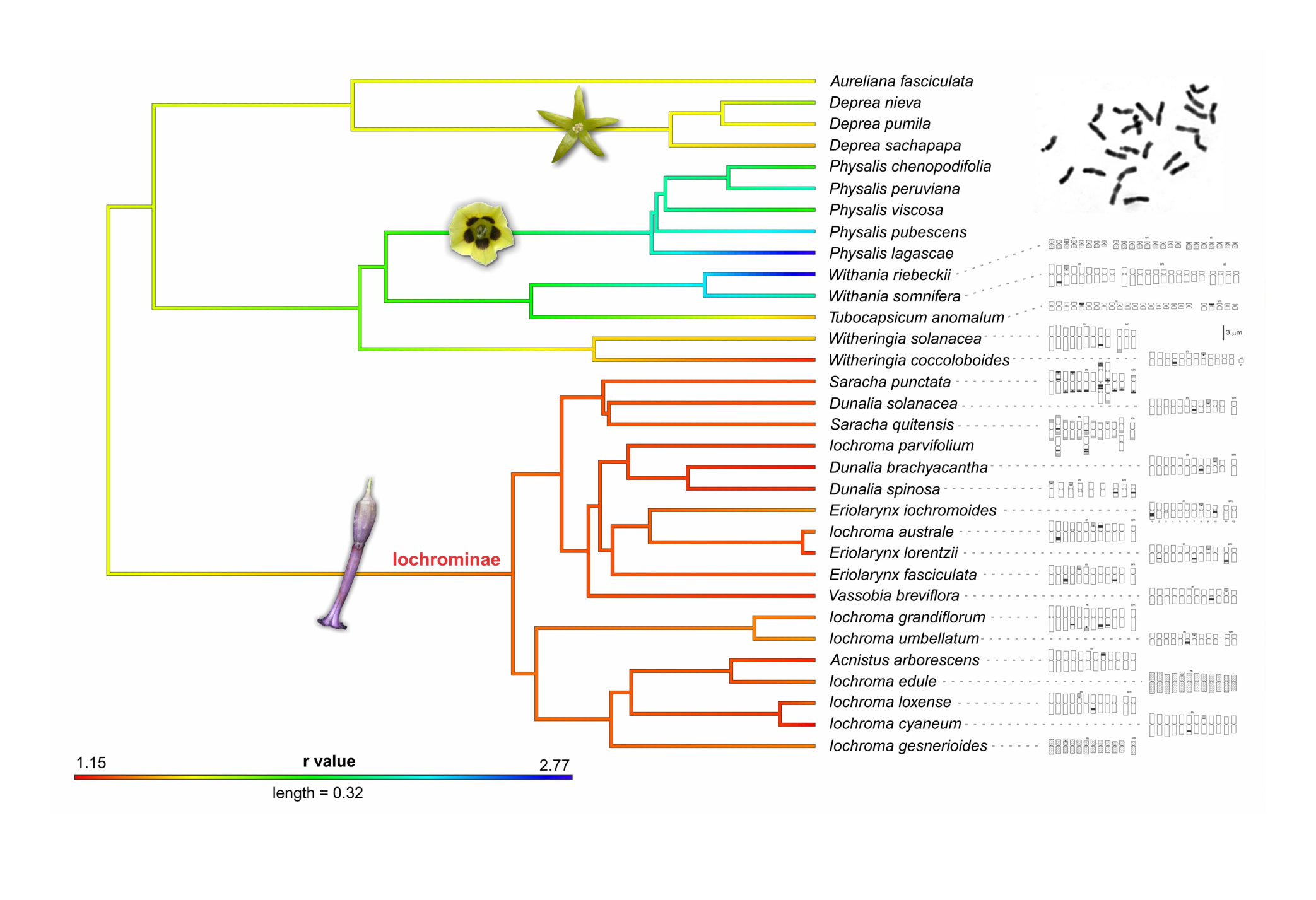
Highlights

- Iochrominae is a largely Andean clade known for its remarkable floral diversity
- Ancestral karyotypes were reconstructed into a molecular phylogeny
- Chromosomal changes are homoplastic, although combinations of traits identify groups within Iochrominae
- Iochrominae comprises species with highly symmetrical karyotypes, mostly 2n = 24
- Diversification of Iochrominae has not been accompanied by strong chromosome barriers


Aureliana fasciculata
Deprea nieva
Deprea pumila
Deprea sachapapa
Physalis chenopodifolia
Physalis peruviana
Physalis viscosa
Physalis pubescens
Physalis lagascae
Withania riebeckii
Withania somnifera
Tubocapsicum anomalum
Witheringia solanacea
Witheringia coccoloboides
Saracha punctata
Dunalia solanacea
Saracha quitensis
Iochroma parvifolium
Dunalia brachyacantha
Dunalia spinosa
Eriolarynx iochromoides
Iochroma australe
Eriolarynx lorentzii
Eriolarynx fasciculata
Vassobia breviflora
Iochroma grandiflorum
Iochroma umbellatum
Acnistus arborescens
Iochroma edule
Iochroma loxense
Iochroma cyaneum
Iochroma gesnerioides
Iochrominae
3 µm
1.15
r value
2.77
length = 0.32

Patterns of chromosomal evolution in the florally diverse Andean clade Iochrominae (Solanaceae)

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ABSTRACT

Iochrominae is a largely Andean clade known for its remarkable diversity of floral forms and colors. Although knowledge of chromosomal changes can provide insights into the processes underlying speciation, such data in Iochrominae are scant. We performed cytogenetic analyses to characterize chromosome number and morphology, CMA/DAPI heterochromatic bands, and distribution of rDNA sites in Iochrominae. Ancestral karyotypes were reconstructed on a newly-estimated molecular phylogeny in order to test congruence between karyotype evolution and clade differentiation. We found that, compared with its closest relatives, Iochrominae comprises species with highly symmetrical karyotypes, with no changes in base chromosome number. The common ancestor of Iochrominae was inferred to be a diploid with 2n = 24, with a karyotype with 0-2 submetacentric chromosomes and the rest metacentric, an arm ratio ca. 1.30, one locus of 45S or NORs, and one locus of 5S. Using phylogenetic comparative methods, we estimated the number of changes for these chromosomal traits, and found the highest for 5S loci. Patterns of character change are largely homoplastic, although combinations of traits can be useful to identify groups within Iochrominae. Asymmetry was the only character that allow us to differentiate this clade among its relatives. Overall, our study suggests that the diversification of Iochrominae has not been accompanied by the formation of strong chromosomal barriers, which may help to explain the crossability of many species and even genera within the group.

Highlights

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Key Words
asymmetry, chromosome, evolution, Iochrominae, karyotype, Solanaceae

1. Introduction

The tomato family Solanaceae includes a diversity of economically important species, such as potatoes, chili peppers, eggplants and tobacco. All of these crops belong to a major lineage within the family informally called the "x = 12 clade" (Olmstead et al., 2008). Its roughly 2300 species share chromosome numbers based on 12 pairs, including the giant genus *Solanum*, with c. 1000-1500 species (Bohs, 2005). The conservation of this base chromosome number suggests that the diversification of this clade across all continents (except Antarctica) over roughly ~~20.9~~21 million years ~~ago~~ (mya; Dupin et al., 2017; De-Silva et al., 2017) was not coupled with significant cytological evolution. Nonetheless, detailed cytological studies remain few, especially for diverse and poorly studied Neotropical groups. For example, there are only c. 52 chromosome counts for the entire tribe of tomatillo and its allies (Physalideae), comprising 29 genera and more than 200 species (Li et al., 2013).

Chromosomes provide valuable information to infer phylogenetic relationships and uncover synapomorphies, since they are hereditary elements of the whole nuclear genome and discrete hereditary units of mutation. The knowledge of the structural and quantitative characteristics of the karyotype has been proven to be useful in evolutionary and taxonomic studies in several angiosperm groups (Stebbins, 1971, 1985; Guerra, 2000; Weiss-Schneeweiss and Schneeweiss, 2013). Karyotype changes are relevant to plant speciation as chromosomal differences establish immediate postzygotic crossing barriers (e.g. Rieseberg, 1997) and are thus expected to be congruent with clade differentiation (e.g. Blöch et al., 2009). Therefore, karyological data provide another source of characters for understanding plant systematics, evolutionary patterns and divergence processes (Stace, 2000; Crawford et al., 2005). Combined with morphology, biogeography and molecular markers, cytogenetic traits can help identifying instances of hybridization and chromosome rearrangements involved in speciation (e.g. Weiss-Schneeweiss et al., 2008; Chiarini, 2014; Baltisberger and Horandl, 2016; Chiarini et al., 2016). Two techniques have been shown to be remarkably useful for such purposes: the FISH procedure, which allows homologous chromosomes in a complement to be differentiated and permits the comparison among related species, and the CMA/DAPI staining, which makes base-specific heterochromatin blocks visible. Both techniques, when combined with other markers, allow the detection of chromosome rearrangements.

Within the large x = 12 clade, there are examples of chromosomal uniformity (e.g. *Lycium* L., Stiefkens and Bernardello, 1996, 2000, 2002; Stiefkens et al., 2010) but also examples of chromosomal heterogeneity, such as in *Jaborosa* Juss. (Chiarini and Barboza 2008; Chiarini et al., 2016). In the latter genus, chromosomal heterogeneity, as well as morphological diversification, is likely related to the Andean uplift (Moré et al., 2015; Chiarini et al., 2016). Iochrominae (Miers) Hunz. is another morphologically diverse clade within Solanaceae whose radiation has been suggested to be related to the Andean orogeny

(Smith and Baum, 2006). According to Olmstead et al. (2008), Fernandez and Smith (2017), and Smith and Kriebel (2018), Iochrominae is a monophyletic subtribe comprising ca. 36 mainly Andean species traditionally assigned to six genera: *Acnistus* Schott, *Dunalia* Kunth, *Eriolarynx* (Hunz.) Hunz., *Iochroma* Benth., *Saracha* Ruiz et Pav., and *Vassobia* Rusby. Iochrominae, together with the subtribes Physalidinae (Miers) Hunz. and Withaninae Bohs & Olmstead, form the large monophyletic tribe Physalideae, which is sister to Capsiceae (De-Silva et al., 2017). Species within Iochrominae can be distinguished by the fact that are all woody shrubs or small trees and often have showy tubular flowers. Iochrominae shows a remarkable floral diversity, spanning a wide range of flower sizes, colors (red, orange, yellow, green, blue, purple, or white) and forms (rotate to tubular) (Shaw, 1998; Hunziker, 2001; Smith and Baum, 2006; Smith and Kriebel, 2018; Dodsworth et al., 2018). On the contrary, most taxa within Physalidinae, Withaninae, and Capsiceae have small, rotate, white or yellow flowers. Thus, the brightly coloured tubular flowers likely represent a derived feature that arose within or at the base of Iochrominae.

Taxonomy of Iochrominae has long been a source of confusion, at least in part due to the high degree of convergence in floral traits. Several authors have discussed the affinities of the genera that belong to the tribe (Olmstead et al., 1999; Sawyer, 2005; Hunziker, 2001; Whitson and Manos, 2005) but a consensus has not been reached. According to Smith and Baum (2006) and Gates et al. (2018), most genera of Iochrominae are not monophyletic. Moreover, Iochrominae has the potential for hybridization among species and across genera (Smith and Baum, 2007), an additional challenge to systematic studies. Such hybridization events are often recognizable by chromosomal rearrangements which could play a primary role in speciation events (White, 1978; Rieseberg, 2001). Several artificial hybrids between Iochrominae species have been generated, and some hybrid populations have been occasionally encountered in nature (Shaw, 1998; Smith et al., 2008). The ease of crossing, the overlapping species ranges, and the observation of natural hybrids suggest that hybridization may have had an important role in the evolutionary history of Iochrominae. Nonetheless, cytological variation has scarcely been explored in this clade beyond traditional chromosome counts [three species of *Iochroma* (Ratera, 1961; Moscone, 1992), *Vassobia* ~~*brevifolia*~~ *breviflora* (Hunziker et al., 1985), *Acnistus arborescens* (Heiser, 1963), three species of *Dunalia* (Dillon and Turner, 1980; Smith and Leiva González, 2005), *Saracha punctata* (Chiarini et al., 2010) and two species of *Eriolarynx* (Moscone, 1992)]. Fluorescent banding and FISH techniques have only been applied to *V. brevifolia* (Rego et al., 2009).

Considering this background, the aims of this work are: 1) to describe and characterize cytogenetically the tribe Iochrominae and related genera, and 2) to test relationships between chromosomal trait evolution and clade differentiation within Iochrominae and Physalideae. In order to do this, ancestral karyotypes were reconstructed using a molecular phylogeny based on plastid and nuclear markers ~~and~~ and this framework was used to examine the congruence between karyotype evolution and the ~~phylogeny~~phylogenetic relationships, as has been observed in other angiosperms (e.g. Blöch et al., 2009; Baltisberger and Hörandl, 2016). In addition, w~~W~~e ~~use this phylogenetic framework~~

~~to~~estimated ~~infer~~ the number of changes in various chromosomal traits, as these features are expected to experience different evolutionary dynamics. Given the important role of hybridization at interspecific and intergeneric level in the evolutionary history of Iochrominae (Smith and Baum, 2006; Shaw, 2018) in contrast to related genera of Physalideae, we predict that karyological features may be more homogeneous among the genera within Iochrominae than among other genera of this tribe. We finally discuss the possible role of karyotype differentiation for establishment of crossing barriers by comparing patterns of hybridization of extant species within Iochrominae.

2. Materials and methods

2.1. Plant material

The provenance of the plant material used for cytogenetic and phylogenetic studies is presented in Table A.1. Voucher specimens were identified by the four authors. The ingroup comprised of 50 species, 36 belonging to Iochrominae subtribe, three species of *Deprea* Raf., one species of *Aureliana* Sendtn., five of *Physalis* L., two of *Withania* Pauq., the monotypic *Tubocapsicum* (Wettst.) Makino, and two species of *Witheringia* L'Hér. The outgroup included three taxa, representing *Lycianthes* (Dunal) Hassl., *Capsicum* L. and *Salpichroa* Miers.

2.2. Karyotype analyses, classical staining

Mitotic chromosomes were examined in root tips obtained from seeds germinated in Petri dishes. Root tips were pre-treated in saturated p-dichlorobenzene in water for 2 h at room temperature, fixed in 3:1 ethanol/acetic acid mixture, washed in distilled water, digested with PECTINEX ® (45 min at 37 °C), and squashed in a drop of 45% acetic acid. Only one root tip was used in each slide. After coverslip removal in liquid nitrogen, the slides were air dried and stored at –20 °C. Some of these slides were used for classical staining with Giemsa. The remaining stored slides were used for determining the location and number of rDNA sites by FISH, and for CMA/DAPI banding.

