratio, and then according to decreasing length within each group. As certain chromosomes showed great similarity, they were grouped in the idiograms. Karyotype asymmetry was estimated using the intrachromosomal and the interchromosomal asymmetry indices (A_1 and A_2, respectively; Romero Zarco 1986), and Stebbins' (1971) karyotype asymmetry categories.

AgNOR banding. Root tips were washed in 0.01 M citrate buffer and macerated according to Schwarzacher *et al.* (1980) using an enzymatic solution of 2% cellulase (w/v) plus 2% pectinase (v/v) at 37º C for 30 minutes. Meristems were squashed in a drop of 45% acetic acid and, after removal of the coverslip, slides were air dried, aged for 1-2 days at room temperature and stored at -20º C until use. AgNOR banding was performed according Bloom and Goodpasture (1976) with the modifications of Kodama *et al.* (1980). The ordering number of NOR (nucleolar organizer region) bearing chromosomes and types were calculated and reported (Table 2).

Results

Karyotype measurements

The *Larnax* and *Deprea* species studied are diploid, with 2n = 24 in all examined cells. The most frequent haploid karyotype formula is 9 m + 3 sm (in *L. pomacochaense*, *L. subtriflora*, *L. toledoana*, *L.* sachapapa, *D. bitteriana*, and *D. zamorae*), whereas *L. glabra* and *Larnax* sp. display 10 m + 2 sm. In addition, karyotype of *L. nieva* and *D. cuyacensis* are remarkable for the highest number of sm chromosomes, with 7 m + 5 sm and 5 m + 7 sm, respectively. Haploid karyotype length (HKL) for individual species ranges from 31.67 µm in *L. nieva* to 46.26 µm in *L. pomacochaense* (Table 2). The shortest chromosome pair measured is no. 12 in *L. glabra* (1.86 µm) and the longest one is no. 1 in *L. pomacochaense* (4.60 µm) (Table 2).

With Feulgen staining, most of the satellites attached to NOR-carrying chromosomes are observed in both members of the respective chromosome pair (Fig. 1), but in some individuals they appear in a single homologue. Most of the species exhibits macrosatellites of constant size (Figs. 1, 2), whereas microsatellites are observed in *L. glabra, L. nieva*, *Larnax* sp., and *D. cuyacensis* (Figs. 1 A, B, G, K; 2). Moreover, the heteromorphic condition is frequent in these cases (*L. glabra*, *L. nieva*, and *Larnax* sp.), with one homologous chromosome bearing a microsatellite and the other one carrying a macrosatellite (Figs. 1 A, B, G; 2). Furthermore, the satellites usually show slight variation in size between individuals, or between cells from the same plant.

In general, karyotypes are symmetrical, considering both centromere position and chromosome size (Table 2, Fig. 3). *Larnax nieva* and *D. cuyacensis* have the karyotypes with the highest intrachromosomal asymmetry index (A_1 = 0.36 and 0.42, respectively), whereas *Larnax* sp. and *L. glabra* show the lowest one (A_1 = 0.22 and 0.23; Fig. 3). Moreover, *L. glabra* shows the highest interchromosomal asymmetry index (A_2 = 0.24) and the highest R index (2.34). Conversely, a population of *L. sahapapa* (2) and *L. nieva*, with the lowest R (1.43 and 1.42, respectively) and A_2 (0.11) index values, display all chromosomes of similar size. According to Stebbins' karyotype asymmetry classification, all of the species fall into 2A category, except *L. glabra*, which falls into 2B category (Table 2). No association between karyotype length and asymmetry can be established (Table 2).

AgNOR banding

AgNOR banding performed in 12 samples showed that the NORs usually have attached satellites that are not always differentially stained with silver staining (Fig. 4). In some cases, NORs appeared terminal after silver staining (Fig. 4 A-D, F, H-J).

In most of the samples (9/12) examined, all individuals have only one pair of chromosomes with AgNORs, with a maximum of two nucleoli in interphase nuclei impregnated with silver (Fig. 5 B-E, I, K, M), although 80-90% cells have only one (Fig. 5 F, J). NOR-bearing chromosomes in metaphase are always two. NORs are located on the short arm on the shortest m chromosome pair, usually having attached macrosatellites (no. 9; Figs. 2; 4 C-E, G-J). The exception is *L. nieva* with one homologous chromosome bearing a microsatellite and the other one carrying a macrosatellite (Figs. 2, 4 B).

On the other hand, *L. glabra* and *Larnax* sp. possess two pairs of NORs. The maximum number of silver-stained nucleoli found is always four in both species (Fig. 5 A, G); however, one is the most frequent number (70-75% cells). In these species, metaphases always show four AgNORs (Fig. 4 A, F), one pair is always located on the short arm of a median m chromosome (no. 5), and the other one is located on the short arm of the shortest sm chromosome (no. 12; Fig. 2). Moreover, both species have particular characteristics: *L. glabra* has a m chromosome pair with a microsatellite attached and the shortest sm chromosome pair with one homologous chromosome bearing a microsatellite and the other one carrying a macrosatellite (Figs. 1 A, 2, 4 A), whereas *Larnax* sp. exhibits this heteromorphic condition in both chromosome pairs (Figs. 1 G, 2, 4 F).

In *D. cuyacensis*, all individuals examined have two pairs of chromosomes with NORs. The maximum number of silver-stained nucleoli found is four (Fig. 5 L); however, the most frequent number is one (81%). It was not possible to observe the metaphases with silver staining; hence, the NOR-bearing pairs were identified by Feulgen staining. One pair of NORs is located on the short arm of a m chromosome (no. 4) with a microsatellite attached, and the other one is located on the short arm of a sm chromosome with a macrosatellite attached (no. 9; Figs. 1 K, 2).

