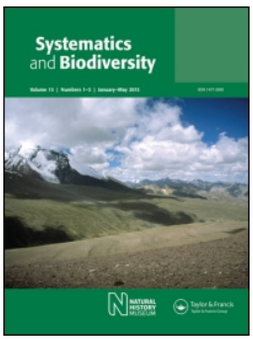

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Research Article

# Insights into the Andean genera *Bridgesia* and *Guindilia* (Sapindaceae): an integrated approach

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Sapindaceae *s.l.* is a moderately large family of trees, shrubs and lianas. The genera *Bridgesia* and *Guindilia* belong to the Thouinieae tribe; however, its circumscription to this tribe is in doubt. This work presents a comparative analysis of pollen morphology between species of both genera. They share the basic spherical tricolporate pollen type for the family, but the features studied do not characterize any of them. In addition, the work intends to characterize the karyotype and genome size evolution of both genera, and elucidate the phylogenetic relationships within the family through maximum likelihood and Bayesian analyses of the ITS, *trn*L and *trn*L-F sequences. Our results show two different patterns regarding karyotype data: *Bridgesia* presents 2n = 2x = 28, with metacentric and submetacentric chromosomes; the basic number of the genus, x = 14, is in agreement with the 25% of the chromosome numbers recorded in Sapindaceae. The genus *Guindilia* exhibits a basic number x = 10; of the three species *G. cristata* and *G. trinervis* present metacentric, submetacentric and subtelocentric chromosomes, whereas *G. dissecta* shows only metacentric and subtelocentric ones. In addition, *G. cristata* is a polyploid species, with DNA content exactly three-fold that of the diploid species, suggesting a recent event of polyploidization in this species. The infra-familial phylogenetic relationship and circumscription of both genera analysed here evidence that *Bridgesia* belongs to *Paullinia* group. The fact that *Guindilia* is grouped in a different clade encouraged us to propose a new informal tribal group, *Guindilia* group, in the current infrafamilial arrangement of Sapindaceae.

**Keywords**: *Bridgesia*, genome size, *Guindilia*, karyotype, phylogeny, pollen morphology, Sapindaceae



## Introduction

Sapindaceae *s.l.* is a moderately large family of trees, shrubs and lianas including *c*. 141 genera and *c*. 1900 species. Most members have a tropical to subtropical distribution, although some genera extend to the temperate regions of Eurasia and North America.

The first complete taxonomic treatment of the family *sensu stricto* was performed by Radlkofer (1931–1934), who identified two subfamilies: Sapindoideae (= Eusapindaceae) and Dodonaeoideae (= Dyssapindaceae). Recently, Harrington, Edwards, Johnson, Chase, and Gadek (2005) using an evolutionary framework based on molecular phylogenetic analysis detected that the infrafamilial groupings were paraphyletic, with the exception of the Paullinieae tribe. They sampled three of the seven genera attributed to Paullinieae, and four of the six in Thouinieae. In the latter tribe were included species of the genera *Bridgesia* and *Guindilia*. This result was later corroborated by Buerki et al. (2009), although no representatives of *Allophylus* and *Guindilia* were included in the analysis, they confirmed that a monophyletic Paullinieae was nested in a paraphyletic Thouinieae. In both contributions was recognized a Paullinia group.

As a result of these uncertainties in evolutionary relationships, this work follows the informal systems of Buerki et al. (2010) for the familial level in which is circumscribed the family Sapindaceae nearly identical to that monographed by Radlkofer (1931–1934); and the Buerki et al. (2009) proposal for the lower levels of taxonomic classification, point of view that is also shared by Acevedo-Rodríguez, van Welzen, Adema, and van der Ham (2011). Although the system proposed by Buerki et al. (2009) is not ideal, at least it reflects the current understanding of evolutionary relationships within the family. With regards to these classifications, the *Paullinia*

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group belongs to the subfamily Sapindoideae and is considered to be monophyletic (Buerki et al., 2009; Harrington et al., 2005). It comprises the tribes Paullinieae *s.s.* and Thouinieae *s.s.*, and the enigmatic *Sapindus oligophyllus* Merr. & Chun.

*Guindilia* Gillies ex Hook. & Arn. (= sub *Valenzuelia* Bertero ex Cambess.) and *Bridgesia* Bertero ex Cambess. belong to the Thouinieae tribe *sensu* Radlkofer (1931−1934); the genera *Athyana* (Griseb.) Radlk., *Thouinia* Poit., *Diatenopteryx* Radlk. and *Allophylus* L. are also included in this tribe. The members of Thouinieae shared similar shrubby or arboreal habit, without stipules or tendrils; flowers have ventral appendages, and the schizocarpic fruits, coconuts finally free, and seeds without aril. The simple leaf would apparently be the only apomorphy for the tribe.

The geographic distribution of the genera of Thouinieae is particular for each genus; *Athyana* and *Diatenopteryx* are present in Peru, Bolivia, Paraguay and Brazil (South America), *Allophylus* shows a pantropical distribution, and *Thouinia* is neotropical, distributed in Mesoamerica, Greater Antilles and the Bahamas. However, *Guindilia* and *Bridgesia* present a limited distribution pattern to the south of South America, along the Andes Mountains.

*Bridgesia* is a monotypic genus, with *B. incisifolia* Bertero ex Cambess. being endemic to Chile. The genus *Guindilia* is composed of three species growing in the Andean regions of Argentina and Chile. *Guindilia dissecta* (Covas & Burkart) Hunz. is endemic to Mendoza (Argentina); *G. cristata* (Radlk.) Hunz. occurs in Catamarca, La Rioja and San Juan (Argentina), and *G. trinervis* Gillies ex Hook. & Arn. presents a disjunct distribution, with populations isolated on both sides of the Andean massif in Chile and Argentina (Martínez Carretero, 1987).

As indicated by Muller and Leenhouts (1976), the pollen grains of Thouinieae are very similar in aperture, shape and wall structure. This tribe exhibits the basal pollen type for the family, spherical tricolporate, and another type, oblate or peroblate tricolporate. However, according to the aforementioned authors, the tribe appears to have a negative correlation between macromorphological level of advancement and pollen evolution.