Permanent mounts were made following the method of Bowen (1956). At least ten metaphases per species were photographed with a phase contrast optic Axiophot microscope. The microphotographs were used to measure for each chromosome pair: s (short arm), l (long arm), and c (mean total chromosome length). The arm ratio (r=l/s) was used to classify the chromosomes as either metacentrics (m), submetacentrics (sm) or subtelocentrics (st), according to Levan et al. (1964). In addition, total haploid chromosome length of the karyotype, based on the mean chromosome length (TL), average chromosome length (c), and average arm ratio (r) were calculated. Idiograms were based on the mean values for each species. Chromosomes were arranged first into groups according to their increasing arm ratio and then according to the decreasing length within each group. Karyotype asymmetry was estimated using the intrachromosomal (A_1) and the interchromosomal (A_2) indices of Romero Zarco (1986). Satellites were designated according to Battaglia (1955) and their lengths were added to those of the corresponding arms.

2.3. CMA/DAPI banding

~~After pre-treatment with para-dichlorobenzene and fixation on 3:1 ethanol:acetic acid mixture, root tips were washed twice in distilled water (10 min each), digested with 2% cellulase 20% pectinase solution (30 min), and squashed in a drop of 45% acetic acid. Only one root tip was used in each slide.~~ After coverslip removal in liquid nitrogen, the slides were aged for three days, stained with chromomycin A_3 (CMA) for 90 min and subsequently with 40-6-diamidino-2-phenylindole (DAPI) for 30 min, and finally mounted in McIlvaine's buffer–glycerol v/v 1:1 (Schweizer, 1976).

Commented [1]: This is already described in the 2.2 section. The mention of the cellulase-pectinase solution was an error.

2.4. Fluorescent in situ hybridization

The location and number of rDNA sites were determined by FISH using two probes: the pTa71 containing the 18-5.8-26S (henceforth 45S) gene of wheat (Gerlach and Bedbrook, 1979) labeled with biotin-14-dATP (BioNick, Invitrogen Carlsbad) and a 5S rDNA fragment obtained by PCR from *Solanum stuckertii* Bitter using the primers 5S rDNA-3 (5′-GTG CTT GGG CGA GAG TAG TA-3′) and 5SrDNA-4 (5′-GGT GCG TTA GTG CTG GTATG-3′; Fulnecek et al., 1998), and then labeled with digoxigenin-11-dUTP (DigNick, Roche). The FISH protocol was according to Schwarzacher and Heslop-Harrison (2000), with minor modifications. The preparations were incubated in 100 µg / ml RNAase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70-100% graded ethanol series, and air-dried. On each slide, 15 µl of hybridization mixture was added (4-6 ng/ µl of probe, 50% formamide, 10% dextran sulfate, 2x SSC and 0.3% SDS), previously denatured at 70°C for 10 min. Chromosome denaturation/hybridization was done at 90°C for 10 min, 48°C for 10 min, and 38°C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany), and slides were placed overnight in a humid chamber at 37° C. The 45S probe was detected with avidin-FITC conjugate (Sigma-Aldrich), the 5S probe was detected with antidigoxigenin-rhodamine (Roche), and then counterstained and mounted with 25 µl antifade Vectashield® (Vector Lab.), containing 1.5 µg / ml of DAPI. At least 10 metaphases of each species, and from at least three different individuals were photographed with a Zeiss Axiophot microscope equipped with epifluorescence and a digital image capture system. The free software ImageJ (http://rsbweb.nih.gov/ij/) was used for merging the images.

2.5. Molecular phylogenetic analyses

Total DNA was extracted for *Physalis* and *Withania* species either from silica-dried young leaves or from herbarium material (MO), whereas the other DNA samples were kindly provided by R. Olmstead and L. Bohs. New sequences were generated according to the protocols of Deanna et al. (2017, 2018) for ITS and *waxy*, and Smith and Baum (2006) for *LEAFY*. Sequence quality was inspected using GENEIOUS v4.6.1 (Drummond et al., 2009). Previously published sequences were incorporated (Table A.1), and alignments were performed in MEGA 6 (Tamura et al., 2013) using the MUSCLE algorithm (Edgar, 2004). Each gene was analyzed individually with maximum likelihood (ML) in RaxML v.8 (Stamatakis, 2014), using GTR + GAMMA model of sequence evolution. All genes were concatenated in SequenceMatrix 1.8 (Vaidya et al., 2011), and then, a partitioned maximum likelihood analysis was also performed in RaxML. Nodal support was assessed

with 1000 ML bootstrap replicates using the rapid Bootstrap (BS) algorithm. Analyses were run on CIPRES Portal to reduce the execution time (Miller et al., 2010). The resulting ML tree was then ultrametricized using semiparametric penalized likelihood with the chronopl function in the {ape} R package and a smoothing parameter of 1 (Sanderson, 2002; Paradis et al., 2004).

2.6. Ancestral States Reconstructions and Phylogenetic Principal Components Analysis

We reconstructed the evolution of four discrete chromosomal features on the combined ultrametricized ML tree, using the ace function from the {ape} package (Paradis et al., 2004) and stochastic mapping using the make.simmap function from the {phytools} R package (Revell, 2012), in R version 3.4.2 (R Core Team, 2017). The features coded as discrete characters were chromosome number, karyotype formulae, number of 45S loci/nucleolar organizer regions (NORs), and number of 5S loci. Given that many of these features had a large number of states, we coded the data in three or fewer states (e.g. one, two or many 5S rDNA loci) in order to limit the number of model parameters for this relatively small clade. For the rDNA loci, we also conducted reconstructions treating the data as continuous in order to visualize increases and decreases in number (Table A.2, Fig. A.5). For discrete variables, character history was traced either under a model where all transition rates were equal ('ER' model) or different ('ARD' model). We used a modified model for chromosome number with the condition that the transition rate from polyploid to diploid is 0 given the low probability of reversions in these shallow timescales. These models of character evolution were fit using the ace function from the {ape} package in R, and compared using a likelihood ratio test (Paradis et al., 2004). Next, we conducted a Bayesian stochastic character mapping (Huelsenbeck et al., 2003; Nielsen, 2001), with 1000 simulations of character histories on the combined ML tree. Data completeness varied across the species, but the mapping was performed for all the species considering the unknown data as ambiguous and inferring the states for these tips during the reconstruction. We estimated median number of changes per transition, generally preferred over means for non-normal distributions, and the 95% credibility interval using the hdr function from the {diversitree} package in R (FitzJohn, 2012).

For chromosome number, we also estimated character history using ChromEvol (Mayrose et al., 2010; Glick and Mayrose, 2014), which was developed precisely to model the evolution of ploidy. As implemented in RASP 3.2. (Yu et al., 2015), we inferred the ancestral haploid chromosome numbers in the tribe, the location of chromosome number changes, and the total number of changes in ploidy across the phylogeny. All models were tested and compared on their likelihood values (AIC, Akaike, 1974). We set the base chromosome number as 12, the rate base number as 1, the maximal chromosome number as 120 (-10 according RASP settings), and the minimal chromosome number as 12 (1 according RASP settings). The base-number was kept fixed and 10,000 simulations were performed.

The remaining cytogenetic characters (percentage of heterochromatin, haploid karyotype length (L), arm ratio (r), and total number of rDNA sites) were coded as continuous characters (for character matrix, see Supplementary data, Table A.2). These characters were mapped and plotted onto the combined ML tree, after pruning the taxa with no data,

and ancestral character states were estimated through a ML-based procedure assuming that characters evolve under a Brownian motion model. The mapping was carried out using ContMap function in the {phytools} package (Revell, 2012) for R version 3.4.2 (R Core Team, 2017).

Phylogenetic signal, as Blomberg et al. (2003)'s K-statistic, was calculated for each continuous trait using phylosignal function from the {picante} R package (Kembel et al., 2010). Higher values of K indicate increasing phylogenetic signal, with a value of one corresponding to the covariance expected under Brownian motion evolution. We tested whether K was significantly different from one comparing to inferred K values to K values from 10,000 simulations of Brownian trait evolution, implemented in the the fastBM function in the {phytools} R package (Revell, 2012). We also tested whether K was different zero (no signal) by comparing the estimated K values from 10,000 null models with tip values shuffled randomly (Kembel et al., 2010).

Finally, we conducted phylogenetic multivariate analysis to visualize variation across the tips and to test for correlations between the four previously mentioned continuous traits. We used phylogenetic PCA (pPCA), with the function phyl.pca and using Pagel's λ in the {phytools} package, which corrects for the non-independence of observations (Revell, 2009).

3. Results

3.1. Phylogenetic relationships

Physalideae and Iochrominae are resolved as a strongly supported monophyletic tribe and a subtribe, respectively (BS = 100). Within Iochrominae, all the relationships were recovered with similar supports to Fernandez and Smith (2017) (Fig. 1) and confirming that, among of the six genera traditionally proposed for the tribe, only *Vassobia* is monophyletic. Sister to Iochrominae, a poorly supported clade (BS = 67) includes Physalidinae and Withaninae species, a relationship recovered with higher support in a more densely sampled phylogeny of Physalideae (BS = 89; Deanna et al., in prep.). Although these two subtribes are not resolved as monophyletic, incongruences with generic classification were not found. One well-supported clade (BS = 87) includes *Deprea* and *Aureliana*, and its sister highly supported group (BS = 100) comprises *Witheringia*, *Tubocapsicum*, *Withania*, and *Physalis*.

3.2. Chromosome numbers and morphology.

Somatic chromosome numbers were assessed for 20 samples and 19 species of Iochrominae and 11 species of the sister clades (Table 1, Fig. A.3, see Supplementary data). Numbers are all based on x =12. Most Iochrominae species are diploids with 2n = 24, except for *D. spinosa*, *I. fuchsioides* and *I. parvifolium*, which are tetraploids with 2n = 48. Polyploids were also found among the sister clades, including two species of *Physalis*, all the *Withania* analysed, and *Tubocapsicum anomalum* (Table 1).

All species showed one chromosome pair with a satellite, except the tetraploid species which presented two pairs. Satellites were always located at the short arm of one of the m pairs with ordering number between 3 and 10 (Fig. A.3, Fig. 1)

Iochrominae species studied were relatively homogeneous in chromosome size (3.44 µm in average), with values of average total chromosome length (c) ~~c values~~ around 2.25 – 4.56 µm (Table 1). The mean smallest chromosomes were found in *I. umbellatum* (2.25 µm) and the largest in *I. grandiflorum* (4.56 µm), which represents a 2.03-fold difference. The absolute largest chromosome was recorded in *I. grandiflorum* (5.46 µm) and the smallest in *D. spinosa* (1.61 µm).

Karyotypes of Iochrominae are remarkably symmetrical, composed entirely by metacentric chromosomes or with one or two submetacentric pairs (Table 1). There are neither marked differences in size among the chromosomes of the same complement (A_1 ranged from 0.116 to 0.246) nor notable differences among arm lengths within single complements (A_2 from 0.083 to 0.135). The overall mean arm ratio (r) was 1.27 (range = 1.15-1.38; Table 1), corresponding to an m chromosome. On the other hand, members of Physalidinae and Withaninae showed moderately to markedly asymmetrical karyotypes (Table 1).

3.3. CMA-DAPI Banding.

Heterochromatin percentage, measured in 14 species of Iochrominae, varied from 1.10 % in *E. lorentzii* to 20.87 % in *S. punctata* with a mean value of 5.60 % (Table 2). Additionally, heterochromatin content for two *Withania*, *Tubocapsicum anomalum*, *Witheringia solanacea*, and *Physalis viscosa* are presented. Chromosome banding revealed three different heterochromatin types: 1) a strong pair of CMA^+ signals (corresponding to GC-rich heterochromatin regions) associated with the secondary constrictions (i.e., NORs) in terminal positions, which were observed in all species recorded, 2) additional $CMA^+/DAPI^-$ heterochromatin blocks not associated with NORs and located in interstitial regions were detected in five species of Iochrominae; the number of these bands varied from one to two pairs (only in *Dunalia brachyacantha*), 3) additional $CMA^+/DAPI^-$ heterochromatin blocks not associated with the NOR and located in terminal or subterminal regions were observed in seven species (Fig. A.4). The number of these bands varied from one pair in *E. fasciculata* to 19 pairs in ~~*Saracha*~~ *S. punctata* (Fig. A.4).

3.4. 45S and 5S rDNA genes.

In the diploid species, two terminal sites (one pair) strongly marked with the 45S rDNA probe were found (Fig. 1-2; Table 2), which coincide with a $CMA^+/DAPI^-$ block and with a secondary constriction, while in the tetraploid species, four sites (two pairs) were found. The exceptions are *S. punctata* and *S. quitensis*, which present dispersion of the 45S signal across several chromosomes (Fig. 2, Table 2). The morphology of NOR-bearing chromosomes and the localization of the 45S loci was variable: the signal was located either in a metacentric or a submetacentric chromosome, and the size of this chromosome also varied among species. The two species of *Saracha* are also peculiar in having heteromorphic chromosome pairs, with some signals just in one of the homologues (Fig. 1-2).

The hybridization signals obtained with the 5S rDNA probe were one pair for most diploid species, except for *E. iochromoides*, *I. australe* and *S. quitensis* (two pairs of signals, Fig. 2*), I. grandiflorum* and *E. lorentzii* (three pairs, Fig. 2), and *S. punctata* (11 pairs, Fig. 2). The position of these signals was subterminal and/or interstitial, and placed either in the

short or in the long arm, in a metacentric or submetacentric chromosome (Fig. 2). *Iochroma edule* and *S. punctata* are remarkable for having signals for the two types of probe in the same chromosome, in the rest of the species the 5S sites are non-syntenic (i.e. located in different chromosomes, according to Tang et al., 2008) with respect to the 45S sites. After the FISH procedure, terminal DAPI$^+$ bands were visualized in almost all species (Fig. 1-2) in both arms of all chromosomes of the complement, but the intensity of such bands varied notably among cells and individuals, and, for this reason, these bands are not represented in the idiograms of figure 1. However, the presence of an interstitial after FISH DAPI$^+$ band was consistently noticed in three species, as shown in Fig. 2.