Discussion

General karyotype features

All *Larnax* and *Deprea* species are diploid with x = 12, certainly the most common basic number in the family, being present in more than half of the species studied until now (Hunziker 2001; Chiarini *et al.* 2010). In general, *Larnax* and *Deprea* species have small chromosomes, as their related taxa within the Physalidae tribe: *Schraderanthus viscosus* (Schrad.) Averett (*sub nom. Leucophysalis viscosa* Schrad.), *Witheringia solanacea* L'Hér. (Chiarini *et al.* 2010), *Aureliana sellowiana* (Sendtn.) Barboza & Stehmann (Barboza *et al.* 2010), and *Vassobia breviflora* (Sendtn.) Hunz. (Rego *et al.* 2009). In addition, the karyotypes analyzed are rather homogeneous in size, with a maximum difference in the average chromosome length of only 1.46-fold among species.

In Solanaceae, most chromosomes are m or sm (e.g. Badr *et al.* 1997; Chiarini *et al.* 2010; Scaldaferro *et al.* 2013). The present study shows that most of the species exhibit a karyotype with predominance of m chromosomes and a low interchromosomal asymmetry index. This is in agreement with previous findings in the most related taxa, such as *W. solanacea*, *S. viscosus*, *Saracha punctata* Ruiz & Pav. (Chiarini *et al.* 2010) and *V. breviflora* (Rego *et al.* 2009). According to the rule in the family, the low asymmetry is also supported by the fact that all the examined species fall into category 2A of Stebbins' karyotype asymmetry classification (1971) for possessing mainly m chromosomes of homogeneous size, except *L. glabra*, which falls into category 2B for having a higher interchromosomal asymmetry. Unfortunately, there are only a few chromosome studies available for Withaninae and Iochrominae clades (Madhavadian 1967; Moscone 1992; Badr *et al.* 1997; Rego *et al.* 2009; Chiarini *et al.* 2010), where *Larnax* and *Deprea* are currently positioned (Olmstead *et al.* 2008; Särkinen *et al.* 2013); hence, additional data are needed for comparative purposes.

The analysis of karyotype variables shows that *D. cuyacensis*, *L. glabra, L. nieva*, and *Larnax* sp. are the most different species. They differ in the asymmetry, in the number and location of NORs, and in the haploid karyotype formula. One dissimilar species is *L. glabra*, whereas *L. nieva* and *D. cuyacensis* are grouped according to the higher number of sm chromosomes and, as a consequence, a higher intrachromosomal asymmetry index.

Nucleolar activity

AgNOR banding was used to reveal active rDNA sites, whose number allowed us to classify the species into two groups: a group with only one NOR and a smaller group composed of three species with two NORs. In the former group the constancy in the location of the NOR is remarkable, since it is always in the short arm of the smallest m chromosome. Within the family, satellites are usually attached to short arms of m or sm chromosomes (e.g. Menzel 1950; Stiefkens and Bernardello 2006; Acosta *et al.* 2005; Bernardello *et al.* 2008; Rego *et al.* 2009; Chiarini *et al.* 2010; Moyetta *et al.* 2013; Scaldaferro *et al.* 2013), as it is also observed in the taxa analyzed in this work.

Another feature noticed in this study and previously recorded in other Solanaceae is the polymorphism in the size of AgNORs among individuals, cells, and even homologous NOR-bearing chromosomes of a single cell (*L. nieva* and *D. bitteriana*; Moscone 1989; Moscone *et al.* 1995). This may be caused by several factors, such as the ribosomal gene repeats, the transcription level, or the effect of pretreatment with spindle inhibitors and the state of chromatin condensation in the NORs (Suda 1975; Jiménez *et al.* 1988; Zurita *et al.* 1999). Structural rearrangements including somatic chromosome mutations that affect satellites could be the mechanisms responsible for such polymorphism (cf. Sato 1981). In addition, the observation of a lower number of nucleoli in all interphase nuclei might be due to their fusion (Nicoloff *et al.* 1977; Sato *et al.* 1981; Lacadena *et al.* 1984), but it could also be a consequence of nucleolar interchromosomic dominance, where NORs of different chromosomes compete to form the nucleoli (Flavell and O`Dell 1979; Nicoloff *et al.* 1979).

In some cases, NORs appear to be terminal after silver staining, probably because microsatellites are no longer recognizable after applying this banding method because of their small size, a phenomenon that has been observed in *Capsicum* and *Solanum* (Moscone *et al.* 1995, Miguel *et al.* 2012).

Karyotype data and systematic considerations

Up to now, there were no evidence of cytogenetic data for *Larnax* and *Deprea* species and the kind of morphological characters that are informative enough to group these species is still unclear. Previous studies suggested the fruiting calyx, either tightly or loosely enveloping the berry, as a primary character to cluster species in *Larnax* (Sawyer 2001). Among the species analyzed, *D. bitteriana, D. cuyacensis, D. zamorae, L. pomacochaense, L. subtriflora,* and *L. toledoana* have the fruiting calyx tightly enveloping the berry (Fig. 6 A-M), but, according to the cytogenetic variables analyzed here, they are mixed with species having a loose fruiting calyx (Figs. 3, 6 N-T).

According to the results presented in this work, karyotype features allow individual species to be distinguished from one another. *Larnax glabra* is one of the most different species of the group considering both morphological and karyological characters. This species has an entirely deep purple corolla, a glabrescent indumentum with scattered short glandular trichomes, and a persistent calyx loosely enveloping the orange berry (Fig. 6 N); all these traits, together with the species' haploid karyotype formula, number and position of satellites, and asymmetry indices, allow us to distinguish it easily from the remaining species. *Larnax* sp. and *L. glabra* share the number and position of satellites and the lower intrachromosal asymmetry, but they differ especially in the satellite size and the interchromosomal asymmetry. Morphologically, they also differ in several traits (Fig. 6 N, O), such as the length calyx lobes (> 1 mm long in *Larnax* sp., < 1 mm long in *L. glabra*), the indumentum (pubescent to sericeous in *Larnax* sp., glabrescent in *L. glabra*), and the berry color (creamy white in *Larnax* sp., orange in *L. glabra*).