In Thouinieae *s.s.* chromosome counts are available in few species of *Allophylus* (x = 14, 2n = 28) and *Diatenopteryx* (x = 15, 2n = 30) (Ferrucci, 2000; Ferrucci & Solís Neffa, 1997). Although the information in the tribe is limited, the basic chromosome numbers recorded correspond to the most frequent in the family (Ferrucci, 2000). These records clearly differ with those reported for the Paullinieae tribe *s.s.*, characterized by x = 12 as the most frequent basic number (Ferrucci, 2000).

Genome size varies tremendously across flowering plants, and this variation has sometimes been used to speculate about the relationships between species as well as to recognize new taxonomic entities or hybrids (Bennett & Leitch, 2012). The information of the C-value is scarce in Sapindaceae and some contributions were made in various genera. The 1C values vary from 0.45 to 2.75 pg (Coulleri, Urdampilleta, & Ferrucci, 2014; Morgan & Westoby, 2005; Ohri, 1996, 2002; Ohri, Bhargava, & Chatterjee, 2004). Some groups, such as *Cardiospermum*, exhibit a great variation in the genome size (Coulleri et al., 2014), which is correlated with variation in chromosome morphology (Urdampilleta, Coulleri, Ferrucci, & Forni-Martins, 2013).

The karyological features of *Bridgesia* and *Guindilia* are unknown, and the molecular knowledge in these genera is scarce; moreover, the infrafamiliar circumscription of *Guindilia* has been questioned.

The limited knowledge about pollen, karyological data and the evolution of Thouinieae and Paullinieae tribes have encouraged us to propose a new insight in the *Bridgesia* and *Guindilia* species from a biosystematic point of view in order to (i) evaluate the pollen morphology in both genera, and (ii) analyse the karyological information, including features, such as genome size and the karyotypic features; and (3) determine the infrafamiliar circumscription of both genera by DNA sequence analysis.

# Materials and methods

## Plant material

The analysed materials are listed in Table 1 and the collection places are shown in Fig. 1. Voucher specimens that were deposited in the following herbaria have been studied: CTES, LIL and MERL; herbarium codes according to Thiers (2015).

## Pollen morphology

**Taxonomic sampling.** For each species, we obtained floral buds for pollen preparation from two herbarium specimens which are indicated in Table 1.

**Pollen morphological analyses.** Observations and photographs of pollen morphology were performed with a Leica DM LB2 binocular microscope (Leica, Wetzlar, Germany) equipped with a digital camera (LM) and scanning electron microscope (SEM) micrographs were obtained with a Jeol JSM-5800LV scanning electron microscope (JEOL USA, Peabody, MA, USA). Pollen grains were acetolysed according to the procedure of Erdtman (1966) and mounted in glycerine jelly. Pollen samples were deposited in the pollen herbarium of the National University of the Northeast, UNNE (PAL-CTES). For light microscopy, 5-8 closed buds per sheet were taken, and measurements of 20 grains per studied specimen were

**Table 1.** Species, collection data and type of analysis to which each sample was subjected [Pal (Palynological), Cyt (Cytogenetic) and Phy (Phylogenetic)].

| | Collection Data | | | Analysis | | |
|---|---|---|---|---|---|---|
| Species | Locality/Coordinates/Date | Collector | Herbaria | Pal | Cyt | Phy |
| *Bridgesia incisifolia* Bertero ex Cambess. | CHILE. IV Region, 10.1 km N of rd to Romeral, N of La Serena, *c.* $^1/_2$ km W of Hwy., S 29°44.75′, W 71° 19.77′, 100 m, 1 Aug 2000. | Landrum L. R. & S. Landrum *9824* | CTES | X | | |
| *B. incisifolia* | IV Region, Coquimbo, from Los Vilos to Caimanes in Pupío estero, Los Vilos commune, 31°53′00,08″S, 71°16′55,40″W, elevación 262 m a.s.l., 'Rumpiato', 23 Dec 2011 | Novoa, P. s.n. | VINAD | | X | |
| *B. incisifolia* | IV Region de Coquimbo, Elqui Dept., Algarrobal, 14 km east of station Diaguitas, along the railroad from La Serena to Rivadavia, rocky slopes, 700 m a.s.l., 'Rompiato', 5 Aug 1939 | Wagenknecht *18410* | LIL | X | | |
| *Guindilia cristata* (Radlk.) Hunz. | ARGENTINA. San Juan. Sarmiento Dept. Behind Ea. El Acequión, Puesto La Quebrada Hermanos Balmaceda, 32°07′0,06″S 68° 51′9,12″W, 1239 m a.s.l., 27 Jan 2010 | Ferrucci et al. *2930* | CTES | X | X | X |
| *G. cristata* | Santa Rosa River, ± 5 km from the mouth of Los Leones River, 24 Jan 1986 | Guaglianone et al. *1494* | CTES | X | | |
| *G. dissecta* (Covas & Burkart) Hunz. | ARGENTINA. Mendoza. Luján Dept. Potrerillos, C° Cabras, 1778 m a.s.l., 29 Nov 1977 | Ambrosetti & Del Vitto *174* | MERL | X | | |
| *G. dissecta* | Las Heras Dept., Uspallata, Quebrada del camino, 32°37′35,94″S 69°29′58.68″W, 1992 m a.s.l., 30 Jan 2010 | Ferrucci et al. *2968* | CTES | X | X | X |
| *G. trinervis* Gillies ex Hook. & Arn. | ARGENTINA. Mendoza. Malargüe Dept., Ruta 222, camino de Las Leñas a Los Molles, 100 m antes del puesto de gendarmería, 35°11′53,22″, 70°02′34.5W, 2036 m a.s.l., 2 Feb 2010 | Ferrucci et al. *3039* | CTES | | X | X |
| *G. trinervis* | San Rafael Dept., Salado Superior River, Rincón de Los Terneros, 7 Dec 1970 | Sosa *27364* | MERL | X | | |
| *G. trinervis* | CHILE. Metropolitana Region. Santiago Prov., San José de Maipo, 950 m a.s.l., 33°38′S 70°21′W, 15 Oct 1939 | Garaventa *4843* | CTES | X | | |



obtained. To gather additional information about sculpture, we obtained microphotographs with scanning electron microscope. The pollen grains were mounted on a metal slide and coated in palladium gold. The terminology used to describe pollen grains follows that of Erdtman (1966) and Punt, Hoen, Blackmore, Nilsson, and Le Thomas (2007).