3.5. Ancestral States Reconstruction

A symmetric diploid karyotype with at least three quarters of metacentric chromosomes and only one pair of 45S and 5S loci was the most likely ancestral state in Iochrominae (Fig. 3, Table 3, Fig. A.6). Stochastic character mapping estimated that the character with the largest number of changes was the amount of 5S loci, with 24 changes, whereas the most static character was the chromosome number. Total time spent per state, median number of changes per transition, 95% credibility interval of number of changes and median total number of changes are presented in Table 3. Results for characters mapping of continuous characters are represented with heatmaps (Fig. 4B, Fig. A.5). In the case of arm ratio (r) it clearly differs among clades, with an estimated value around 1.30 for the ancestor to all Iochrominae (Fig. 4B). For two of the four traits (average arm ratio and heterochromatin percentage), the Blomberg's K was significantly different from zero, but not from one, indicating phylogenetic signal in the pattern of asymmetry and heterochromatin amount (Table 4). The remarkable symmetric karyotypes of Iochrominae in comparison to the members of sister clades of Physalideae are shown in Fig. 1 and asymmetry indices in Table 1.

3.6. Trait correlation

The phylogenetic PCA included Physalideae species with available information on total haploid chromosome length (LT), average arm ratio (r), ribosomal DNA loci amount (rDNA), and total heterochromatin percentage (het) and, illustrates the karyological-cytogenetic variation within the tribe (Fig. 4A). The first two PC axes account for 93% of the total variation. Variation along the first pPC is highly correlated with TL (loading = 0.997), while spread along the second pPC relates to variation in het and rDNA (loadings= -0.953 and -0.812, respectively). By contrast, r varies little across the species (Table 1) and does not load significantly on either PC axis.

4. Discussion

Chromosome numbers. Our data in Iochrominae confirm the meiotic numbers previously found in *E. iochromoides* and *E. lorentzii* (Moscone, 1992), *V. breviflora* (Ratera, 1943; Hunziker et. al, 1985; Rego et al., 2009), *D. brachyacantha* (Moscone, 1992), *I. australe* (Moscone, 1992) and *A. arborescens* (Heiser, 1963; Diers, 1961), whereas the remaining species are reported for the first time. These numbers are consistent

with other species of the clade: *D.~~unalia~~ obovata* (Ruiz Pav.) Dammer n = 12 (Dillon and Turner, 1980), *D. tubulosa* (Benth.) J. F. Macbr., n = 12 (Mehra and Bawa, 1969) and *D. spathulata* (Ruiz Pav.) Braun Bouché, n = 12 (Smith and Leiva González, 2005). Numbers of the Physalidinae and the Withaninae species also confirm previous reports and are diploids or polyploids based on x =12 (Table 1). These patterns support the conclusion that x = 12 is the basic number of the tribe (Badr et al., 1997; Rego et al., 2009; Barboza et al., 2010; Chiarini et al., 2010, Deanna et al., 2014). Polyploidy, the only numerical alteration found, seems not to be abundant in Iochrominae: it was found in three species, which represents 13 % of the total of species with chromosome numbers reported ~~up~~ to date. This pattern differs from the Withaninae, since *Tubocapsicum anomalum* and most *Withania* species are polyploids. Polyploidy seems to be also frequent in Physalidinae: eight species of *Physalis* of the 25 species examined in this or previous studies are polyploids (tetraploids or hexaploids, Menzel, 1950, 1951). Also in this clade, *Quincula lobata* (x = 11) has diploid and tetraploid populations with 2n = 22 or 2n = 44 (Menzel, 1950). Thus, the available data suggest that Iochrominae is more conservative with 2n = 24 compared to its close relatives.

~~The effects of chromosome number and nuclear DNA content also manifest at the ecological and evolutionary scales (Henry et al., 2015). Zenil-Ferguson et al. (2017) found that, although polyploidy occurs in woody species, rates of chromosome doubling are over six times higher in herbaceous species across eudicots. This could be due to the slower rates of molecular evolution or longer average generation time in woody species (e.g., Smith and Donoghue, 2008). Moreover, a focused study on *Solanum* inferred~~

Rates of chromosome doubling. Our results show more polyploidy events in Withaninae and Physalidinae than in Iochrominae. Considering that Iochrominae are woody perennials, whereas Withaninae and Physalidinae are mostly herbs, we support the idea that polyploidy is more frequent in herbaceous rather than in woody species, as proposed by Zenil-Ferguson et al. (2018). Further studies in sister clades of Iochrominae, such as chromosome counts in other woody and herbaceous Physalideae (*Aureliana*, *Deprea, Nothocestrum* vs. *Chamaesaracha*, *Physalis*, respectively), will provide stronger insights into this evolutionary pattern.

Chromosome size. Solanaceae is not a family that stands out for strong differences in chromosomal size, or in genome size, which are directly related. Other families of angiosperms show up to 20-fold differences between and within genera (Greilhuber et al., 2006). In the context of flowering plants, the mitotic chromosomes of Iochrominae are small (Guerra, 2000), but relative to other genera of Solanaceae, they are intermediate (Badr et al., 1997). In fact, the lengths found are between the records for *Solanum* (1–3.5 µm: Bernardello and Anderson, 1990; Acosta et al., 2005; Chiarini and Bernardello, 2006) and *Cestrum* (c= 6–10 µm: Badr et al., 1997; Sykorova et al., 2003). There were no large increases in chromosomal size during the differentiation of the Physalideae analyzed. The range of chromosome size recorded here for Withaninae (1.48–5.47µm) and Physalidinae (2.36–4.08 µm) overlaps with that of Iochrominae (2.24–4.56 µm). Thus, size does not appear to be a useful feature for distinguishing the clades.

Although chromosome size has been predicted to co-vary with life history, the data in Solanaceae do not seem to follow that pattern (Stebbins, 1971). ~~For example, Stebbins~~

~~(1971) proposed that perennial species are likely to have smaller chromosomes, but without a solid explanation to account for this phenomenon. Indeed, p~~Previous studies have found substantial variation in chromosome size within woody Solanaceae (~~e.g. small in *Lycium*,~~ Stiefkens and Bernardello, 1996, 2000, 2002; ~~*Lycianthes*,~~ Acosta et al., 2005 ~~or medium-sized in *Saracha punctata*, *Latua pubiflora*~~, Chiarini et al., 2010, 2018). ~~Also, within *Solanum*, small to medium-sized chromosomes were found among annuals, perennials, herbs, trees and vines (Chiarini et al., 2018).~~ A range of ~~other~~ factors, such as the rate of DNA replication, the duration of the life cycle and recombination rates, may ~~also~~ contribute to this variation (Soltis and Soltis, 1987; Turney et al., 2004; Nakazato et al., 2006) but further studies of Solanaceae are needed to test their importance individually and in combination.

Karyotype features. Iochrominae, like the genus *Lycium* (Stiefkens and Bernardello, 1996, 2000, 2002; Stiefkens et al., 2010), comprises woody perennial species with constant and little diversified karyotypes, all features formerly regarded as ancestral (Stebbins, 1958, 1971; Brandham, 1983). Some authors have proposed a "karyotype orthoselection" for the maintenance of complements formed by chromosomes of approximately the same length, with median or submedian centromeres (Brandham and Doherty, 1998; Moscone et al., 2003). However, it is not an easy task to establish the direction of karyotype evolution, since many reversals of character states might have occurred (Stace, 2000; Mandáková and Lysák, 2008). In Solanaceae, when data of karyotype symmetry were interpreted in relation to the later phylogenetic hypotheses, the resulting picture is complicated, with values of symmetry changing back and forth (Chiarini et al., 2018). Within the x = 12, various clades have followed different evolutionary paths, with examples of uniform and relatively asymmetrical formulae (*Capsicum*, *Physalis*, Menzel, 1950, 1951; Chiarini, unpublished data); uniform, symmetrical formulae (*Lycium*, Stiefkens and Bernardello, 1996, 2000, 2002; Stiefkens et al., 2010); or heterogeneous asymmetrical formulae (*Jaborosa*, Chiarini et al., 2016).

Subtelocentric and telocentric chromosomes are relatively unusual in the Solanaceae (e.g. Goodspeed, 1954; Bernardello and Anderson, 1990; Acosta et al., 2005; Chiarini et al., 2018). The presence of these chromosomes in the five species of *Physalis* here studied is remarkable and constitutes a distinctive feature. In a general survey of the family Solanaceae, Badr et al. (1997) reported values of r ranging from 1.17 to 2.78, while our records for *Physalis* (r =1.87–2.77), together with those recovered from the literature (Menzel, 1950, 1951; Rodríguez and Bueno, 2006), showed ~~remarkable~~ even higher r values, as also asymmetry indices. This observed pattern ~~on~~ of intrachromosomal asymmetry showed strong phylogenetic signal, where ~~clade~~ Iochrominae presents karyotypes highly symmetrical in comparison to its sister clade, suggesting that asymmetry evolution is congruent with clade differentiation in this group. In fact, the common ancestor reconstructed for Physalidinae and Withaninae had an asymmetrical formula, while the ancestor of the Iochrominae had a symmetrical one. Hence, karyotype differentiation among the clades of Physalideae would have taken place early in the evolutionary history, with karyotype evolution possibly being a significant factor of speciation and differentiation of clades within this tribe.

Karyotypes and hybridization. There is evidence pointing out a relationship between karyotypes and crossability in various plant clades (Baltisberger and Hörandl, 2016). Extant species with divergent karyotypes should not be able to cross, whereas species with the same karyotype should be able to produce hybrids. A review of crossing experiments and interspecific homoploid hybridization in sympatric species of *Ranunculus* (Baltisberger and Hörandl, 2016), showed enhanced crossability of species with the same karyotype and strong crossing barriers between those with different karyotypes and concluded that karyotype evolution is a major driver of diversification. In Solanaceae, two species of *Lycium* with markedly different corolla shapes, but with the same karyotype formula, were able to cross, producing a hybrid with intermediate morphology (Bernardello et al., 1995). Species of Iochrominae are known for their capacity to produce fertile hybrids, which makes the group ~~a~~ popular for breeding and gardening. It appears that many species have the potential of crossing with each other: of 21 reciprocals pairwise crosses involving seven different species, only two failed to yield viable seed (Smith and Baum, 2007). The similarity in the karyotypes would allow two species to cross, without the need for a subsequent chromosomal duplication to establish the resulting hybrid. The existence of introgression among species with the same chromosome number and the production of homoploid hybrids in the nature has been demonstrated at least in one case (Smith et al., 2008). Thus, the diversification of Iochrominae has not been accompanied by the formation of strong chromosome barriers. Rather, post-germination factors, such as reduced hybrid fitness, and/or pre-mating factors, such as allopatry and ethological isolation, might have acted to maintain the morphological and evolutionary cohesiveness of Iochrominae species (Muchhala et al. 2014).

rDNA loci. In Physalideae, as well as in other angiosperms (Moreno et al., 2015; Van Lume et al., 2017) and Solanaceae (Chiarini et al., 2016, 2018), the number and position of rDNA loci are highly homoplastic. However, in a survey of 45S rDNA loci number and distribution, Roa and Guerra (2012) concluded that the most frequent numbers of sites per diploid karyotype were two and four, and that they often occur at terminal positions (45%), usually within the short arms. According to our data, Physalideae follows this general trend, with the exception of *S. punctata* and *S. quitensis*, which have small terminal signals dispersed in most chromosomes of the complement. This dispersion type has also been observed in other Solanaceae, such *Jaborosa* (Chiarini et al., 2016) and *Cestrum* (Urdampilleta et al., 2015). In the other hand, 5S rDNA sites seem to have a different behavior: Roa and Guerra (2015) found that, in most karyotypes (54.5%, including polyploids), two 5S rDNA sites (a single pair) are present, with 58.7% of all sites occurring in the short arm. Karyotypes with multiple sites and small chromosomes (<3 µm) often display proximal sites, while medium-sized (between 3 and 6 µm) and large chromosomes (>6 µm) more commonly show terminal or interstitial sites. Within Iochrominae, most species present a single pair, located either in terminal or in interstitial position of the medium-sized chromosomes. The amount of these rDNA sites within the Physalideae analyzed showed the highest number of changes, where transitions directed to increase the number of sites were the most common.

Synteny. Roa and Guerra (2015) found that adjacent 5S and 45S rDNA sites are frequently found in the short chromosome arm, reflecting the preferential distribution of

both sites in this location. Given the high frequency of genera with at least one species with adjacent rDNA sites, they suggested that this association arose several times during angiosperm evolution, but ~~it~~ has been maintained only rarely as the dominant array. Some groups within Asteraceae (Garcia et al., 2010; Mazzella et al., 2010) and mosses (Sone et al., 1999) are exceptional in having the two rDNA loci physically linked. However, in general terms, both the number and localization of 45S and 5S rDNA loci are largely independent from one another (Małuszyńska et al., 1998). The phylogenetic distribution of the linked arrangement suggests its recurrent origin and/or reversal (Garcia et al., 2010). Iochrominae follow the general pattern, with the two rDNA sites in different chromosomes, with the only exception being *I. edule*. The degree of synteny is a function of the time since their divergence, with translocation, inversion, and transposition being the main mechanisms of chromosome rearrangement. Disruption in conserved syntenic segments can be used to deduce the mechanisms of chromosome rearrangements that accompanied species divergence (Frary et al., 2016).

In the other hand, the dispersion of both rDNA genes in the *Saracha* species suggests profuse chromosomal rearrangements. A similar situation has been detected in other Solanaceae, such as *Jaborosa* (Chiarini et al., 2016) and *Cestrum* (Urdampilleta et al., 2015). Mobile elements, which are activated by certain kinds of stress, may be responsible for such dispersion (Raskina et al., 2008; Chénais et al., 2012). The situation is probably transient, since, genomes tend to eliminate redundant sequences (Kotseruba et al., 2010). An analysis of the 45S rDNA of *Nicotiana* showed that parental loci were maintained in newly formed polyploids, although the sequences within a locus might be subject to concerted evolution, and over time periods of one million years or more, individual loci would disappear (Kovařík et al. 2008).

Chromosome evolution. Different chromosomal traits may present contrasting evolutionary patterns, suggesting different underlying ~~evolutionary~~ dynamics (e.g. Volkov et al., 2017). Our results in Physalideae reveal such different patterns of evolution across the rDNA genes, with the 45S site being more stable than the 5S. In the span of at least 6 mya (~~according to~~ De-Silva et al., 2017), there were six dispersion events of 45S sites, whereas the number and position of the 5S sites underwent more frequent changes. A similar situation was observed in *Jaborosa* (Chiarini et al., 2016) and *Solanum* (Chiarini et al., 2018), whereas in *Lycium* both rDNA sites seem to be stable (Blanco et al., 2012). While most changes in chromosomal features (e.g. chromosome number, karyotype formula, and 45S rDNA loci) presented similar number of changes (seven total changes per trait, Table 3), the 5S rDNA loci stood out as having higher number of gains (11 gains, considering from one to two or more pairs, Table 3). The bias towards gains of rDNA loci could relate to processes including unequal recombination, transposition, and conversion/homogenization of repeats among loci (Hemleben et al., 2004; Raskina et al., 2008; Volkov et al. 2017) or the multiplication of transposable elements (Raskina et al., 2004; Datson & Murray, 2006; Evtushenko et al., 2016).