Two other cytogenetically very different species are *D. cuyacensis* and *L. nieva*, both with numerous cytogenetic and morphological peculiarities. They have a higher intrachromosomal asymmetry and a higher number of sm chromosomes than the other species analyzed. *Deprea cuyacensis* and *L. nieva* differ in the number, position and size of satellites; in addition, they do not share any morphological trait: *L. nieva* is a very tall shrub (2.5-3 m vs. 1.2-1.8 in *D. cuyacensis*), having a creamy green corolla without a ring of trichomes inside (vs. pale purple with a ring of trichomes inside, Fig. 6 C, P), heterodynamous stamens (vs. homodynamous), mucronate anthers (vs. non-mucronate), and a green berry (vs. orange berry in *D. cuyacensis*, Fig. 6 C, Q). Both species are endemic and restricted to small areas in Peru (Sawyer 2001; Leiva González and Lezama Asencio 2003), being part of stable and strongly isolated islands, as occurs in the seasonally dry tropical forests of the Andes and the more mesic mid-elevation montane forests (Särkinen *et al.* 2012). Consequently, they have reached a high intrachromosomal asymmetry probably by parallel evolution.

Larnax sachapapa and *L. subtriflora* are species with two populations analyzed because they are more widely distributed than the remaining species (Sawyer 1999; Leiva González S., Deanna R., Barboza, G.E., unpubl. data). On the one hand, samples of *L. sachapapa* belong to two conspicuous and nearby Ecuadorian populations which differ from the remaining populations of this species by some characters (corolla 10-14 mm long vs. 17-22 mm long; anthers longer than filaments vs. anthers shorter than filaments; fruiting calyx loosely enveloping the berry vs. tightly enveloping the berry, and leaves 20-32 cm long vs. 2.5-16.5 cm long). Both populations studied also have some morphological differences which are reflected in the karyological variability observed, especially in the interchromosomal asymmetry index (Fig. 3). On the other hand, samples of *L. subtriflora* were taken from the distribution extremes: northern Peru (sub 1, Fig. 6 H, I) and Bolivian Yungas (sub 2, Fig. 6 J, K). These populations do not significantly differ in the karyological variables analyzed or in morphological characters, with the exception that the Bolivian population has a trichome ring inside the corolla and an abundant glandular indumentum, whereas the Peruvian population lacks the trichome ring and has poorly developed glandular pubescence.

Lastly, there is a group composed of seven species (*D. bitteriana, D. zamorae, L. pomacochaense, L. sachapapa, L. subtriflora,* and *L. toledoana*) that have the same haploid karyotype formula, similar asymmetry, and an equal number and position of satellites. Differences among them involve other characters, such as haploid karyotype length or satellite size. However, these species do not share any morphological characters; the group includes species with anthocyanins in their infundibular to campanulate corolla (*D. bitteriana* and *D. zamorae*, Fig. 6 A, D), and others with stellate or slightly campanulate corolla without anthocyanins (*L. pomacochaense, L. sachapapa,* and *L. subtriflora,* Fig. 6 F, H, J, S); a species with mucronate anthers, very long calyx lobes, and yellowish green berry (*L. sachapapa*, Fig. 6 R-T) and others with non-mucronate anthers, short calyx lobes, and orange berry (*D. bitteriana*, *D. zamorae*, *L. subtriflora* and *L. toledoana*, Fig. 6 A, B, D, E, H-M); and species with a fruiting calyx tightly or loosely enveloping the berry (*D. zamorae*, *D. bitteriana, L. pomacochaense*, *L. subtriflora*, and *L. toledoana* vs. *L.* sachapapa, respectively).

Hence, *Larnax* and *Deprea* cannot be karyologically differentiated as two different groups considering all the variables here analyzed, especially through asymmetry indices (Fig. 3), consistent with previous phylogenetic results (Särkinen *et al.* 2013). *Larnax* and *Deprea* have been controversial genera since some species have been transferred from one genus to another (*Withania* Pauquy, *Athenaea* Sendtn., *Deprea* Raf.) by different authors (Dunal 1852; Hemsley 1882; Zahlbruckner 1892; D´Arcy 1973, 1993). Despite this, Hunziker (1977) and Sawyer (2005) defined and differentiated *Larnax* from *Deprea*, its most closely related genus, based on several synapomorphies (Deanna *et al.* 2014). Recently, however, the limits between both genera have become confusing due to the continuous description of species with intermediate features of both genera (cf. Sawyer 2001; Leiva González and Rodríguez Rodríguez 2006; Leiva González *et al.* 2008). According to the recent molecular phylogenetic tree of Särkinen *et al.* (2013), *Larnax* and *Deprea* are placed in an unnamed clade closer to the Withaninae subtribe. However, previous phylogenetic analysis, although poorly supported, placed *Larnax* closer to the Iochrominae clade (cf. Olmstead *et al.* 2008); therefore, *Larnax* and *Deprea* are now in an unclear position. In addition, *Larnax* and *Deprea* could be a monophyletic clade as a whole, but they are not monophyletic clades independently (cf. Särkinen *et al.* 2013).

Karyological studies using conventional and AgNOR stains in species of *Larnax* and *Deprea* support their position within Physalideae tribe; however, no association with any of its three subtribes (Physalidineae, Withaninae and Iochrominae) can be proposed because detailed cytogenetic information (as here presented) is restricted to isolated genera of each subtribe (Rego *et al.* 2009; Barboza *et al.* 2010; Chiarini *et al.* 2010).