## Statistical analyses

Pollen measurements obtained with LM are presented in Table 2, for the 12 variables analysed minimum, maximum and mean values are indicated, the latter inside parentheses. Statistical differences between the four taxa were estimated by ANOVA at a 5% significance level ($P \leq 0.05$) for all evaluated morphological variables (MVs). When ANOVA test indicated significant differences ($P \leq 0.05$), means were compared using the Fischer LSD test. Principal component analysis (PCA) was performed to explore associations between the set of MVs and the four taxa analysed. Analyses were performed using the statistical software InfoStat (Di Rienzo et al., 2011).

*Abbreviations*. P = polar axis, E = equatorial axis, P/E = polar to equatorial diameter ratio, A = apocolpium size, A/E = apocolpium index, CL = colpus length, CW = colpus width, PL = pore length, PW = pore width, S = sexine, N = nexine.

## Genome size estimation

The genome size was estimated in species cited in Table 1, using a PA flow cytometer (Partec GmbH, Germany). Each sample was measured three times. For nucleus extraction and release, a leaf portion was chopped with a sharp razor in 0.5 mL of the extraction buffer (CyStain® UV Precise P), after addition of staining buffer containing propidium iodide. The suspension of isolated nuclei was

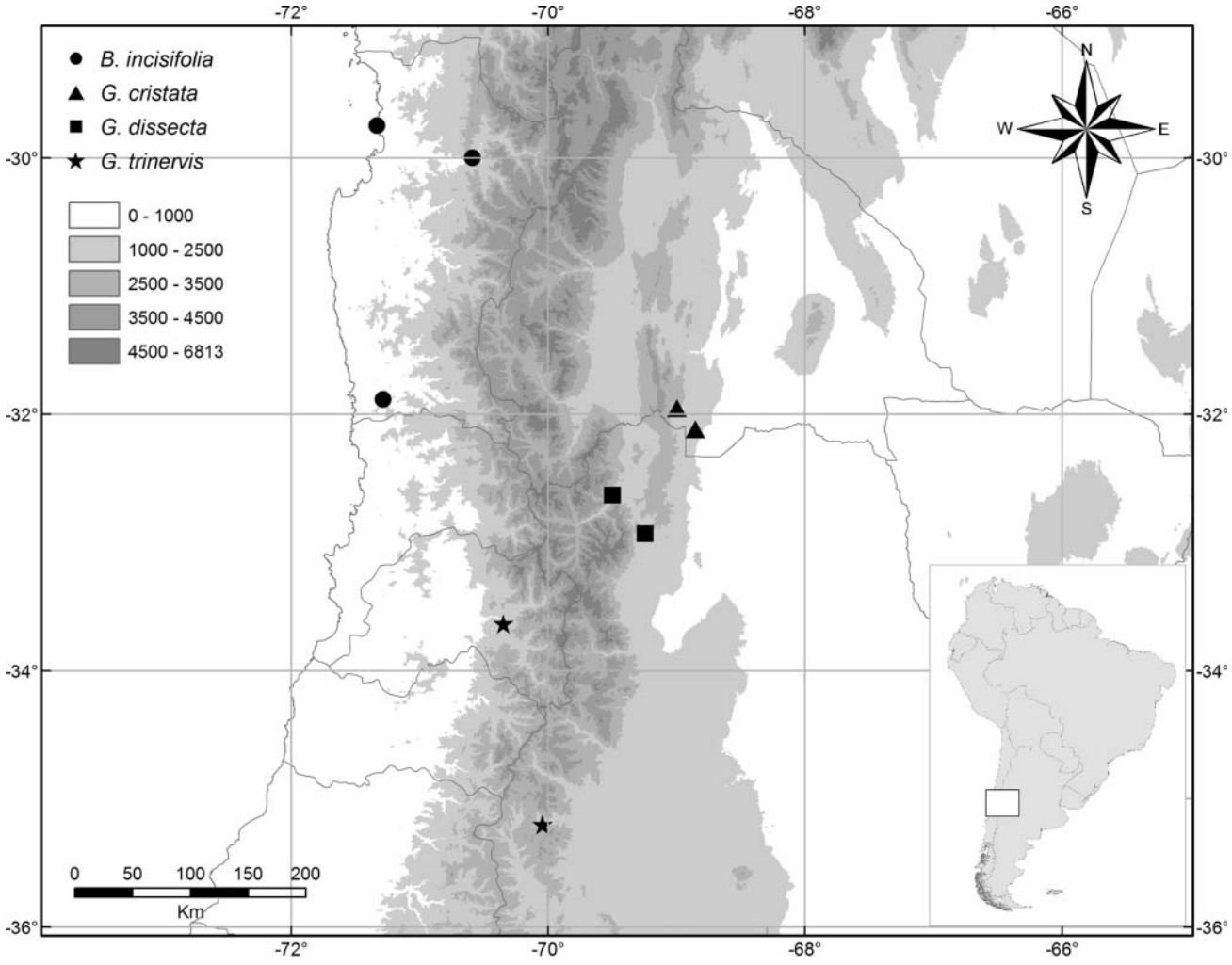


**Fig. 1.** Geographic distribution of *Bridgesia* and *Guindilia* species analysed. The greyscale indicates altitude (m a.s.l.).