Karyotype evolution is congruent with major morphological features. Specific karyotypes characterize the subtribe Iochrominae, which is separated from Physalidinae and Withaninae based on morphological characters and their phylogenetic position. Iochrominae are woody shrubs or treelets, with a calyx slightly or non-accrescent in the

fruit, while the sister clades are herbs, with different degrees of calyx accrescence in the Withaninae and with a dramatic inflated calyx in *Physalis*. Ecological preferences and geographical ranges also separate these groups (Smith and Baum, 2006). Although a cause-effect relationship cannot be drawn, karyotype differentiation of major clades might prevent hybridizations and allow the fixation of character combinations specific to each clade.

Taxonomic implications. As previously demonstrated, only *Vassobia* of the six traditional genera of Iochrominae is monophyletic (Smith and Baum, 2006). In addition, karyological features are very homogeneous, and hybridization among genera is probably occurring in nature (Smith and Leiva, 2011). In contrast, the sister clade includes monophyletic genera (such as *Deprea*), longer branches on the tree, and the karyotype pattern is more diverse. While the delimitation of natural groups in Iochrominae could be achieved by transferring species among the genera or recognizing new genera (Shaw, 2016, 2018), the comparative lack of karyological variation and the crossability among genera suggest that combining the genera into a single monophyletic Iochroma may be the most stable solution. Additionally, the latter approach would provide easier diagnosis, as the genera within Iochrominae (as currently delimited) do not possess clear morphological or cytogenetic synapomorphies. Smith and Baum (2006) note that the clades within Iochrominae reflect geographical structure of the Andes (Smith and Baum 2006), but this factor is not sufficient to discriminate taxonomic groups.

Concerning the whole family Solanaceae, the chromosome number seems to be a more conserved character than the karyotype formula, and this in turn is more conserved than the number and position of the rDNA genes. This suggests that, despite there were profuse chromosomal rearrangements (evidenced by banding and FISH techniques), somehow these do not greatly affect the morphology of the karyotype, let alone the chromosome number. The causes of the conservation of a determined chromosome number (in this case x = 12, shared by a large number of species in the family) is a matter of discussion (Chiarini et al., 2018). However, the differences in chromosomal characteristics could be useful to define clades: the chromosome number for higher taxonomic hierarchies (e.g. subfamily) and the karyotype formula for lower hierarchical levels (tribes or subtribes).

5. Conclusions

The present study provides new insights into the genomic evolution in Iochrominae at the level of chromosomal traits. Clades can often be distinguished by their karyotype features (Urdampilleta et al., 2012; Hidalgo et al., 2017), and here we find that Iochrominae differs from other Physalideae in having remarkably symmetrical chromosomes. Within Iochrominae, however, chromosomal traits show weak correspondence to phylogenetic relatedness. Although some features, like the proliferation of 5S rDNA loci were restricted to subclades, all of the traits exhibited varying degrees of homoplasy, with multiple gains and losses across the group. Comparing across traits, we find a gradation from more to less conservative, as follows: chromosome number; number of 45S sites or NORs; karyotype formula; number of 5S loci, consistent with previous findings in *Solanum* (Chiarini et al., 2018). Ongoing chromosome studies on more members of Physalidinae and Withaninae

will provide further insights into karyological evolutionary patterns of the Physalideae tribe.

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No potential conflict of interest was reported by the authors.

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TABLES

Table 1. Chromosome data of the studied Physalideae taxa: sporophytic number (2n); karyotype formula; ordering number of the satellited pair (SAT); total haploid chromosome length of the karyotype in μm (TL); average total chromosome length in μm ± standard deviation (c); average arm ratio ± standard deviation (r); intrachromosomal asymmetry index (A_1); interchromosomal asymmetry index (A_2). *Data from a previous publication.

Species	2n	Karyotype formula	SAT	TL	c	r	A_1	A_2
Iochrominae								
Acnistus arborescens	24	12m	5	49.19 ± 3.37	3.85 ± 0.967	1.32 ± 0.05	0.218	0.111
Dunalia brachyacantha	24	11m + 1sm	10	40.42 ± 3.60	3.37 ± 0.131	1.18 ± 0.06	0.129	0.135
Dunalia solanacea	24	11m + 1sm	9	32.60 ± 0.18	2.80 ± 0.24	1.26 ± 0.05	0.179	0.084
Dunalia spinosa	48	22m + 2sm	14;15	77.82 ± 2.12	3.24 ± 0.38	1.23 ± 0.06	0.161	0.106
Eriolarynx iochromoides	24	10m + 2sm	9	37.29 ± 3.71	3.11 ± 0.71	1.38 ± 0.05	0.242	0.094
Eriolarynx lorentzii	24	10m + 2sm	9	38.64 ± 3.38	3.22 ± 0.27	1.32 ± 0.06	0.207	0.120
Eriolarynx fasciculata	24	11m + 1sm	4	42.74 ± 4.27	3.56 ± 0.35	1.26 ± 0.05	0.184	0.091
Iochroma australe	24	11m + 1sm	7	43.36 ± 3.37	3.62 ± 0.39	1.21± 0.05	0.156	0.120
Iochroma edule	24	12m	8	48.46 ± 4.55	4.04 ± 0.38	1.21 ± 0.03	0.148	0.112
Iochroma cyaneum	24	12m	8	53.76 ± 4.23	4.48 ± 0.35	1.15 ± 0.02	0.116	0.095
Iochroma fuchsioides	48							
Iochroma gesnerioides	24	11m + 1sm	3	32.32 ± 2.11	2.69 ± 0.18	1.34 ± 0.07	0.224	0.090
Iochroma grandiflorum	24	11m + 1sm	6	54.73 ± 10.51	4.56 ± 0.86	1.32 ± 0.05	0.221	0.126
Iochroma loxense	24	10m + 2sm	5	47.94 ± 3.87	3.99 ± 0.32	1.30 ± 0.06	0.191	0.101
Iochroma parvifolium	48	22m + 2sm	14;15	71.09 ± 2.60	2.97 ± 0.39	1.23 ± 0.03	0.163	0.135
Iochroma umbellatum (4796)	24	10m +2sm	4	32.37± 4.56	2.70± 0.38	1.36 ± 0.07	0.235	0.083
Iochroma umbellatum (4711)	24	10m +2sm	6	26.98 ± 3.21	2.25 ± 0.27	1.38 ± 0.06	0.246	0.098
*Saracha punctata**	24	11m + 1sm	-	49.75 ± 3.38	4.15 ± 0.33	1.26 ± 0.07	0.173	0.107
Saracha quitensis	24	11m + 1sm	9	44.86 ± 5.98	3.74 ± 0.50	1.27 ± 0.07	0.183	0.096
Vassobia breviflora	24	12m	9	35.74 ± 3.60	2.98 ± 0.20	1.21 ± 0.03	0.160	0.091
Withaninae and								

Physalidinae								
*Aureliana fasciculata**	24	9m + 2 sm + 1st	6	65.68 ± 12.65	5.47 ± 1.05	1.53 ± 0.10	0.272	0.159
Physalis chenopodifolia	24	6m + 4sm + 2st	12	38.05 ± 2.78	3.17 ± 0.23	1.87 ± 0.08	0.356	0.152
Physalis lagascae	24	9sm + 3st	8	30.00 ± 5.33	2.50 ± 0.44	2.77 ± 0.16	0.576	0.133
Physalis peruviana	48	12m + 10sm + 1st +1t	20	56.66 ± 6.57	2.36 ± 0.27	2.23 ± 0.32	0.338	0.186
Physalis pubescens	48	13m + 9sm + 1st +1t	19	60.27 ± 5.49	2.51 ± 0.23	2.36 ± 0.29	0.320	0.193
Physalis viscosa	24	6m + 4sm + 2st	12	38.95 ± 3.36	3.25 ± 0.28	1.87 ± 0.11	0.40	0.135
Tubocapsicum anomalum	48	19m + 5sm	17	35.41 ± 6.82	1.48 ± 0.28	1.44 ± 0.05	0.215	0.173
Withania riebeckii	48	8m + 9sm + 7st	3	44.01 ± 5.32	1.83 ± 0.22	2.76 ± 1.02	0.415	0.157
Withania somnifera	48	9m + 11sm +4st	3	80.54 ± 13.72	3.36 ± 0.57	2.19 ± 0.11	0.471	0.251
Witheringia coccoloboides	24	12m + B	1	29.32 ± 11.59	2.44 ± 0.40	1.21 ± 0.07	0.173	0.127
Witheringia solanacea	24	9m + 3 sm	10	49.00 ± 10.53	4.08 ± 0.88	1.46 ± 0.04	0.27	0.13

Table 2. Cytogenetic features in Physalideae species studied with fluorescent techniques. SC= secondary constriction; Int = intercalary band; T = terminal band. Parentheses indicate heteromorphic bands. * An extra CMA/DAPI band found only in one of the homologues.

	Pairs of $CMA^{+}/DAPI^{-}$ Bands			Total of bands pairs	Total of chromosome pairs with bands	Heterochromatin Percentage	Pairs of FISH signals			Dispersion of 45S	Sintenty of rDNA genes
	SC	Int	T				Main 45S loci	5S loci	DAPI after FISH bands		
Iochrominae											
Acnistus arborescens	1	-	10	11	8	7.73					
Dunalia brachyacantha	1	2	9	12	9	9.81	1	1	24	no	no
Dunalia solanacea	1	1	-	2	2	3.17	1	1	-	no	no
Dunalia spinosa	2	-	-	2	2	1.82	2	2	11	no	no
Eriolarynx fasciculata	1	-	1	2	2	3.76	1	2	22	no	no
Eriolarynx iochromoides							1	2	1	no	no
Eriolarynx lorentzii	1	-	-	1	1	1.10	1	3		no	no
Iochroma australe	1	-	-	1	1	1.77	1	2	18	no	no
Iochroma cyaneum							1	1	20	no	no
Iochroma edule	1	-	-	1	1	1.34	1	1	20	no	yes
Iochroma gesnerioides	1	-	-	1	1	1.17					

Iochroma grandiflorum							1	3	20	no	no
Iochroma loxense							1	1	23	no	no
Iochroma parvifolium	2	1	6	9	5	5.97					
Iochroma umbellatum 4711	1	1	-	2	2	2.56	1	1	19	no	no
Iochroma umbellatum 4796	1	1	-	2	2	2.41					
Saracha punctata	1	-	19	20	11	20.87					
Saracha quitensis	1	-	17*)	18	12	16.50	1	2		yes	yes
Vassobia breviflora	1	1	7	9	8	4.02 ±1.6	1	1	20	no	no
Withaninae and Physalidinae											
Physalis angulata							1	1		no	no
Physalis chenopodifolia							1	1		no	no
Physalis peruviana							1	2		no	no
Physalis pubescens							2	3		no	no
Physalis viscosa						22.89	1	1		no	no
Tubocapsicum anomalum	2	-	-	2	2	3.05	1	2		no	no
Withania frutescens	1	-	-	1	1	0.905					
Withania riebeckii	1	-	-	1	1	2.435					
Withania somnifera							1	1		no	no
Witheringia coccoloboides							1	1		no	no
Witheringia solanacea	1	-	-	1	1	0.875	1	1		no	no

Table 3. Summary of the Stochastic Character Mapping for discrete chromosomal traits. MT= percentage mean total time spent in each state, TC = median number of total changes, C = median number of changes per transition, (95% CI) = 95% credibility interval of number of changes, m = methacentric, sm = submethacentric, t = telocentric, st = subtelocentric. Most frequent transitions and most persistent states are in bold. *Modified model where transition rate from 1 to 0 is fixed to 0 (see methods).

Trait	Model	Character states	MT	TC	TC (95% CI)	Transition	C	C (95% CI)	State at the Iochrominae root
Chromosome number	MOD*	0 =diploid	88.58	7	3.10–9.14	0-> 1	7	3.10–9.14	0
		1 = polyploid	11.42						
Karyotype formula	ARD	0 = none, one or two sm chromosomes and the rest m	57.27	7	4.14-11.84	0-> 1	0	2.93–13.51	0
						0-> 2	0	-126.7–6.14	
		1 = more than two pairs sm and the rest m	17.66			1-> 0	1	-8.07–2.95	
						1-> 2	1	1.08–3.92	
		2 = one or more st or t, and the rest m or sm	25.07			2-> 0	1	-51.28–3.43	
						2-> 1	2	-3.97–2.41	
5S loci	ARD	0 = one pair	62.56	24	12.57-36.63	0-> 1	5	-0.70–12.09	0
		1 = two pairs	25.22			0-> 2	6	-1.32–11.44	
		2 = more than two pairs	12.22			1-> 0	4	-0.76–11.95	
						1-> 2	2	-0.48–7.83	
						2-> 0	2	-2.02–10.21	
						2-> 1	1	-9.37–6.80	

45S loci	ARD	0 = one pair	91.59	7	2.95-13.72	0-> 1	6	1.74–9.05	0
		1 = two or more pairs	8.41			1-> 0	1	-21.52–6.52	

Table 4. Summary of phylogenetic signal (Blomberg's K) for single continuous chromosomal traits. PICs: phylogenetically independent contrasts relative to tip shuffling randomization. P-values indicate whether the K-value is significantly different from zero (no phylogenetic signal) and/or from one (signal expected under Brownian Motion). P-values less than 0.05 are bolded.

Trait	Blomberg's K	P-value of observed vs. random variance of PICs (<0.05 means K significantly different to zero)	P-value of observed vs. variance of PICs fitted to Brownian motion evolution (<0.05 means K significantly different to one)
Total haploid chromosome length of the karyotype in μm (LT)	0.291	0.289	**0.004**
Average arm ratio (r)	1.052	**1e-4**	0.923
Heterochromatin percentage (het)	0.605	**0.020**	0.343
Number of ribosomal DNA loci (rDNA)	0.520	0.052	0.166

Appendix A

Table A.1. List of Iochrominae species and related genera included in this study, including details of voucher specimen, provenance, and GenBank accession numbers. GenBank numbers in bold are new for this study; "na" indicates either no voucher analyzed for cytogenetic/phylogenetic analyses or no sequence available for this region for this accession.