In several plant groups, the appearance of macrosatellites instead of microsatellites are associated with advanced taxa (Stebbins 1971; Moscone 1989) and, in this work, we postulate it as a synapomorphic condition according to the molecular phylogenetic tree of Särkinen *et al.* (2013). Presence of macrosatellites is remarkable in most of *Larnax* and *Deprea* species compared with species of other related genera, such as *A. sellowiana* and *W. solanacea*, which have one pair of microsatellites (Barboza *et al.* 2010; Chiarini *et al.* 2010) and belong to Withaninae and Physalidinae clades, respectively (Särkinen *et al.* 2013). As a consequence, the appearance of macrosatellites could be a synapomorphic trait for the *Larnax* and *Deprea* group, but more karyological studies of tribe Physalideae members are needed to confirm this. Besides, the haploid karyotype formula 9 m + 3 sm is found in *A. sellowiana* (subtribe Withaninae; Barboza *et al.*, 2010), *W. solanacea* (subtribe Physalidinae; Chiarini *et al.* 2010), *V. breviflora* (subtribe Iochrominae; Rego *et al.* 2009), and in an important group of *Larnax* and *Deprea* species; therefore, we consider this haploid karyotype formula as a plesiomorphic trait.

As it was demonstrated, the different karyotype parameters obtained, especially karyotype asymmetry indices and number, size, position and heteromorphic condition of satellites, are useful to single out the studied species. At present, an integral treatment of both genera is being accomplished in an attempt to identify new morphological characters which, together with additional cytogenetic traits (heterochromatin amount, position and type, and number and position of rDNA sites) allow us to group species according to our ongoing phylogenetic studies.

Additional karyotype analyses in a higher number of members of the Physalideae tribe are desirable to enhance the knowledge about possible karyoevolutionary trends in the studied genera and in genera belonging to the subtribes Physalidineae, Withaninae and Iochrominae.

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Figure Legends

FIGURE 1. Somatic metaphases of *Larnax* and *Deprea* species (2n = 24) after conventional staining (Feulgen). **A**, *L. glabra*; **B**, *L. nieva*; **C**, *L. pomacochaense*; **D**, *L. subtriflora* 1; **E**, *L. subtriflora* 2; **F**, *L. toledoana*; **G**, *Larnax* sp.; **H**, *L. sachapapa* 1; **I**, *L. sachapapa* 2; **J**, *D. bitteriana*; **K**, *D. cuyacensis*; **L**, *D. zamorae*. Arrows indicate satellites. Scale bar = 5 µm.

FIGURE 2. Idiograms of *Larnax* and *Deprea* species. Chromosomes showing high similarity were grouped. Scale bar = 5 µm.

FIGURE 3. Diagram showing the intrachromosomal asymmetry index (A_1) plotted against the interchromosomal asymmetry index (A_2). Species codes are given in Table 1. Solid circles (●) and empty squares (□) indicate species with a fruiting calyx loosely or tightly enveloping the berry, respectively.

FIGURE 4. Somatic metaphases of *Larnax* and *Deprea* species (2n = 24) after AgNOR banding. **A**, *L. glabra*; **B**, *L. nieva*; **C**, *L. pomacochaense*; **D**, *L. subtriflora* 2; **E**, *L. toledoana*; **F**, *Larnax* sp.; **G**, *L. sachapapa* 1; **H**, *L. sachapapa* 2; **I**, *D. bitteriana*; **J**, *D. zamorae*. Arrows indicate AgNORs. Scale bar = 5 µm.

FIGURE 5. Interphase nuclei of *Larnax* and *Deprea* species after AgNOR banding. **A**, *L. glabra*; **B**, *L. nieva*; **C**, *L. pomacochaense*; **D**, *L. subtriflora* 1; **E**, *L. subtriflora* 2; **F**, *L. toledoana*; **G, H**, *Larnax* sp.; **I**, *L. sachapapa* 1; **J**, *L. sachapapa* 2; **K**, D. *bitteriana;* **L,** *D. cuyacensis*; **M,** *D. zamorae*. Scale bar = 10 µm.

FIGURE 6. *Larnax* and *Deprea* species cytogenetically analyzed. **A, B.** *D. bitteriana*. **A.** Flower. **B.** Fruits. **C.** *D. cuyacensis*, flower and mature fruit. **D, E.** *D. zamorae*. **D.** Flower. **E.** Fruits. **F, G.** *L. pomacochaense*. **F.** Flower. **G.** Fruits. **H, I.** *L. subtriflora* 1 (Peru). **H.** Flower. **I.** Fruit. **J, K.** *L. subtriflora* 2 (Bolivia). **J.** Flower. **K.** Fruit. **L, M.** *L. toledoana.* **L.** Flower. **M.** Fruit. **N.** *L. glabra,* flower and fruit. **O.** *Larnax* sp., flower, buds, and fruit. **P, Q.** *L. nieva.* **P.** Flower. **Q.** Fruits. **R.** *L. sachapapa* 1, fruits. **S, T.** *L. sachapapa* 2. **S.** Flower. **T.** Fruit. Photographs A, B by M. T. Cosa; C, F-I, P, Q, S, T by R. Deanna; D, E, J-M, O by S. Leiva; N, R by G. E. Barboza.