filtered through a nylon filter (40 μm pore size) and immediately examined. The reference standard used for analysis was *Paspalum intermedium* Munro ex Morong and Britton (2n = 20 = 2x = 1.417 pg) for all species except for *Guindilia cristata*, whose reference was *P. dilatatum* Chirú (2n = 60 = 6x = 3.57 pg) (Vaio et al., 2007). A minimum of 5000 nuclei were measured in each sample and nuclear DNA content was calculated as: (Sample peak mean/Standard peak mean) × 2C DNA content of the standard (in pg). Cx values, representing the

**Table 2.** Pollen grain measurements in species of the genera *Bridgesia* and *Guindilia* (minimum, maximum and mean values in μm, the latter inside parentheses).

| Species | P | E | P/E | A | E | A/E | CL | CW | PL | PW | S | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Bridgesia incisifolia* | 27.5-50 (35) | 25-37.5 (32.37) | 0.85-1.8 (1.08) | 7.5-12.5 (9.37) | 30-40 (33) | 0.2-0.42 (0.28) | 22.5-35 (28.5) | 2.5-7.5 (4.37) | 5-7.5 (6.25) | 5-7.5 (6.25) | 1-2 (1.5) | 1-1 (1) |
| *Guindilia cristata* | 32-47.5 (38.3) | 27.5-42.5 (34.02) | 0.76-1.31 (1.13) | 5-12.5 (6.91) | 27.5-40 (33.75) | 0.13-0.35 (0.2) | 25-37.5 (32.08) | 2.5-5 (3.41) | 7.5-10 (8.08) | 7.5-12.5 (8.16) | 1-2 (1.31) | 1-1.5 (1.12) |
| *G. dissecta* | 25-50 (34.43) | 22.5-37.5 (29.28) | 1-1.8 (1.17) | 5-17.5 (7.68) | 22.5-40 (30.93) | 0.13-0.58 (0.24) | 17.5-35 (28.43) | 2.5-7.5 (4.09) | 5-7.5 (6.68) | 5-7.5 (6.06) | 1-2 (1.4) | 1-1 (1) |
| *G. trinervis* | 30-50 (35.52) | 25-35 (28.44) | 1.08-1.6 (1.25) | 5-12.5 (8.96) | 25-35 (29.06) | 0.14-0.42 (0.27) | 25-32.5 (26.43) | 2.5-7.5 (4.18) | 5-10 (6.75) | 5-7.5 (6.8) | 1-1.5 (1.38) | 1-1 (1) |

P = polar axis, E = equatorial axis, P/E = polar to equatorial diameter ratio, A = apocolpium size, A/E = apocolpium index, CL = colpus length, CW = colpus width, PL = pore length, PW = pore width, S = sexine, N = nexine.

DNA content of one non-replicated monoploid genome with the chromosome number x (Greilhuber, Lysák, Dolezel, & Bennett, 2005), were calculated as the 2C nuclear DNA amount divided by ploidy level. The mean and standard errors were determined for each set of measured tissues of each species.

### Chromosome count

Chromosome preparations were obtained from root tips taken from germinating seeds in the species listed in Table 1. After a pretreatment with 2 mM 8-hydroxyquinoline at 15 °C for 4−5 h, they were fixed in ethanol: acetic acid (3:1, v:v) for 12 hours and stored at −20 °C until use. For conventional chromosome analysis, the HCl/Giemsa technique (Guerra, 1983) was used. Permanent mounts were prepared with Entellan (Merck) and photographed with a phase contrast optic Zeiss Axiophot microscope (Jena, Germany) and a Leica DFC300FX camera (Wetzlar, Germany).

### DNA isolation, amplification, and sequencing

Total genomic DNA of *Bridgesia incisifolia*, *Guindilia cristata*, *G. dissecta* and *G. trinervis* of the samples listed in the Table 1 were isolated from leaf tissue using the CTAB II protocol (Weising, Nybom, Wolff, & Kahl, 2005). The internal transcribed spacer region (ITS) was amplified with the PCR technique (Mullis & Faloona, 1987) using ITS4/ITS5 (White, Bruns, Lee, & Taylor, 1990) primer pair. PCR amplifications were performed using a MasterCycler (Eppendorf, Hamburg, Germany) in a total volume of 50 μl, containing 1.5 mM MgCl2, 0.1 μM each primer, 0.2 mM dNTP and 1.25 U GoTaq DNA polymerase (Promega, Madison, WI, USA). PCR products were purified with a polyethylene glycol 8000 mixture (PEG 8000 20%, NaCl 2.5 M), and fluorescence sequencing was performed in Macrogen (Seoul, South Korea) with the same primers used for PCR amplification.

### Alignment and phylogenetic analysis

Sequences were *manually* edited and aligned with MEGA 6 with Muscle algorithms choice (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013), also added to the analysis were some sequences of species of the phylogenetic analysis performed by Harrington et al. (2005) and Buerki et al. (2009). Phylogenetic reconstruction was performed using maximum likelihood (ML) (Felsenstein, 1973) as implemented in RAxML GUI 1.0 (Silvestro & Michalak, 2012), and the best-fit model chosen was that used by Buerki et al. (2009), that is, a general time-reversible model with the addition of invariant sites and a gamma distribution of rates across sites (GTR+G+I). Statistical support for nodes was assessed by 1000 ML bootstrap replicates (Felsenstein, 1985) with the same model. Bayesian Inference (BI) analysis was performed with MrBayes v. 3.2 (Ronquist et al., 2012) software, constituted of two independent parallel runs of four Markov chains each and run for 10 million generations and sampled every 1000 generations. The chains and convergence of the two runs were monitored by Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). A total of 25% of the samples were discarded as burn-in and the resulting data were used to generate a 50% Bayesian majority-rule consensus tree.

The congruence between ML and BI trees obtained with different markers was estimated by calculating $I_{cong}$ associated with *P*-value, according to Vienne, Giraud, and Martin (2007). The trees were analysed using FigTree 1.4.2 (http://tree.bio.ed.ac.uk/) and TreeView 1.6.6 (Page, 1996). Both in maximum likelihood and in Bayesian analysis the chosen outgroup was *Harrisonia abyssinica* Oliv.