Species	Provenance of voucher specimen		GenBank accession numbers		
	Cytogenetic analyses	Phylogenetic analyses	ITS	LEAFY	waxy
Acnistus arborescens (L.) Schltdl.	BRAZIL. Paraíba, Pico do Jabre, Agra et al. 7079 (JPB) BRAZIL. Río de Janeiro, Barboza 800 (CORD).	COSTA RICA. Puntarenas, Las Cruces B. S., Bohs 2428 (UT).	DQ314173	DQ301528	DQ309483
Aureliana fasciculata (Vell.) Sendtn.	BRAZIL. Paraná, Morretes, La Graciosa, Barboza et al. 1630 (CORD).	BRAZIL. Paraná, Morretes, La Graciosa, Barboza et al. 1630 (CORD).	na	na	EF537144
		BRAZIL. São Paulo, Paraíso, Serra do Japi, Stehmann et al. 4790 (BHCB).	KC832786	na	na
Capsicum lycianthoides Bitter	na	ECUADOR. Pichincha, Smith 203 (WIS).	DQ314158	DQ309518	DQ309468
Deprea nieva (S.Leiva & N.W.Sawyer) Barboza &	PERU. Amazonas, Bongará, Deanna & Leiva González 43	PERU. Amazonas, Bongará, Deanna & Leiva González 46	KP267769	**MH304887**	KP267763

Deanna	(CORD, HAO).	(CORD).			
Deprea pumila (S.Leiva, Barboza & Deanna) S.Leiva	ECUADOR. Pastaza, Mera, camino al río Anzú, Orozco et al. 3890 (COL, CORD, QCA).		KX557320	**MH304886**	KX690189
Deprea sachapapa (Hunz.) S.Leiva & Deanna	ECUADOR. Cotopaxi, Reserva de Otonga, Orozco et al. 3983 (COL, CORD, QCA).	ECUADOR. Pichincha, Smith 205 (WIS).	DQ314166	DQ301519	DQ309476
Dunalia brachyacantha Miers	ARGENTINA. Córdoba, between Cruz del Eje and Punilla, Chiarini 708 (CORD). ARGENTINA. Tucumán, Chicligasta, Urdampilleta et al. 740 (CORD).	ARGENTINA. Jujuy, Nee & Bohs 50811 (NY).	DQ314172	DQ301527	DQ309482
Dunalia obovata (Ruiz & Pav.) Dammer	na	PERU. Junin, Smith 458 (WIS).	DQ314192	DQ301547	DQ309499
Dunalia solanacea Kunth	COLOMBIA. Huila, La Plata, Finca Meremberg, Orejuela & Deanna 2580 (JBB, CORD).	ECUADOR. Pichincha, Smith 211 (WIS).	DQ314174	DQ301529	DQ309484
Dunalia spathulata (Ruiz & Pav.) Braun & Bouché	na	PERU. Huanuco, Smith 452 (WIS).	DQ314198	DQ301554	DQ309506
Dunalia spinosa (Meyen) Dammer	PERÚ. La Libertad, Salpo, Leiva 5664 (CORD).	BOLIVIA. Potosi, Smith 379 (WIS).	DQ314188	DQ301543	DQ309495
Eriolarynx fasciculata (Miers) Hunz.	BOLIVIA. Chuquisaca, Zudañez, Moreno et al. 264	BOLIVIA. Cochabamba, Smith 432 (WIS).	DQ314196	DQ301552	DQ309504

	(CORD).				
Eriolarynx iochromoides (Hunz.) Hunz.	ARGENTINA. Catamarca, Andalgalá, Rio Potrero, Barboza et al. 1966 (CORD). Ibidem. Urdampilleta et al. 730 (CORD)	ARGENTINA. Catamarca, Andalgalá, Río Potrero, Barboza et al. 1966 (CORD).	KP267802	**MH304888**	KP267816
Eriolarynx lorentzii (Dammer) Hunz.	ARGENTINA. Catamarca, Andalgalá, Rio Potrero, Hunziker et al. 24905 (CORD). Ibid. Barboza et al. 1967, 1968 (CORD). ARGENTINA. Catamarca, Chiarini 1295 (CORD).	ARGENTINA. Tucumán, Hawkes et al. 3452 (BIRM).	DQ314171	DQ301525	DQ309481
Iochroma amicorum M.Cueva, S.D.Sm. & S.Leiva	na	PERU. Oxapampa, Huancabamba, PN Yanachaga-Chemillen, Smith 542 (HOXA).	KM514683	KM514684	KM521199
Iochroma australe Griseb.	ARGENTINA. Salta, Candelaria, El Maray, Barboza et al. 317 (CORD). ARGENTINA. Tucumán, Tafí del Valle, Urdampilleta et al. 775 (CORD).	BOLIVIA. Chuquisaca, Smith 390 (WIS).	DQ314189	DQ301544	DQ309496

Iochroma baumii S.D.Sm. & S.Leiva	na	ECUADOR. Napo, Papallacta, Smith 476 (WIS).	DQ314202	DQ301558	DQ309513
Iochroma calycinum Benth.	na	ECUADOR. Pichincha, Smith 471 (WIS).	DQ314201	DQ301557	DQ309512
Iochroma confertiflorum (Miers) Hunz.	na	ECUADOR. Loja, Smith 237 (WIS).	DQ314176	DQ301531	DQ309486
Iochroma cornifolium (Kunth) Miers	na	ECUADOR. Loja, Smith 242 (WIS).	DQ314177	DQ301532	DQ309487
Iochroma cyaneum (Lindl.) M.L.Green ex G.H.M.Lawr. & J.M.Tucker	Grown from seed at UW-Madison. Original collection by W.G. D'Arcy, grown at Missouri Botanical Gardens, Smith 265 (WIS)	ECUADOR. Loja, Smith 223 (WIS).	DQ314180	DQ301535	DQ309490
Iochroma edule S.Leiva	PERU. Otuzco, 7.94837˚W 78.56065˚S, Smith 359 (MO)	PERU. La Libertad, Smith 300 (WIS).	DQ314193	DQ301548	DQ309500
Iochroma ellipticum (Hook. f.) Hunz.	na	ECUADOR. Galápagos, Jager 622 (CDS).	DQ314199	DQ301555	DQ309507
Iochroma fuchsioides (Bonpl.) Miers	ECUADOR. Pichincha, Quito. 0.15˚S, 78.483˚W, Smith 219 (QCNE, MO, WIS)	ECUADOR. Azuay, Smith 488 (WIS).	DQ314203	DQ301559	DQ309514
Iochroma gesnerioides (Kunth) Miers	Cultivated at the Botanical and Experimental Garden,	ECUADOR. Pichincha, Smith 200 (WIS).	DQ314179	DQ301534	DQ309489

	Radboud University Nijmegen. Accession numbers NLD020 984750203 and 884750081. Grown from seed at UW-Madison. Origin, Leipzig Bot. Garden, Nijmegen accession 934750129, Smith 266 (WIS).				
Iochroma grandiflorum Benth.	PERÚ. Huancabamba, Carmen de la Frontera, Barboza & Leiva González 4838 (CORD).	PERU. Cajamarca, Smith 320 (WIS).	DQ314170	DQ301523	DQ309480
Iochroma lehmannii Dammer ex Bitter	na	ECUADOR. Cañar, Smith 484 (WIS).	DQ314200	DQ301556	DQ309511
Iochroma loxense Kunth (Miers)	ECUADOR. Loja, 3.999˚S 79.306˚W, Smith 220 (WIS, MO).	ECUADOR. Loja, Smith 220 (WIS).	DQ314175	DQ301530	DQ309485
Iochroma nitidum S.Leiva & Quip.	na	PERU. Amazonas, Smith 371 (WIS).	DQ314168	DQ301521	DQ309478
Iochroma parvifolium (Roem. & Schult.) D'Arcy	PERU. La Libertad, Julcan, Agallpampa, Leiva González & Oberti 4696 (CORD).	PERU. La Libertad, Smith 303 (WIS).	DQ314195	DQ301551	DQ309503
Iochroma peruvianum (Dunal) J.F. Macbr.	na	PERU. Cajamarca, Smith 353 (WIS).	DQ314197	DQ301553	DQ309505

Iochroma salpoanum S.Leiva & Lezama	na	PERU. La Libertad, Smith 364 (WIS).	DQ314187	DQ301542	DQ309509
Iochroma squamosum S.Leiva & Quip.	na	PERU. Piura, Smith 330 (WIS).	DQ314186	DQ301541	DQ309494
Iochroma tingoanum S.Leiva	na	PERU. Amazonas, Smith 370 (WIS).	DQ314167	DQ301520	DQ309477
Iochroma tupayachianum S.Leiva	na	PERU. La Libertad, Smith 526 (MO, WIS).	KC290442	KC290441	KC243428
Iochroma umbellatum (Ruiz & Pav.) Hunziker ex D'Arcy	PERU. Ancash, Sihuas, Särkinen 4711 (BM). PERU. Ancash, Huari, Särkinen 4796 (BM).	PERU. La Libertad, Smith 301 (WIS).	DQ314169	DQ301522	DQ309479
Lycianthes inaequilatera Bitter	na	ECUADOR. Pichincha, Smith 210 (WIS).	DQ314159	DQ309519	DQ309469
Physalis chenopodifolia Lam.	MEXICO. Estado de México, Pirámides de Teotihuacan, Chiarini 1277 (CORD).	MEXICO. Chiarini et al. 1277 (CORD).	na	**MH304893**	**MH304879**
		Cultivated. Whitson 1287 (DUKE).	AY665883	na	na
Physalis lagascae Roem. & Schult.	PERU. Cajamarca, Cutervo, Särkinen 4548 (BM).	PERU. Särkinen 4548 (BM).	na	**MH304892**	**MH304880**
		MEXICO. Nayarit, Flores 1810 (MO).	AY665898	na	na

Physalis peruviana L.	Cultivated, seeds from commercial source. Deanna 178 (CORD)	ECUADOR. Pichincha, Gardens of Herbario Nacional (QCNE), Smith 217 (WIS).	DQ314161	DQ301514	DQ309471
Physalis pubescens L.	PERU. Ancash, Pallasca, Knapp 10609 (BM).	MEXICO. Morelos, Lagunas de Zempoala, Chiarini et al. 1281 (CORD).	na	**MH304895**	**MH304881**
		COSTA RICA. La Selva Biological Station, Whitson 3 (DUKE).	AY665916	na	na
Physalis viscosa L.	ARGENTINA. Chaco, Presidencia de La Plaza, Chiarini et al. 911 (CORD). ARGENTINA. Cordoba, Bialet Masse, Chiarini 1285 (CORD).	ARGENTINA. Córdoba, Calamuchita, Deanna & Tamborini 179 (CORD).	na	**MH304894**	**MH304882**
		Cultivated, Whitson 1282 (DUKE).	AY665870	na	na
Salpichroa tristis Walp.	na	BOLIVIA. Potosí, Smith 382 (WIS).	DQ314160	DQ309520	DQ309470
Saracha andina Rob.Fernandez, I. Revilla & E. Pariente	na	PERU. Ayacucho, Lucanas, Smith & Fernandez 594 (COLO, F, MO, USM).	KY172041	KY172040	KY172039

Saracha punctata Ruiz & Pav.	PERU. Cajamarca, Cutervo, La Capilla, Leiva González et al. 3992 (HAO).	BOLIVIA. La Paz, Nee 51804 (NY).	DQ314182	DQ301537	DQ309492
Saracha quitensis (Hook.) Miers	ECUADOR. Zamora Chinchipe, Orozco et al. 3935 (COL, QCA).	ECUADOR. Napo, Smith 257 (WIS).	DQ314178	DQ301533	DQ309488
Tubocapsicum anomalum (Franch. & Sav.) Makino	Cultivated at Radboud University, Nijmegen. Accession number NLD162, 904750027. Origin: Long Wulu, Shanghai, People's Republic of China.	Chen 231 (MO).	DQ314163	DQ301516	DQ309473
Vassobia breviflora (Sendtn.) Hunz.	ARGENTINA. Catamarca, Paclín, Huacra river, Hunziker et al. 24834 (CORD). ARGENTINA. Jujuy, Dr Manuel Belgrano, Chiarini 1226 (CORD). URUGUAY. Rocha, La Pedrera, Chiarini 675 (CORD).	BOLIVIA. Chuquisaca, Smith 412 (WIS).	DQ314190	DQ301545	DQ309497
Vassobia dichotoma (Rusby) Bitter	na	BOLIVIA. La Paz, Smith 440 (WIS).	DQ314191	DQ301546	DQ309498
Withania aristata Pauq.	SPAIN. Canary Islands, Punta	na	na	na	na

	de Teno, Santos Guerra 5034, 5037 (ORT). SPAIN. Canary Islands, Taganana, Santos Guerra 5055 (ORT).				
Withania coagulans (Stocks) Dunal	Cultivated at Radboud University, Nijmegen. Accession numberNLD162, 914750053. Origin unknown.	na	na	na	na
Withania frutescens (L.) Pauq.	Cultivated at Radboud University, Nijmegen. Accession number NLD162, 924750086. Origin unknown.	na	na	na	na
Withania riebeckii Balf. f.	Cultivated at Radboud University, Nijmegen. Accession number NLD162, 904750221. Origin unknown.	Cultivated plants from the Missouri Botanical Garden, D'Arcy 17803 (MO).	na	**MH304891**	**MH304883**
Withania somnifera (L.) Dunal	Cultivated at IMBIV, Commercial source https://www.asklepios-seeds.de/gb/, Chiarini 1361 (CORD).	Whitson 1262 (KNK).	na	**MH304890**	**MH304884**
		Lester S. 0960.	KC832797	na	na
Witheringia coccoloboides	Cultivated at Radboud University, Nijmegen.	COSTA RICA. Bohs	**MH281826**	**MH304889**	**MH304885**

(Dammer) Hunz.	Accession number NLD162, 814750081. Origin: Cajarca, Quindío, Colombia.	2568 (UT).			
Witheringia solanacea L'Hér.	PERU. Cajamarca, San Ignacio, San José de Lourdes, Leiva González et al. 3812 (HAO).	PANAMA. D'Arcy 16399 (MO).	DQ314164	DQ301517	DQ309474

Table A.2. Characters matrix of the chromosome traits employed in the ancestral state reconstructions. "na" indicates no information available for this trait.

traits	discrete				continuous			
species	number	formula	5S	45S	LT	r	het	rDNA sites
Acnistus arborescens	0	0	na	na	49.19	1.32	7.73	na
Aureliana fasciculata	0	2	na	na	65.68	1.53	na	na
Deprea nieva	0	1	0	0	31.67	1.67	10.78	2
Deprea pumila	0	1	0	0	na	na	13.7	2
Deprea sachapapa	0	1	1	0	34.87	1.42	14.9	3
Dunalia brachyacantha	0	0	0	0	40.42	1.18	9.81	2
Dunalia obovata	0	na	na	na	na	na	na	na
Dunalia solanacea	0	0	0	0	32.6	1.26	3.17	2
Dunalia spathulata	0	na	na	na	na	na	na	na
Dunalia spinosa	1	0	1	1	77.82	1.23	1.82	4
Eriolarynx fasciculata	0	0	1	0	42.74	1.26	3.76	3
Eriolarynx iochromoides	0	0	1	0	37.29	1.38	na	3