568 **Table 1. List of *Larnax* and *Deprea* species, samples studied, code, voucher specimen and provenance.**

Species[A]	Code	Voucher specimen	Provenance[B]
L. glabra (Standl.) N.W. Sawyer (9, 31)	gla	*Orozco C. I. et al. 3812* (COL, CORD, QCA).	COLOMBIA. Cauca Dept., El Tambo Munic., Munchique Natural National Park
L. nieva S. Leiva & N.W. Sawyer (9, 65)	nie	*Deanna R. & Leiva S. 43* (CORD, HAO).	PERU. Amazonas Dept., Bongará Prov., km 384, Nueva Cajamarca – Pomacochas (Florida) roadsides.
L. pomacochaense S. Leiva (8, 95)	pom	*Deanna R. & Leiva S. 34* (CORD, HAO).	PERU. Amazonas Dept., Bongará Prov., km 328-329, Bongará-Nuevo Cajamarca road.
L. sachapapa Hunz.1 (16, 102)	sac1	*Orozco C. I. et al. 3983* (COL, CORD, QCA).	ECUADOR. Cotopaxi Prov., San Francisco de las Pampas.
L. sachapapa 2 (8, 104)	sac2	*Deanna R., Leiva S. & Cerón C. 142* (CORD, HAO, QUSF).	ECUADOR. Pichincha Prov., Quito, km 45, Calacalí-Nanegalito road.
L. subtriflora (Ruiz & Pav.) Miers 1 (2, 7)	sub1	*Deanna R. & Leiva S. 71* (CORD, HAO).	PERU. Cajamarca Dept., Cutervo Prov., km 1542-1543, Cutervo - La Capilla roadsides.
L. subtriflora 2 (3, 29)	sub2	*Barboza G. E. & Carrizo García C. 3663* (CORD).	BOLIVIA. La Paz Dept., Nor-Yungas Prov., route from Chuspipata to Coroico.
L. toledoana Barboza & S. Leiva (11, 28)	tol	*Orozco C. I. et al. 3949* (COL, CORD, QCA).	ECUADOR. Loja Prov., Cerro Toledo.
Larnax sp. (5, 10)	sp	*Orozco C. I. et al. 3908* (COL, CORD, QCA).	ECUADOR. Zamora-Chinchipe Prov., boundaries of Podocarpus National Park.

D. bitteriana (Werderm.) N.W. Sawyer & Benítez (9, 50)	bit	*Orozco C. I. et al. 3871* (COL, CORD)	COLOMBIA. Cundinamarca Dept., Subachoque, El Tablazo moorland.
D. cuyacensis (N.W. Sawyer & S. Leiva) S. Leiva & Lezama (9, 79)	cuy	*Barboza G. E., Leiva S. & Basso A. V. 3367* (CORD)	PERU. Piura Dept., Ayabaca Prov., Ayabaca locality, Cuyas forest.
D. zamorae Barboza & S. Leiva (8, 29)	zam	*Orozco C. I. et al. 3926* (COL, CORD, HAO, QCA)	ECUADOR. Zamora-Chinchipe Prov., boundaries of Podocarpus National Park.

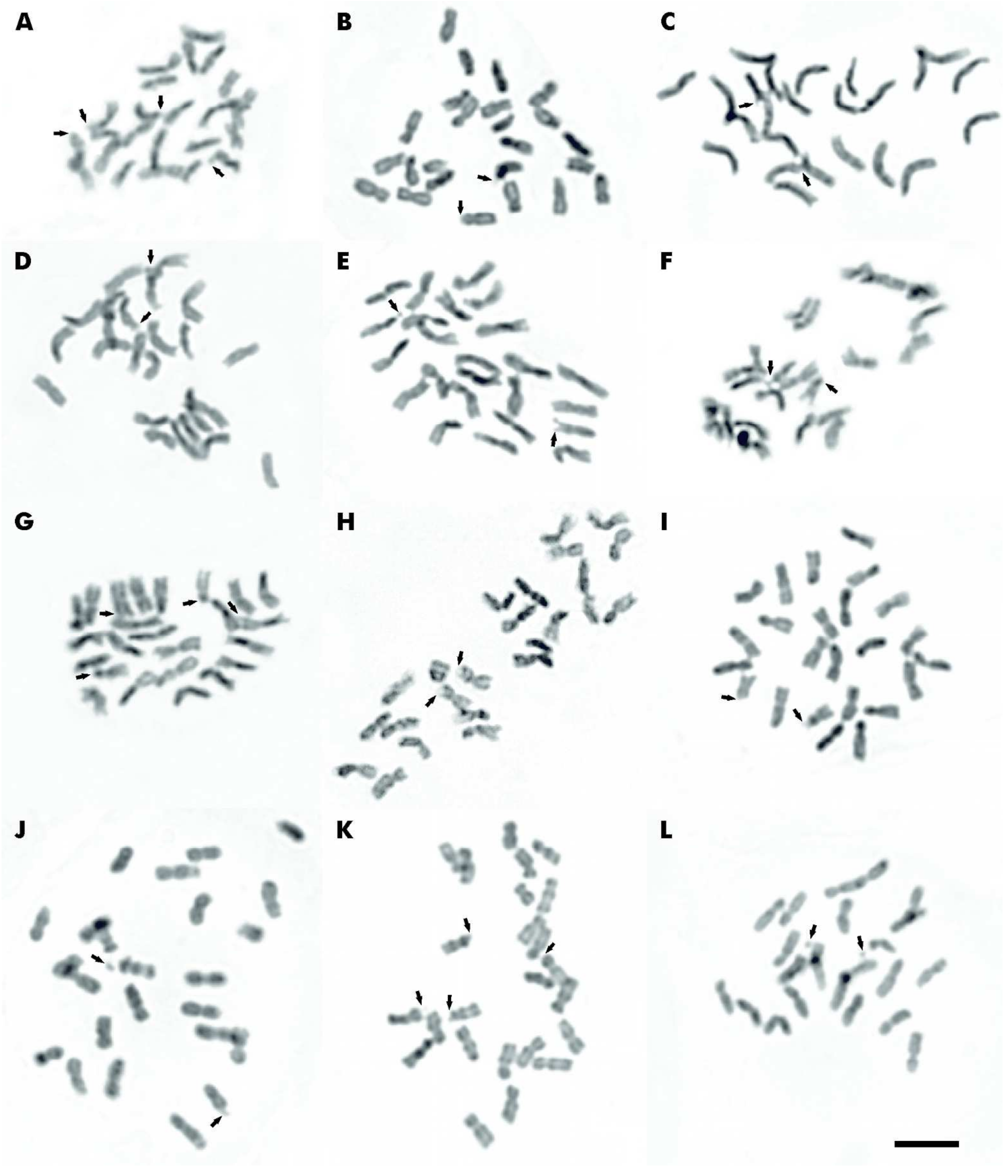
[A] In parentheses, number of seedlings and somatic metaphases analyzed per species with Feulgen staining, in this order.