## Results

### Pollen morphology and statistical analysis

All the species studied have pollen grains, which are isopolar, radially symmetrical, 3−4 colporate, subspheroidal-suboblate to prolate spheroidal or prolate, medium-sized, colpi long (in *G. dissecta* frequently syncolporate grains), endoapertures circular, subcircular, lolongate or lalongate. The ambitus is subcircular to circular. The exine is tectate, sexine twice as thick as nexine, or about the same thickness. In *Guindilia* species the surface is always striate, whereas in *B. incisifolia* it can be striate-perforate, or striate-perforate and rugulate in the same sample. The colpus membrane exhibits granules or rugulate (Fig. 2−23).

The measurement range and the mean value of the 12 morphological variables analysed are presented in Table 2. A multivariate analysis (ANOVA) was carried out to explore correlations between morphological variables and the four taxa analysed. Mean values for morphological variables are listed in Table 2, different letters indicate significant differences ($P \leq 0.05$). Significant differences were observed among the four species for all morphological variables, except for colpus width and sexine (Table 3). *Guindilia cristata* differs from the remaining taxa in presenting higher values of polar axis, colpus length, pore length and nexine; for these variables, the remaining species did not differ from one another. *Guindilia cristata* is also characterized by exhibiting the highest values of equatorial diameter and pore size, and the lowest A:E ratio of all species. Therefore, these variables may be enough to discriminate *G. cristata* from the other species.

The biplot obtained from the first two principal components (PC1 and PC2) explained 95.2% of total variability in the data (Fig. 24) and revealed that *Guindilia cristata* was positively correlated with pore length, pore width,

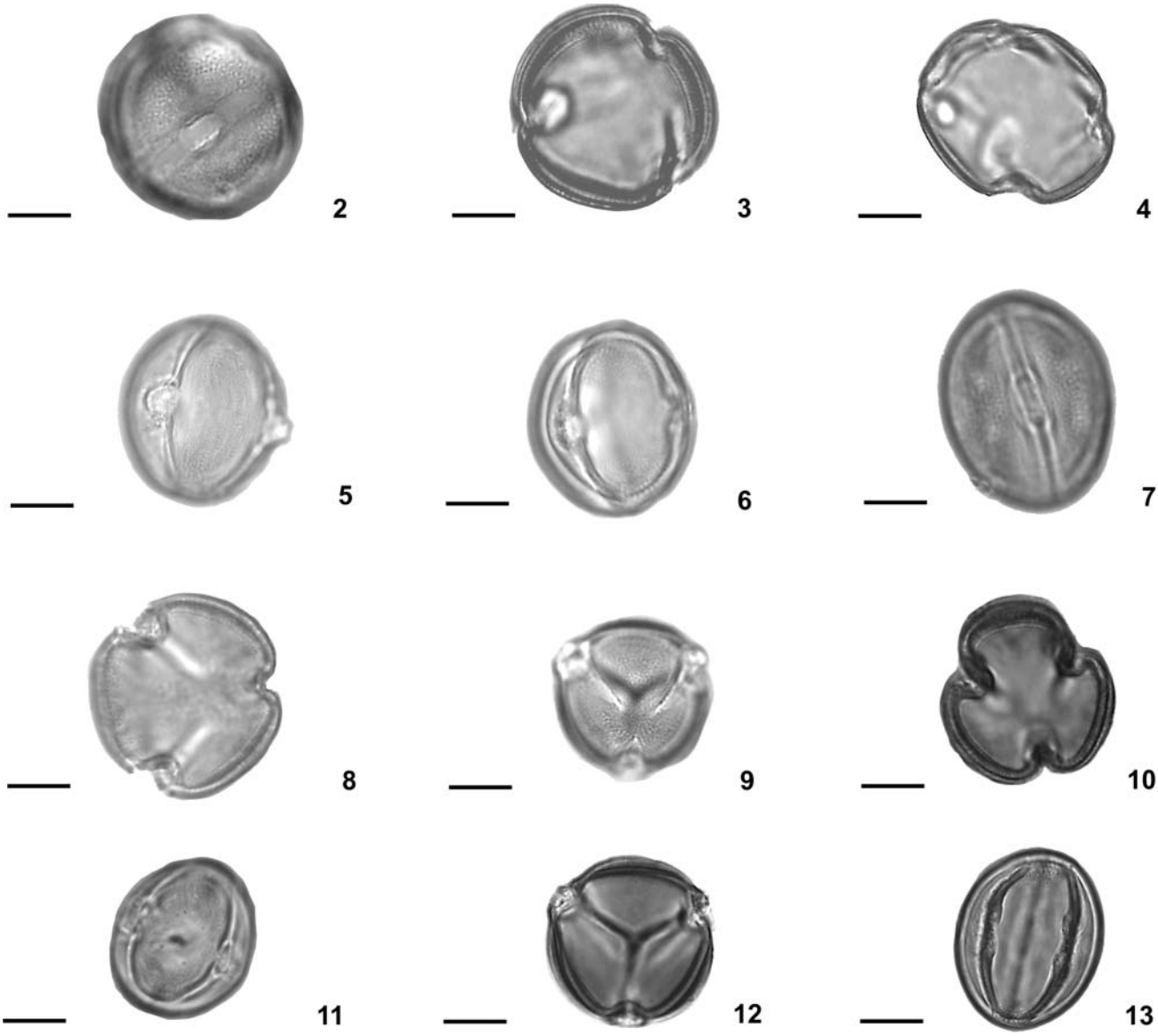


**Fig. 2–13.** Light microscope (LM) micrographs of acetolysed pollen grains. (2–4) *Bridgesia incisifolia* No. 9824: (2) equatorial view; (3) polar view, 3 aperturate; (4) polar view, 4 aperturate; (5, 8, 11) *Guindilia cristata* No. 2930: (5) equatorial view; (8) polar view; (11) equatorial view, cross-section; (6, 9, 12) *Guindilia dissecta* No. 2968. (6) equatorial view; (9) polar view; (12) polar view, cross-section, syncolporate grain; (7, 10, 13) *G. trinervis* No. 4843: (7) equatorial view; (10) polar view; (13) equatorial view, cross-section. Scale bars: 20 μm in (2–13).

polar axis, nexina thickness, and colpus length, whereas *G. trinervis* and *G. dissecta* were placed on the opposite site of the plane, and were positively associated with colpus width and apocolpium index. *Bridgesia incisifolia* was positively correlated with apocolpium size and sexine thickness (Fig. 24).