Eriolarynx lorentzii	0	0	2	0	38.64	1.32	1.1	4
Iochroma australe	0	0	1	0	43.36	1.21	1.77	3
Iochroma cyaneum	0	0	0	0	53.76	1.15	na	2
Iochroma edule	0	0	0	0	48.46	1.21	1.34	2
Iochroma fuchsioides	1	na	na	na	na	na	na	na
Iochroma gesnerioides	0	0	0	0	32.32	1.34	1.17	2
Iochroma grandiflorum	0	0	2	0	54.73	1.32	na	4
Iochroma loxense	0	0	0	0	47.94	1.3	na	2
Iochroma parvifolium	1	0	na	na	71.09	1.23	5.97	na
Iochroma umbellatum	0	0	0	0	26.98	1.38	2.56	2
Phyalis lagascae	0	2	0	0	30	2.77	na	2
Physalis chenopodifolia	0	2	0	0	38.05	1.87	na	2
Physalis peruviana	1	2	2	0	56.66	2.23	na	4
Physalis viscosa	0	2	0	0	38.95	1.87	na	2
Physalis pubescens	1	2	2	1	60.27	2.36	na	5
Saracha punctata	0	0	2	1	49.75	1.26	20.87	22
Saracha quitensis	0	0	1	1	44.86	1.27	16.5	19
Tubocapsicum anomalum	1	1	1	0	35.41	1.44	3.05	3
Vassobia breviflora	0	0	0	0	35.74	1.21	4.02	2
Witheringia solanacea	0	1	0	0	49	1.46	0.875	2
Withania riebeckii	1	2	na	na	44.01	2.76	2.435	na
Witheringia coccoloboides	0	0	na	na	29.32	1.21	na	na
Withania somnifera	1	2	0	0	80.54	2.19	na	2

Figure A.3. Metaphase chromosomes of Physalideae (Iochrominae, Withaninae, Physalidinae) species stained with classical technique. **A.** *Acnistus arborescens*. **B.** *Dunalia brachyacantha*. **C.** *Dunalia spinosa*. **D.** *Dunalia solanacea*. **E.** *Eriolarynx iochromoides*. **F.** *Eriolarynx lorentzii*. **G.** *Iochroma cyaneum*. **H.** *Iochroma australe*. **I.** *Iochroma umbellatum* (4711). **J.** *Saracha quitensis*. **K.** *Iochroma parvifolium*. **L.** *Iochroma gesnerioides*. **M.** *Vassobia breviflora*. **N.** *Tubocapsicum anomalum*. **O.** *Iochroma loxense*. **P.** *Iochroma edule*. **Q.** *Eriolarynx fasciculata*. **R.** *Iochroma grandiflorum*. **S.** *Witheringia coccoloboides*.

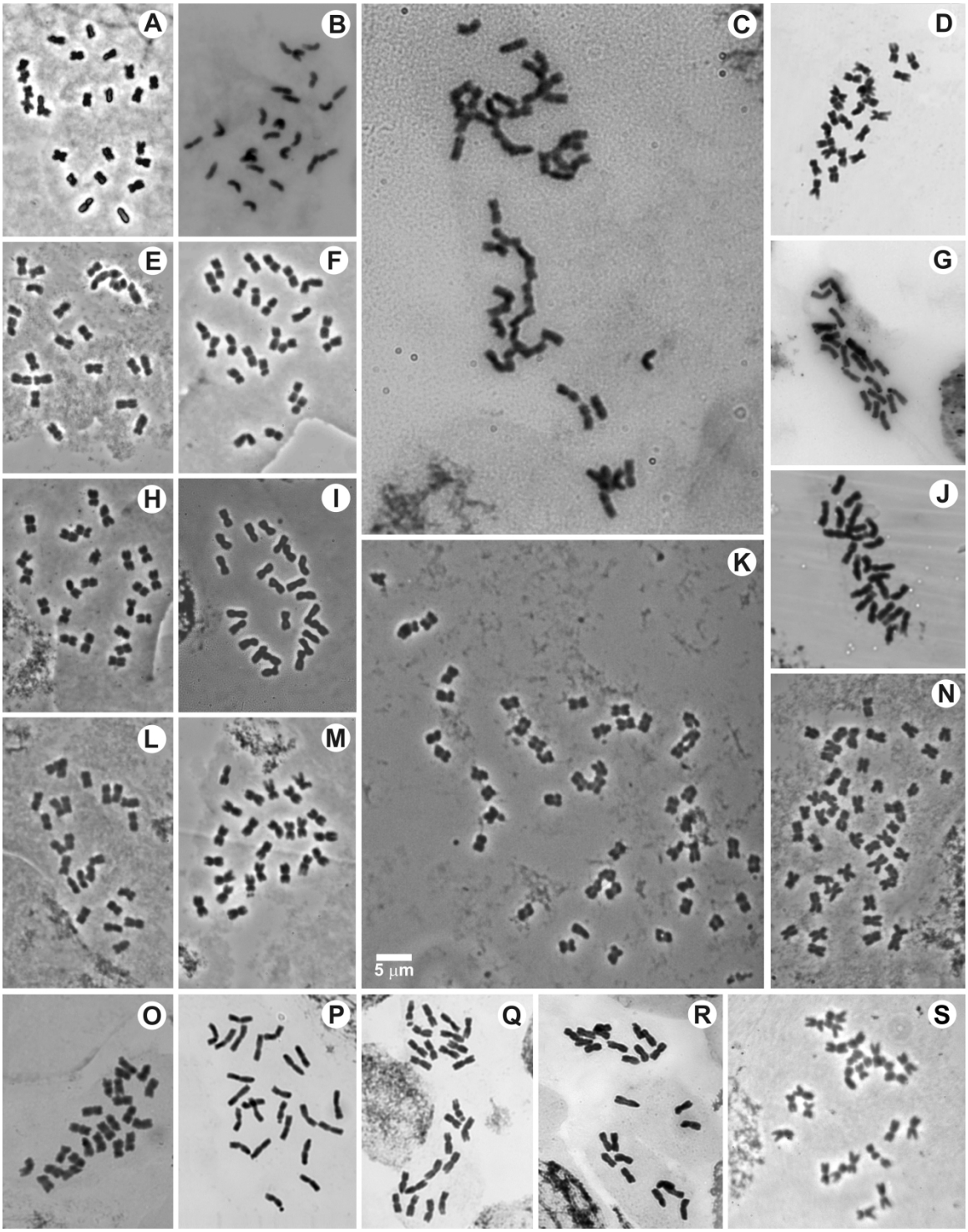


Figure A.4. Metaphase chromosomes of Physalidae (Iochrominae, Withaninae, Physalidinae) species stained with CMA/DAPI technique. **A.** *Acnistus arborescens*. **B.** *Dunalia brachyacantha*. **C.** *Dunalia spinosa*. **D.** *Iochroma australe*. **E.** *Eriolarynx lorentzii*. **F.** *Iochroma gesnerioides*. **G.** *Iochroma parvifolium*. **H.** *Iochroma umbellatum* (4711). **I.** *Iochroma umbellatum* (4796). **J.** *Saracha punctata*. **K.** *Witheringia solanacea*. **L.** *Tubocapsicum anomalum*. **M.** *Withania riebeckii*.

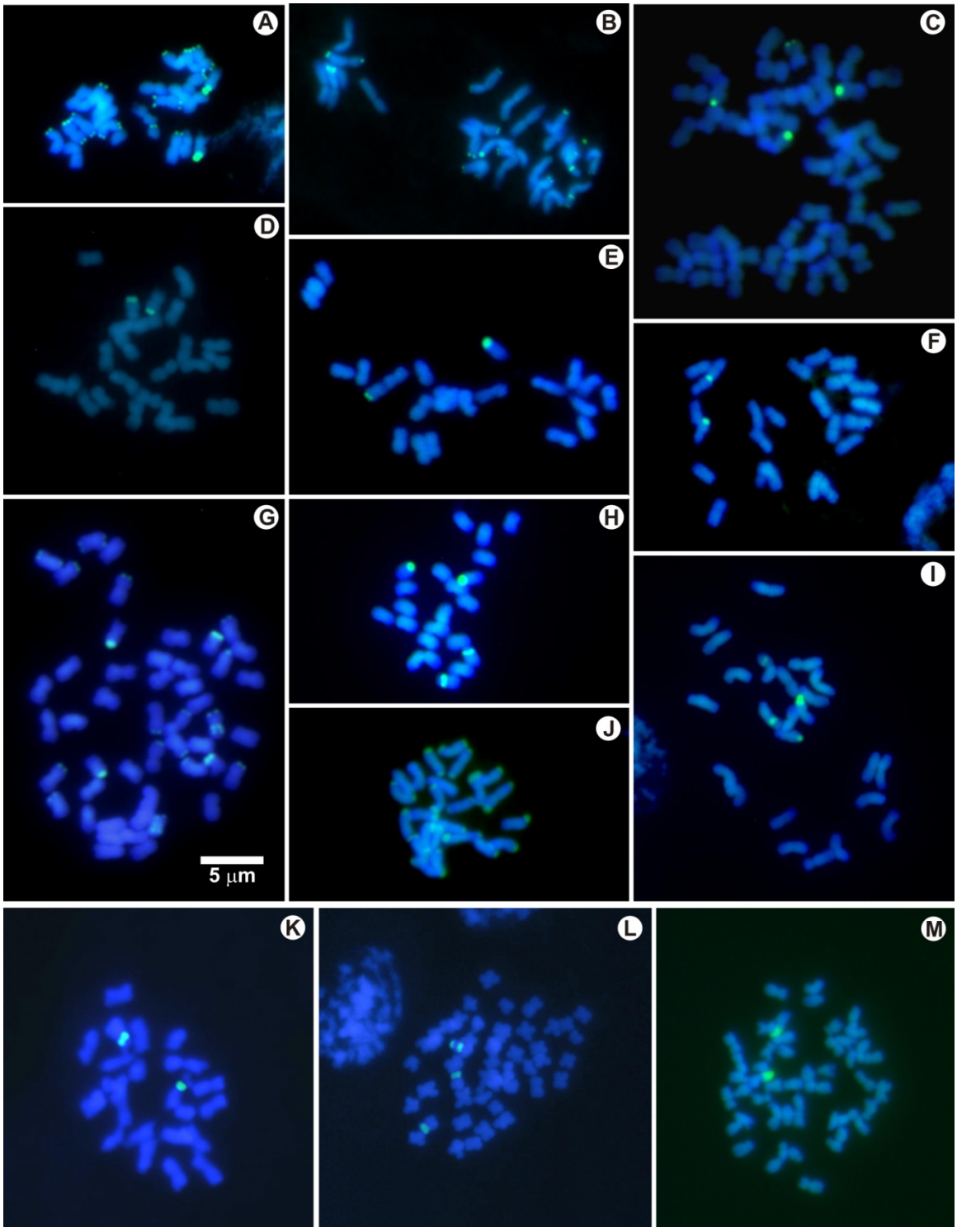
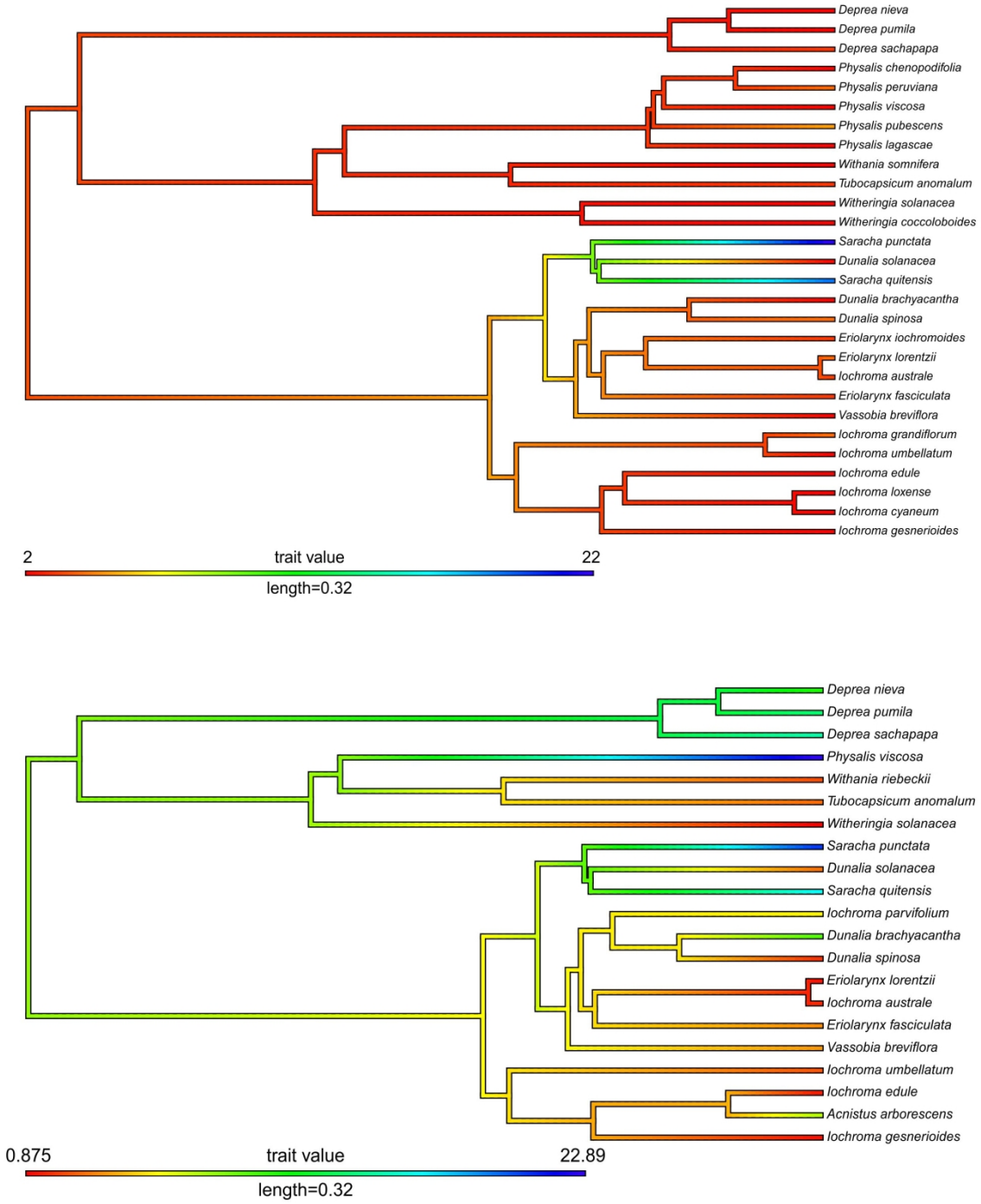


Figure A.5. Heatmaps, in order, of amount of rDNA loci, heterochromatin content, and total haploid chromosome length of the karyotype reconstructed on Iochrominae and relatives. Scales below indicate values of arm ratio and its color guides.