[B] Abbreviations: Prov. = province, Dept. = department, Munic. = municipality (town).

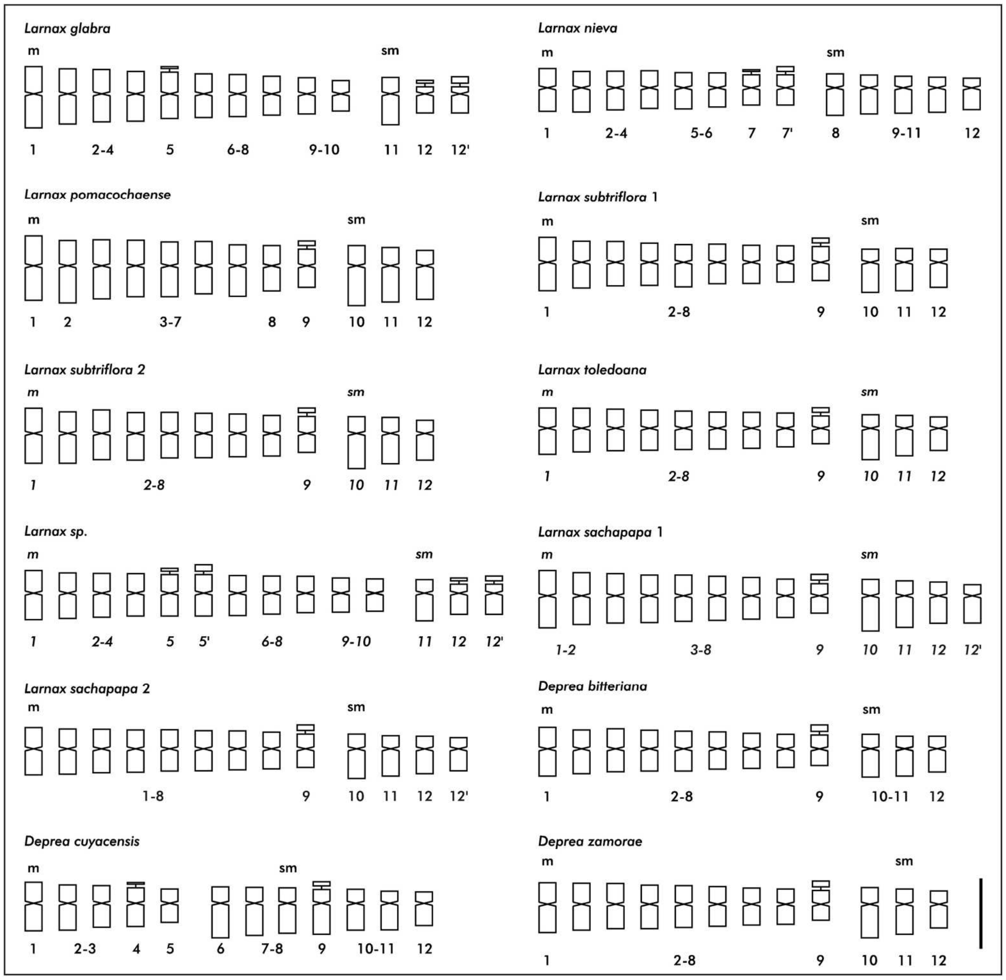
Table 2. Karyotype features of eight species of *Larnax* and three species of *Deprea*, all of them with 2n = 24.

Species are listed following the order in Table 1. Abbreviations: HKF = haploid karyotype formula; m = metacentric; sm = submetacentric; NOR = nucleolus organizing region; chr-NOR = ordering number of the NOR-bearing chromosome and type; HKL = haploid karyotype length in µm, - mean (sd); sd = standard deviation; c = mean chromosome length; chr-l = range of chromosome length; r = mean arm ratio; R = ratio between the longest and the shortest chromosome pair; A_1 = intrachromosomal asymmetry index; A_2 = interchomosomal asymmetry index; AT = Stebbins' karyotype asymmetry type.

Species	HKF	chr-NOR	HKL	c (sd)	chr-l	r (sd)	R	A_1	A_2	AT
L. glabra	10 m + 2 sm	5 (m); 12 (sm)	37.77 (2.15)	3.15 (0.73)	1.86-4.36	1.39 (0.46)	2.34	0.23	0.24	2B
L. nieva	7 m + 5 sm	7 (m)	31.67 (2.21)	2.64 (0.27)	2.15-3.05	1.67 (0.45)	1.42	0.36	0.11	2A
L. pomacochaense	9 m + 3 sm	9 (m)	46.26 (4.71)	3.85 (0.54)	2.69-4.60	1.49 (0.37)	1.71	0.30	0.15	2A
***L. sachapapa* 1**	9 m + 3 sm	9 (m)	38.97 (7.61)	3.25 (0.52)	2.12-4.11	1.46 (0.42)	1.94	0.28	0.17	2A
***L. sachapapa* 2**	9 m + 3 sm	9 (m)	34.87 (4.94)	2.91 (0.31)	2.37-3.38	1.42 (0.37)	1.43	0.26	0.11	2A
L. subtriflora 1	9 m + 3 sm	9 (m)	35.49 (3.40)	2.96 (0.36)	2.42-3.77	1.44 (0.42)	1.56	0.26	0.12	2A
L. subtriflora 2	9 m + 3 sm	9 (m)	39.07 (5.57)	3.26 (0.40)	2.56-3.91	1.42 (0.38)	1.53	0.26	0.13	2A
L. toledoana	9 m + 3 sm	9 (m)	32.88 (3.28)	2.74 (0.38)	1.97-3.29	1.47 (0.42)	1.67	0.28	0.15	2A
***Larnax* sp.**	10 m + 2 sm	5 (m); 12 (sm)	34.12 (6.67)	2.84 (0.40)	2.16-3.52	1.34 (0.37)	1.63	0.22	0.15	2A
D. bitteriana	9 m + 3 sm	9 (m)	33.44 (3.69)	2.79 (0.36)	2.18-3.47	1.42 (0.36)	1.59	0.26	0.14	2A
D. cuyacensis	5 m + 7 sm	4 (m); 9 (sm)	36.11 (5.72)	3.01 (0.40)	2.36-3.62	1.79 (0.36)	1.53	0.42	0.14	2A
D. zamorae	9 m + 3 sm	9 (m)	35.45 (2.56)	2.95 (0.44)	2.10-3.57	1.43 (0.31)	1.70	0.27	0.16	2A