## Chromosomal analysis and genome size

The somatic numbers of the *Bridgesia* and *Guindilia* species were obtained for the first time (Table 4 and Figs 25–28). Chromosome numbers of both genera were significantly different. The basic chromosome number in *B. incisifolia* is x = 14 (2n = 28) and the three taxa studied of *Guindilia* share the basic number x = 10. *Guindilia dissecta* and *G. trinervis* are diploid with 2n = 20, whereas *G. cristata* is hexaploid with 2n = 60.

A summary of cytogenetic results, including chromosome complement, karyotypic formula, morphometric chromosome values, karyotype asymmetry data and genome size is presented in Table 4.

Mitotic chromosomes of *Bridgesia* and *Guindilia* are small to medium-sized (Table 4; Figs 25–32). Chromosome length varies from 1.2 to 3 μm, and *G. cristata* and

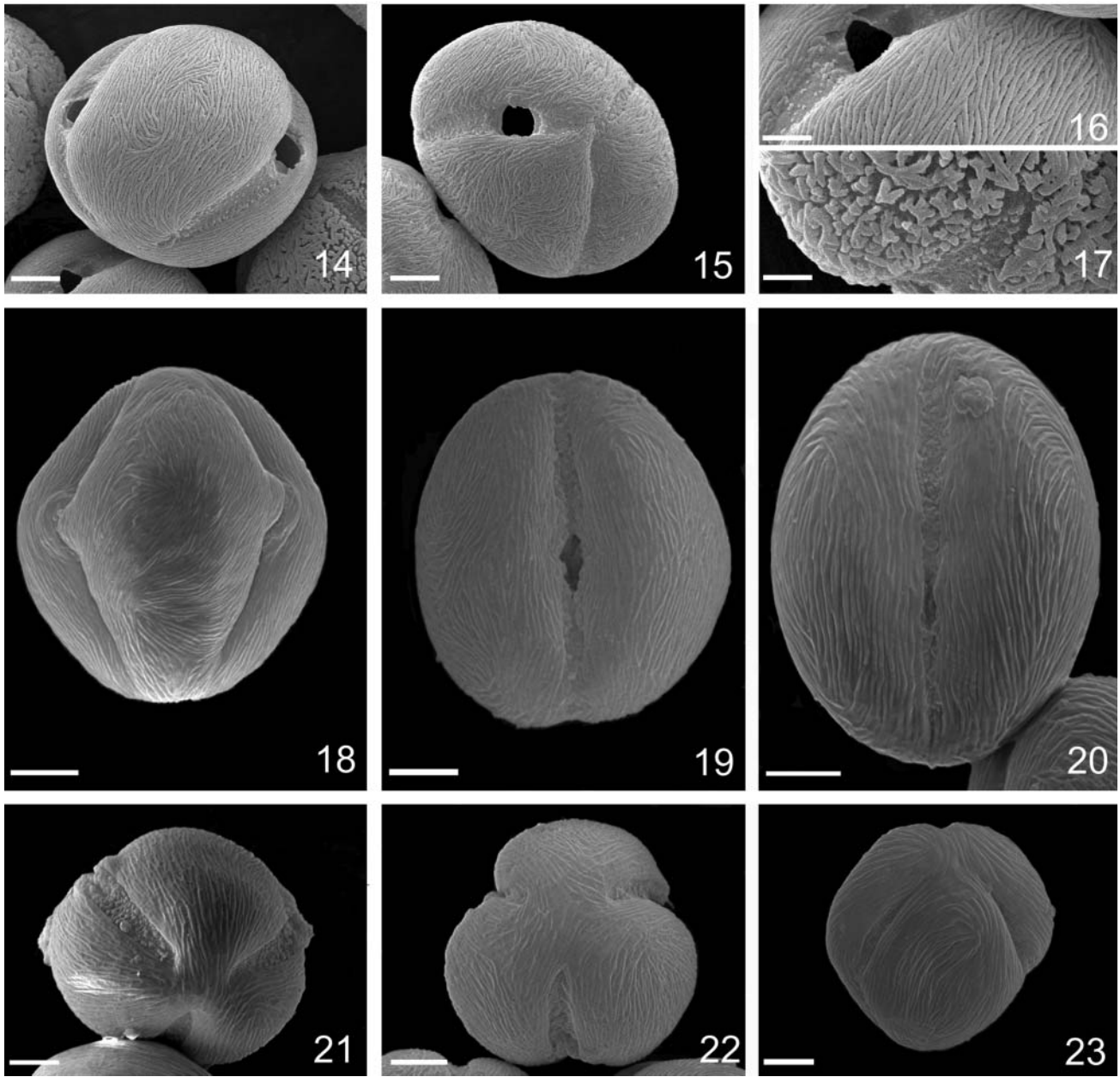


**Fig. 14–23.** Scanning electron microscope (SEM) micrographs of pollen grains. (14–17) *Bridgesia incisifolia* No. 9824: (14) equatorial view; (15) subpolar view; (16–17) detail of mesocolpium and apertural membrane; (18–19) *Guindilia cristata* No. 2930: (18) equatorial view; No. 1494: (19) polar view; (20–21) *G. dissecta* No. 2968: (20) equatorial view; (21) polar view; (22–23) *G. trinervis* No. 4843: (22) equatorial view; (23) polar view. *Scale bars*: 6 μm in (14–15); 2 μm in (16–17); 8 μm in (18); 4 μm in (19); = 6 μm in (20–23).