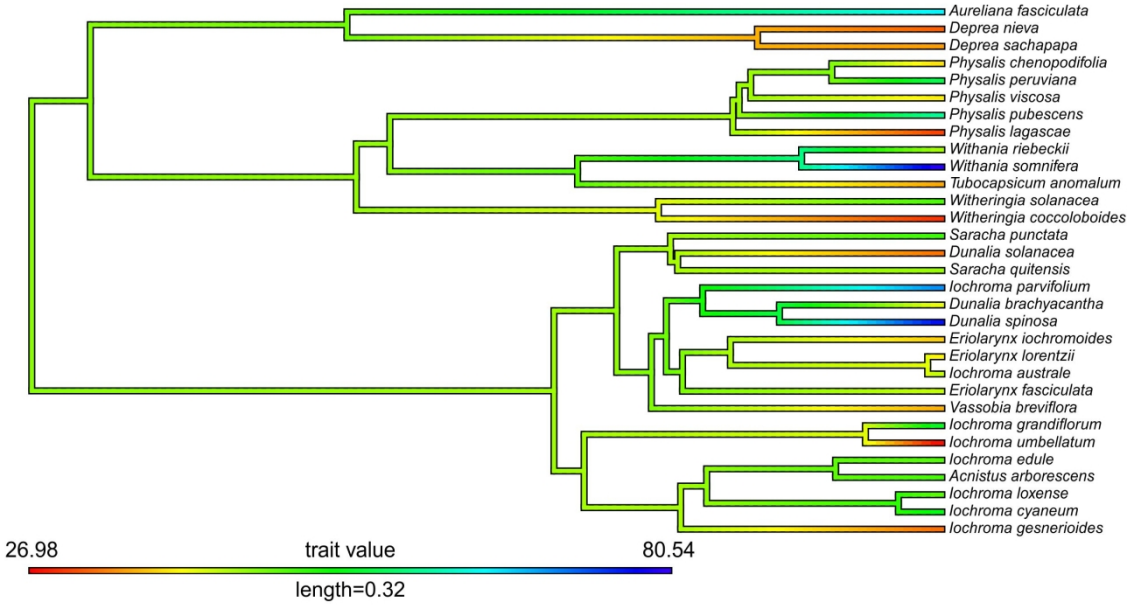

Aureliana fasciculata
Deprea nieva
Deprea sachapapa
Physalis chenopodifolia
Physalis peruviana
Physalis viscosa
Physalis pubescens
Physalis lagascae
Withania riebeckii
Withania somnifera
Tubocapsicum anomalum
Witheringia solanacea
Witheringia coccoloboides
Saracha punctata
Dunalia solanacea
Saracha quitensis
Iochroma parvifolium
Dunalia brachyacantha
Dunalia spinosa
Eriolarynx iochromoides
Eriolarynx lorentzii
Iochroma australe
Eriolarynx fasciculata
Vassobia breviflora
Iochroma grandiflorum
Iochroma umbellatum
Iochroma edule
Acnistus arborescens
Iochroma loxense
Iochroma cyaneum
Iochroma gesnerioides
26.98
trait value
80.54
length=0.32

Figure A.6. Chromosome haploid number reconstruction with ChroEvol in RASP. "CONST_RATE" model (the lowest AIC scoring model) was selected and used in the analyses. Haploid chromosome numbers for the extant species are shown next to the species name, and 'X' means no data for that species. Pies at nodes represent frequencies of node states across 10000 simulations of character evolution.

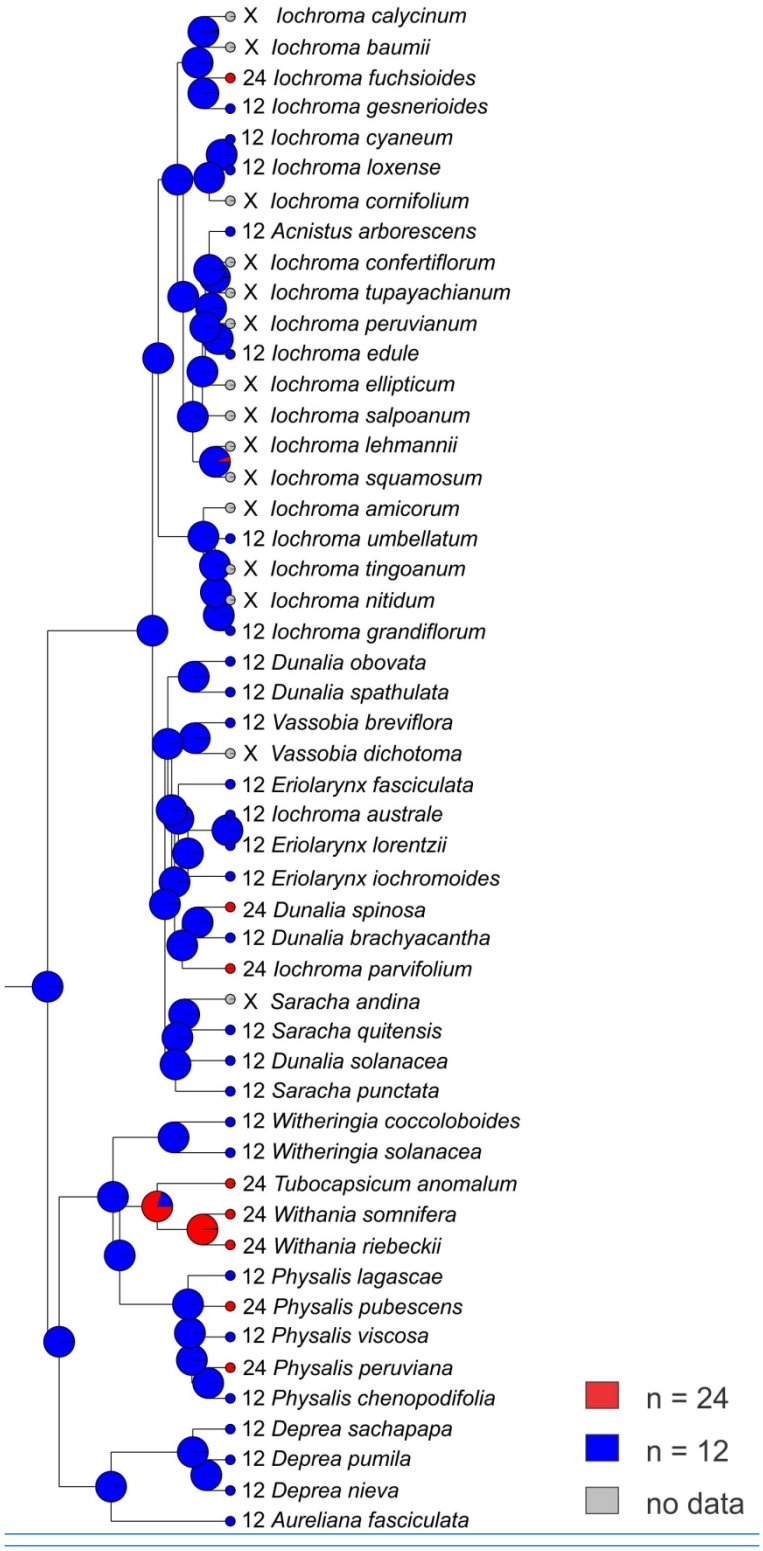


FIGURE CAPTIONS

Figure 1. Haploid idiograms of Physalideae species based on mean chromosome values (all at the same scale) placed onto the best ML tree based on two low copy nuclear markers (*waxy* and *LEAFY*) and one ribosomal nuclear marker (ITS). Chromosomes are ordered from longest to shortest within each category, from m to st, with an ordering number indicated below each one (these numbers do not stand for homologies). Gray blocks indicate 45S loci, circles indicate positive pyknosis by DAPI staining after FISH, black blocks are 5S loci. Idiograms diagonally striped represent species studied only with classical technique. Both homologues are represented when species have heteromorphic pairs. Bootstrap support > 60 are given above the branches; bold branches indicate bootstrap support > 80.

Figure 2. Fluorescence in situ hybridization with 5S (red signals) and 45S rDNA (green signals) probes in Physalidae (Iochrominae, Withaninae, Physalidinae) species. The rest of the signals correspond to dispersion of the rDNA loci. **A.** *Tubocapsicum anomalum*. **B.** *Witheringia coccoloboides*. **C.** *Witheringia solanacea*. **D.** *Dunalia brachyacantha*. **E.** *Dunalia spinosa*. **F.** *Dunalia solanacea*. **G.** *Eriolarynx fasciculata*. **H.** *Iochroma umbellatum* (4711). **I.** *Iochroma edule*. **J.** *Iochroma grandiflorum*. **K.** *Iochroma cyaneum*. **L.** *Iochroma australe*. **M.** *Eriolarynx lorentzii*. **N.** *Eriolarynx lorentzii*. **O.** *Saracha punctata*. **P.** *Saracha quitensis*. **Q.** *Iochroma loxense*. **R.** *Eriolarynx iochromoides*. All pictures at the same scale.

Figure 3. Ancestral character state reconstruction of chromosome features in Iochrominae and related taxa on the best combined ML tree, using stochastic mapping of rDNA loci, chromosome number and karyotype formula. Pies at nodes represent frequencies of node states across 1000 simulations of character evolution.

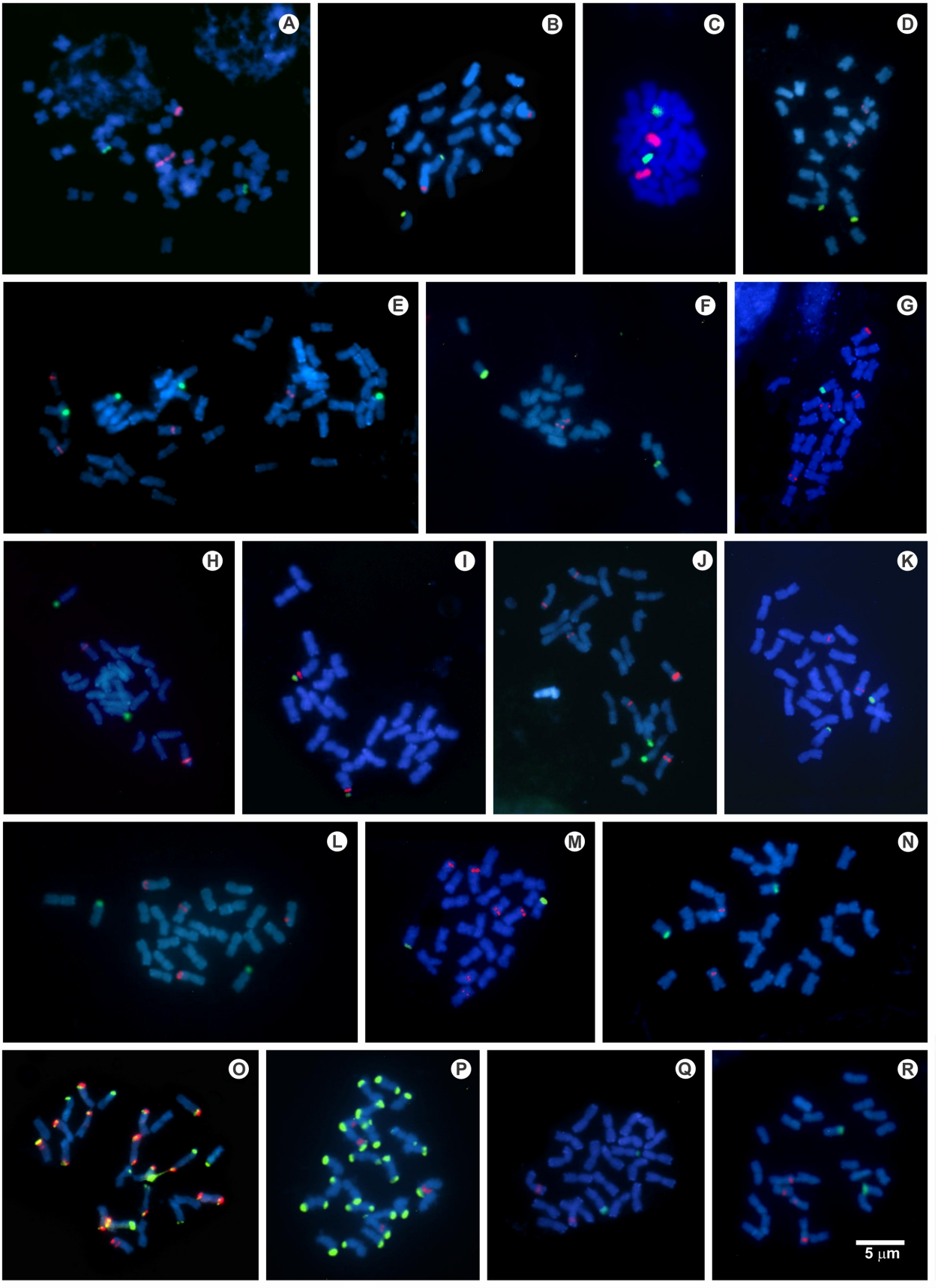
Figure 4. Phylogenetic PCA and heatmap of continuous karyological features. **A.** Species scores on pPC1 and pPC2, with scale on bottom and left axes. Red arrows show loadings for each variable on the PC axes, with scale shown on top and right axes, except r that did not load significantly on either PC axis and hence has no associated arrow. Purple circles indicate diploid species and grey squares show polyploids. **B.** Maximum likelihood reconstruction of mean arm ratio (r) values on the best combined ML tree.