Somatic metaphases of Larnax and Deprea species (2n = 24) after conventional staining (Feulgen). A, L. glabra; B, L. nieva; C, L. pomacochaense; D, L. subtriflora 1; E, L. subtriflora 2; F, L. toledoana; G, Larnax sp.; H, L. sachapapa 1; I, L. sachapapa 2; J, D. bitteriana; K, D. cuyacensis; L, D. zamorae. Arrows indicate satellites. Scale bar = 5 µm.
175x203mm (300 x 300 DPI)



Idiograms of Larnax and Deprea species. Chromosomes showing high similarity were grouped. Scale bar = 5 µm.
171x166mm (300 x 300 DPI)

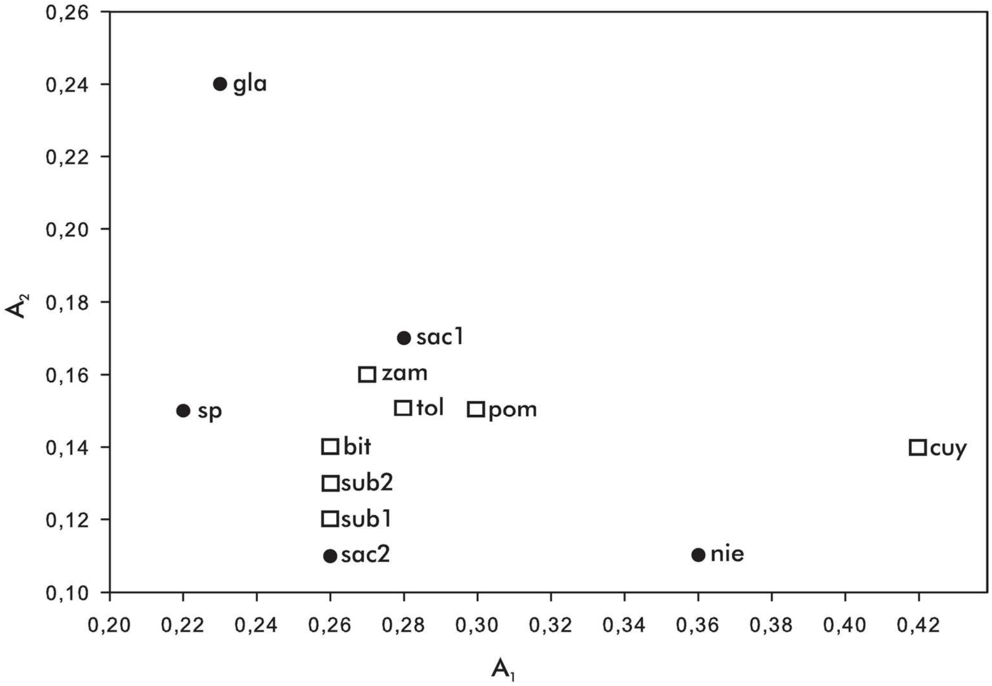
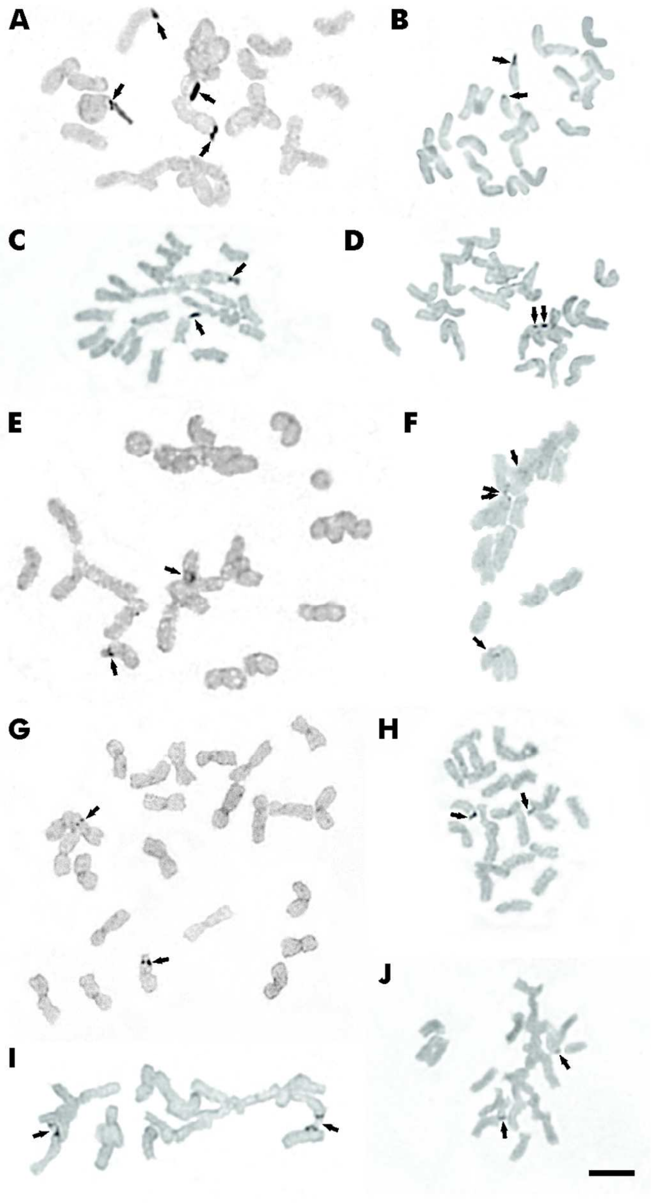
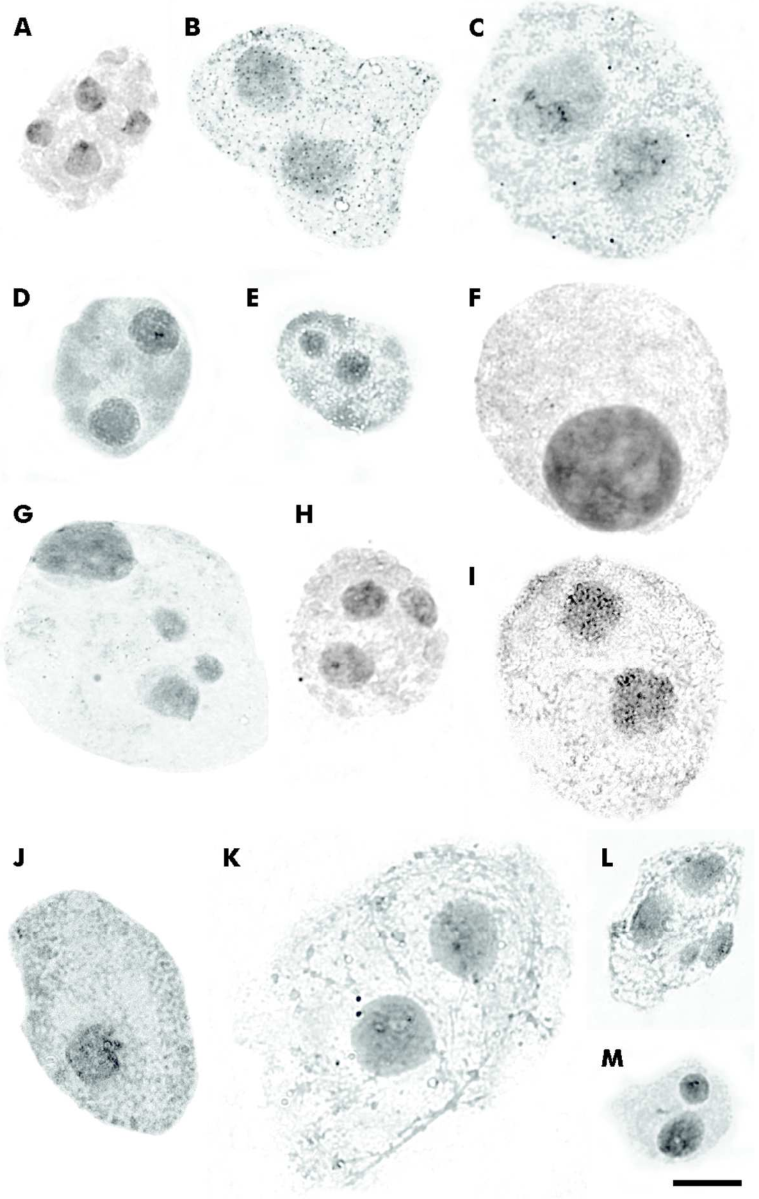