*G. dissecta* show the larger chromosomes than *B. incisifolia* and *G. trinervis*. Figures 29–32 show the idiograms representing the chromosome morphology and karyotype of each taxa analysed.

In the species studied, the karyotypic formulae show great variability, in the *Guindilia* species this feature is characterized by the presence of three *st*-chromosomes; this chromosome type is absent in *Bridgesia* (Table 4; Figs 25–32). The karyotypes are slightly asymmetrical, with the asymmetry index ranges being A1 = 0.37–0.44 and A2 = 0.14–0.19 (Table 4).

Regarding the DNA content (2C-value), in *Bridgesia* and *Guindilia* species varied from 2.32 to 7.08 pg; however, the 1C-value ranged from 1.16 to 1.20 pg in *Guindilia* and was 1.28 pg in *Bridgesia* (Table 4, Figs 33–36).

**Table 3.** Mean values of morphological variables measured in four taxa: *Bridgesia incisifolia*, *Guindilia cristata*, *G. dissecta* and *G. trinervis.*

| Variables | *B. incisifolia* | *G. cristata* | *G. dissecta* | *G. trinervis* |
|---|---|---|---|---|
| Polar axis | 35[a] | 38.31[b] | 34.44[a] | 35.52[a] |
| Equatorial diameter | 32.38[b] | 34.03[c] | 29.28[a] | 28.44[a] |
| P:E | 1.08[a] | 1.13[ab] | 1.17[b] | 1.25[c] |
| Apocolpium index | 9.38[c] | 6.92[a] | 7.69[ab] | 8.06[b] |
| E (Equat. diam.) | 33[c] | 33.75[c] | 30.94[b] | 29.06[a] |
| A:E | 0.29[b] | 0.20[a] | 0.25[b] | 0.28[b] |
| Colpus length | 28.5[a] | 32.08[b] | 28.44[a] | 26.44[a] |
| Colpus width | 4.38[a] | 3.42[a] | 4.09[a] | 4.19[a] |
| Pore length | 6.25[a] | 8.08[b] | 6.69[a] | 6.75[a] |
| Pore width | 6.25[ab] | 8.17[c] | 6.06[a] | 6.81[b] |
| Sexina | 1.50[a] | 1.32[a] | 1.40[a] | 1.39[a] |
| Nexina | 1.00[a] | 1.12[b] | 1.00[a] | 1.00[a] |

Different superscript letters represent significant differences ($P \leq 0.05$) among species.

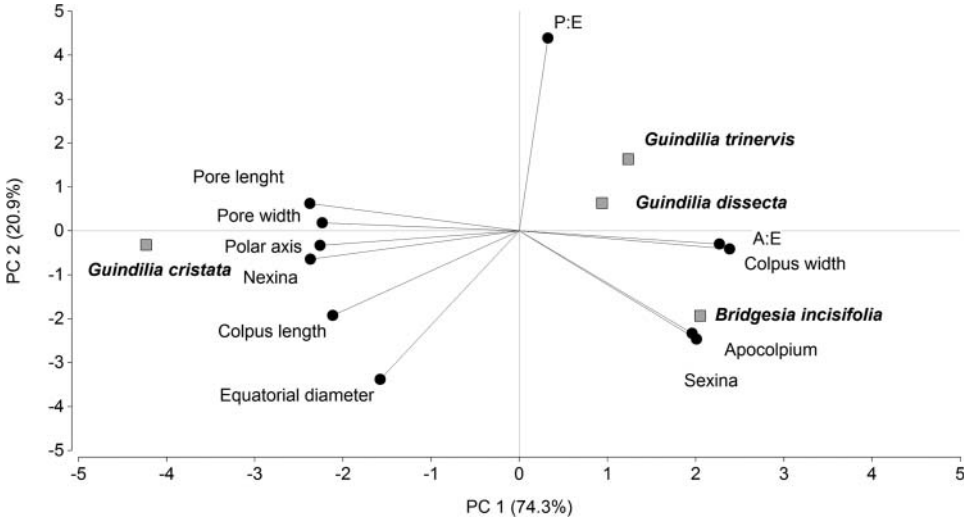


**Fig. 24.** Biplot showing relationships between morphological variables (black circles) and the four taxa analysed (grey squares): *Guindilia cristata*, *G. trinervis*, *G. dissecta* and *Bridgesia incisifolia*.

## Molecular phylogenetic analysis

The ITS sequences of the *Guindilia* species, *Paullinia pinnata* and *Urvillea ulmacea* were successfully amplified (Table 5), with the sequences obtained ranging from 672 to 686 bp. PCR amplifications resulted in single bands and the sequence files showed no problematic double peaks.

The ITS sequences lengths ranged from 565 bp in *Xanthoceras sorbifolium* to 732 bp in *Melicoccus lepidopetalus*. The aligned ITS region comprised 886 pb, of which

**Table 4.** Chromosome number, genome size and voucher specimen data of *Bridgesia* and *Guindilia* species.

| Species | 2n | KF | Lt | C | V | A1 | A2 | 2C (pg) | Mpb (*) | 1C | Voucher specimen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Bridgesia incisifolia* | 28 | 7m + 7sm | 21,5 | 1,5 | 2,0-1,2 | 0,41 | 0,15 | 2.57 | 2513,46 | 1,28 | P. Novoa s.n. (VINAD) |
| *Guindilia cristata* | 60 | 19m + 2sm + 9st | 57,6 | 1,9 | 2,6-1,2 | 0,37 | 0,14 | 7.08 | 6924,24 | 1,18 | M.S. Ferrucci *2930* (CTES) |
| *G. dissecta* | 20 | 7m + 3st | 20,8 | 2,1 | 3,0-1,7 | 0,4 | 0,19 | 2.32 | 2268,96 | 1,16 | M.S. Ferrucci *2968* (CTES) |
| *G. trinervis* | 20 | 6m + 1sm + 3st | 13,8 | 1,4 | 1,9-1,2 | 0,44 | 0,15 | 2.40 | 2347,2 | 1,20 | M.S. Ferrucci *3039* (CTES) |