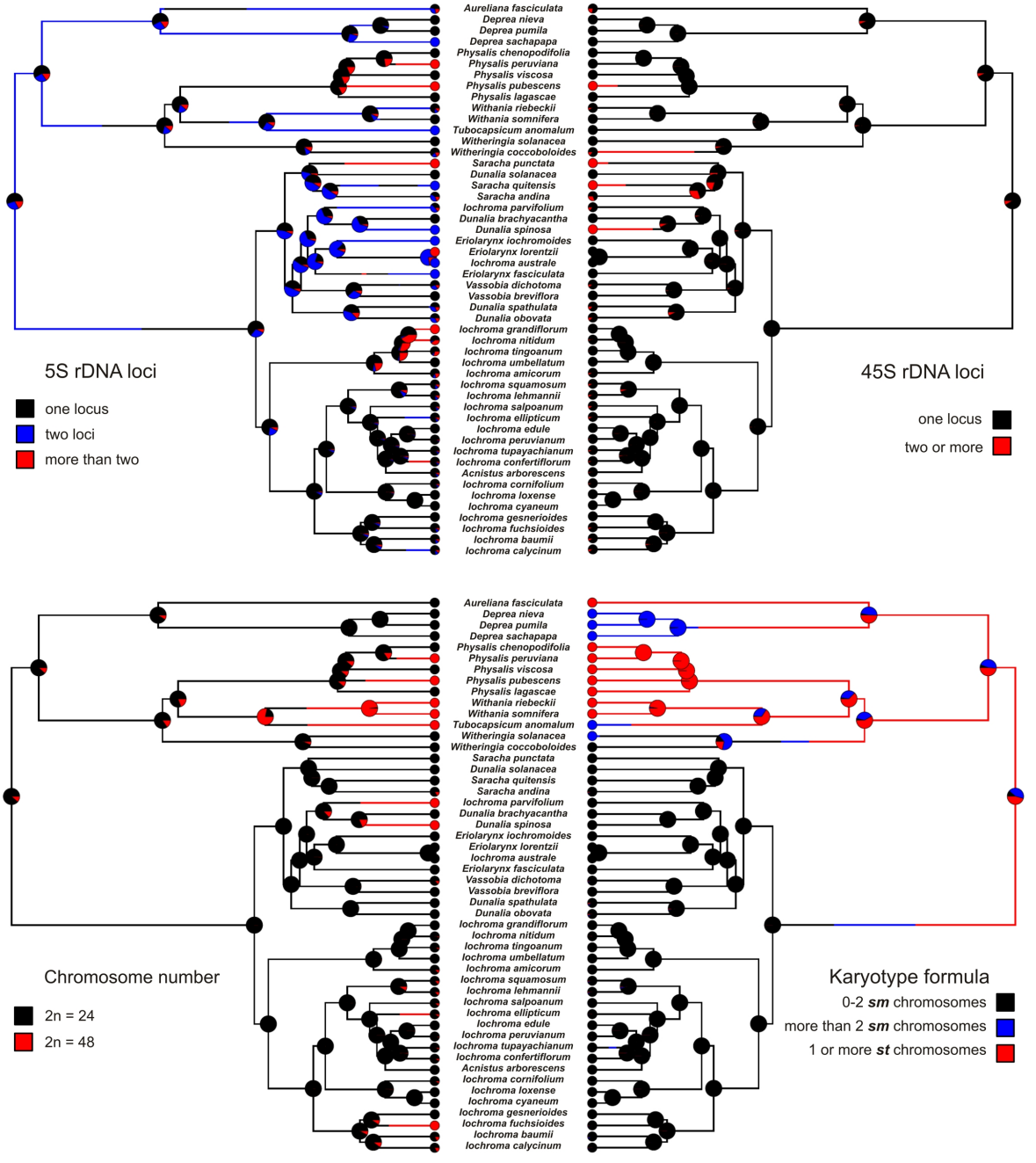
Iochromeae

Physalideae

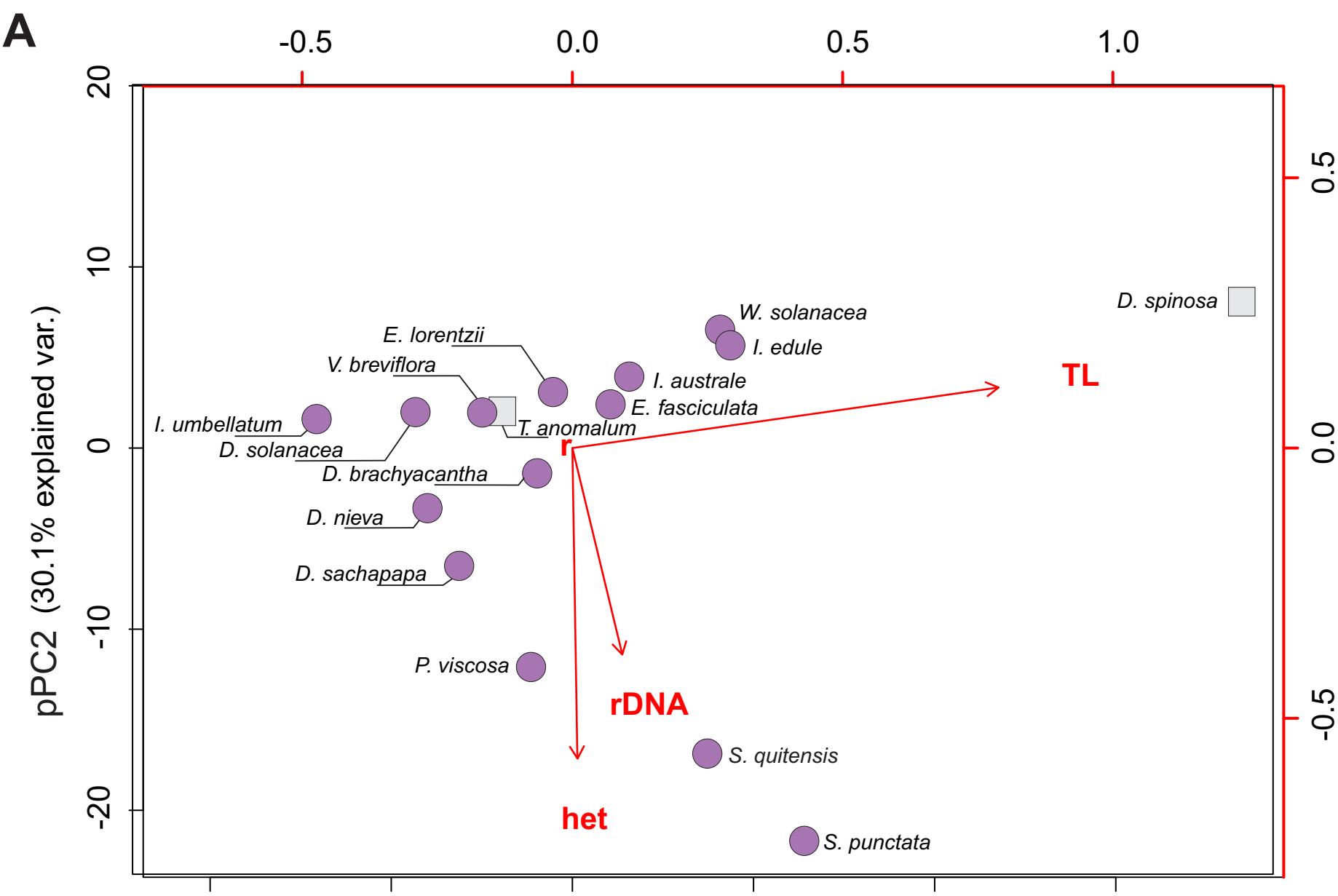
lochroma calycinum
lochroma baumii
lochroma fuchsioides
lochroma gesnerioides
lochroma cyaneum
lochroma loxense
lochroma cornifolium
Acnistus arborescens
lochroma confertiflorum
lochroma tupayachianum
lochroma peruvianum
lochroma edule
lochroma ellipticum
lochroma salpoanum
lochroma lehmannii
lochroma squamosum
lochroma amicorum
lochroma umbellatum
lochroma tingoanum
lochroma nitidum
lochroma grandiflorum
Dunalia obovata
Dunalia spathulata
Vassobia breviflora
Vassobia dichotoma
Eriolarynx fasciculata
lochroma australe
Eriolarynx lorentzii
Eriolarynx iochromoides
Dunalia spinosa
Dunalia brachyacantha
lochroma parvifolium
Saracha andina
Saracha quitensis
Dunalia solanacea
Saracha punctata
Witheringia coccoloboides
Witheringia solanacea
Tubocapsicum anomalum
Withania somnifera
Withania riebeckii
Physalis lagascae
Physalis pubescens
Physalis viscosa
Physalis peruviana
Physalis chenopodifolia
Deprea sachapapa
Deprea pumila
Deprea nieva
Aureliana fasciculata
Lycianthes inaequilatera
Capsicum lycianthoides
Salpichroa tristis

3 µm

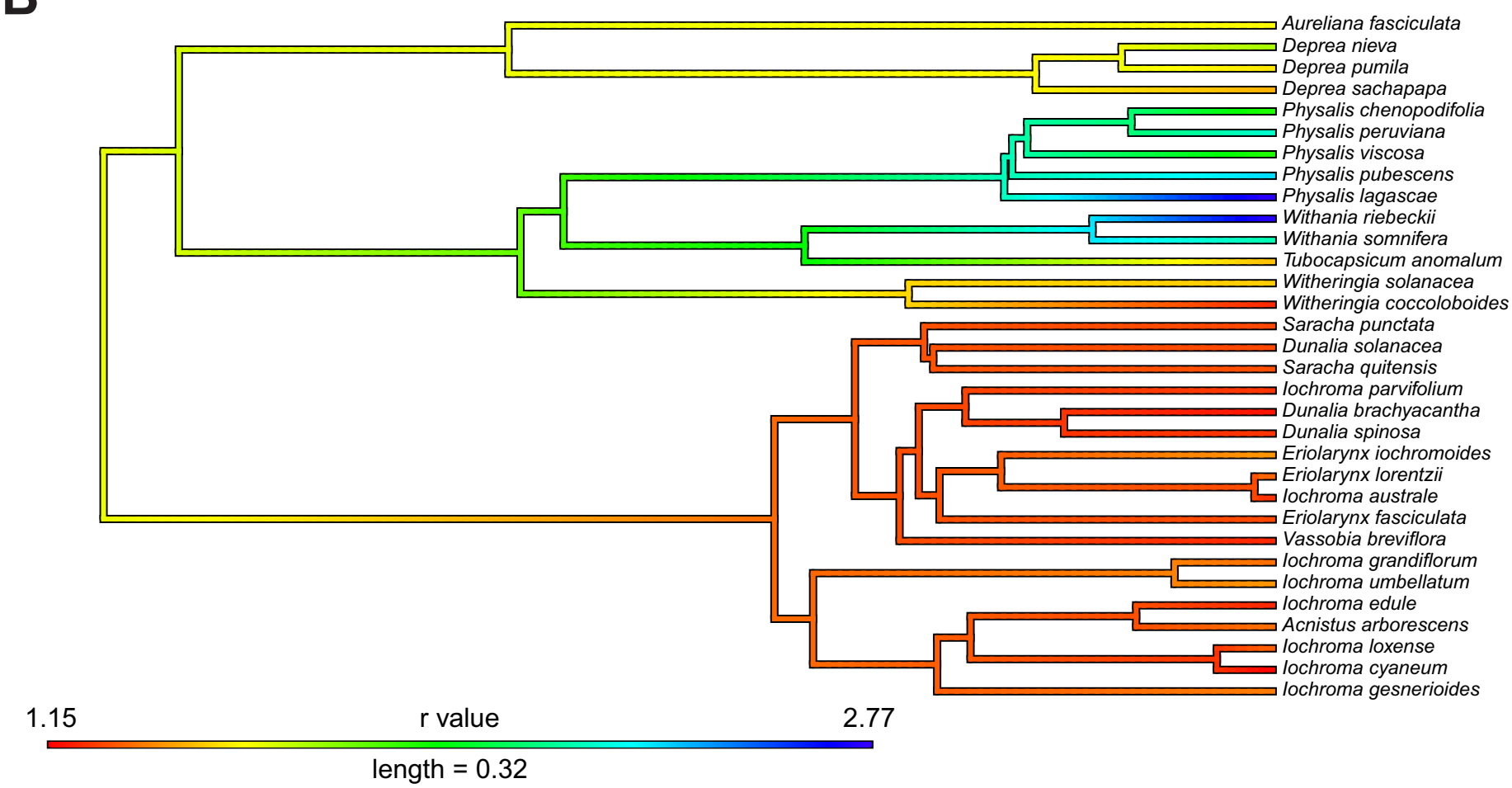

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
5 μm


Aureliana fasciculata
Deprea nieva
Deprea pumila
Deprea sachapapa
Physalis chenopodifolia
Physalis peruviana
Physalis viscosa
Physalis pubescens
Physalis lagascae
Withania riebeckii
Withania somnifera
Tubocapsicum anomalum
Witheringia solanacea
Witheringia coccoboloides
Saracha punctata
Dunalia solanacea
Saracha quitensis
Saracha andina
Iochroma parvifolium
Dunalia brachyacantha
Dunalia spinosa
Eriolarynx iochromoides
Eriolarynx lorentzii
Iochroma australe
Eriolarynx fasciculata
Vassobia dichotoma
Vassobia breviflora
Dunalia spathulata
Dunalia obovata
Iochroma grandiflorum
Iochroma nitidum
Iochroma tingoanum
Iochroma umbellatum
Iochroma amicorum
Iochroma squamosum
Iochroma lehmannii
Iochroma salpoanum
Iochroma ellipticum
Iochroma edule
Iochroma peruvianum
Iochroma tupayachianum
Iochroma confertiflorum
Acnistus arborescens
Iochroma cornifolium
Iochroma loxense
Iochroma cyaneum
Iochroma gesnerioides
Iochroma fuchsioides
Iochroma baumii
Iochroma calycinum
5S rDNA loci
one locus
two loci
more than two
45S rDNA loci
one locus
two or more
Chromosome number
2n = 24
2n = 48
Karyotype formula
0-2 sm chromosomes
more than 2 sm chromosomes
1 or more st chromosomes

A


-0.5
0.0
0.5
1.0
20
10
0
-10
-20
0.5
0.0
-0.5
pPC2 (30.1% explained var.)
D. spinosa
W. solanacea
I. edule
E. lorentzii
V. breviflora
I. australe
E. fasciculata
I. umbellatum
T. anomalum
D. solanacea
r
TL
D. brachyacantha
D. nieva
D. sachapapa
P. viscosa
rDNA
S. quitensis
het
S. punctata
-20
-10
0
10
20
30
pPC1 (67.6 % explained var.)

B


Aureliana fasciculata
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Eriolarynx lorentzii
Iochroma australe
Eriolarynx fasciculata
Vassobia breviflora
Iochroma grandiflorum
Iochroma umbellatum
Iochroma edule
Acnistus arborescens
Iochroma loxense
Iochroma cyaneum
Iochroma gesnerioides
1.15
r value
2.77
length = 0.32