Diagram showing the intrachromosomal asymmetry index (A1) plotted against the interchromosomal asymmetry index (A2). Species codes are given in Table 1. Solid circles (●) and empty squares (□) indicate species with a fruiting calyx loosely or tightly enveloping the berry, respectively.
152x114mm (300 x 300 DPI)



Somatic metaphases of Larnax and Deprea species (2n = 24) after AgNOR banding. A, L. glabra; B, L. nieva; C, L. pomacochaense; D, L. subtriflora 2; E, L. toledoana; F, Larnax sp.; G, L. sachapapa 1; H, L. sachapapa 2; I, D. bitteriana; J, D. zamorae. Arrows indicate AgNORs. Scale bar = 5 µm.
85x155mm (300 x 300 DPI)



Interphase nuclei of Larnax and Deprea species after AgNOR banding. A, L. glabra; B, L. nieva; C, L. pomacochaense; D, L. subtriflora 1; E, L. subtriflora 2; F, L. toledoana; G, H, Larnax sp.; I, L. sachapapa 1; J, L. sachapapa 2; K, D. bitteriana; L, D. cuyacensis; M, D. zamorae. Scale bar = 10 µm.
85x134mm (300 x 300 DPI)

Larnax and Deprea species cytogenetically analyzed. A, B. D. bitteriana. A. Flower. B. Fruits. C. D. cuyacensis, flower and mature fruit. D, E. D. zamorae. D. Flower. E. Fruits. F, G. L. pomacochaense. F. Flower. G. Fruits. H, I. L. subtriflora 1 (Peru). H. Flower. I. Fruit. J, K. L. subtriflora 2 (Bolivia). J. Flower. K. Fruit. L, M. L. toledoana. L. Flower. M. Fruit. N. L. glabra, flower and fruit. O. Larnax sp., flower, buds, and fruit. P, Q. L. nieva. P. Flower. Q. Fruits. R. L. sachapapa 1, fruits. S, T. L. sachapapa 2. S. Flower. T. Fruit. Photographs A, B by M. T. Cosa; C, F-I, P, Q, S, T by R. Deanna; D, E, J-M, O by S. Leiva; N, R by G. E. Barboza.
175x216mm (300 x 300 DPI)