(*) 1 pg DNA = 978 Mbp (Dolezel, Bartos, Voglmayr, & Greilhuber, 2003)

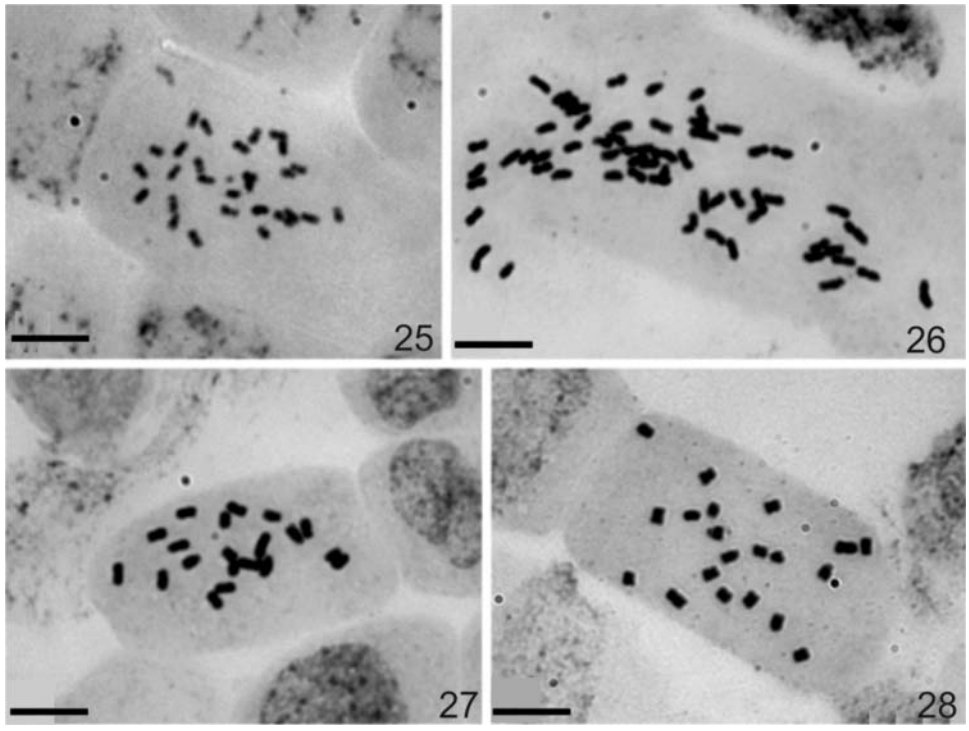


**Fig. 25–28.** Metaphase chromosome plates of *Bridgesia* and *Guindilia* species stained with HCl-Giemsa. (25) *B. incisifolia* No. Novoa *without number*: 2n = 28; (26) *G. cristata* No. 2930: 2n = 60; (27) *G. dissecta* No. 2968: 2n = 20; (28) *G. trinervis* No. 3039: 2n = 20. Scale bars: 5 μm.

319 pb were constant and 313 pb were potentially parsimony-informative. The comparisons of pairwise distances of ITS sequences between *Guindilia* and *Bridgesia* indicated a divergence of 16.9–17.6%; however, comparisons between *Guindilia* species ranged from 1.6% to 2.4%.

As with the ITS sequences, the *trn*L-F sequences of the *Guindilia* species were successfully PCR amplified, ranging from 386 to 424 bp. The *trn*L-F sequences lengths ranged from 250 bp in *Allophylus africanus* to 434 bp in *Harrisonia abyssinica*. The matrix aligned for *trn*L-F

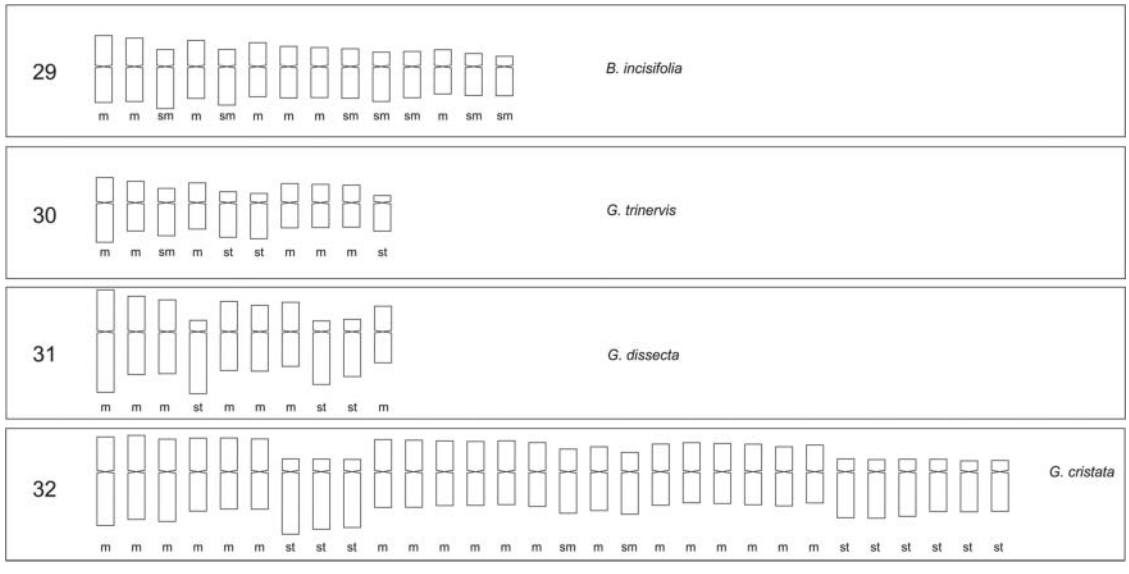


**Fig. 29–32.** Haploid complement of *Bridgesia* and *Guindilia* species. (29) *B. incisifolia*; (30) *G. cristata*; (31) *G. dissecta*; (32) *G. trinervis*. Scale bar: 5 μm.

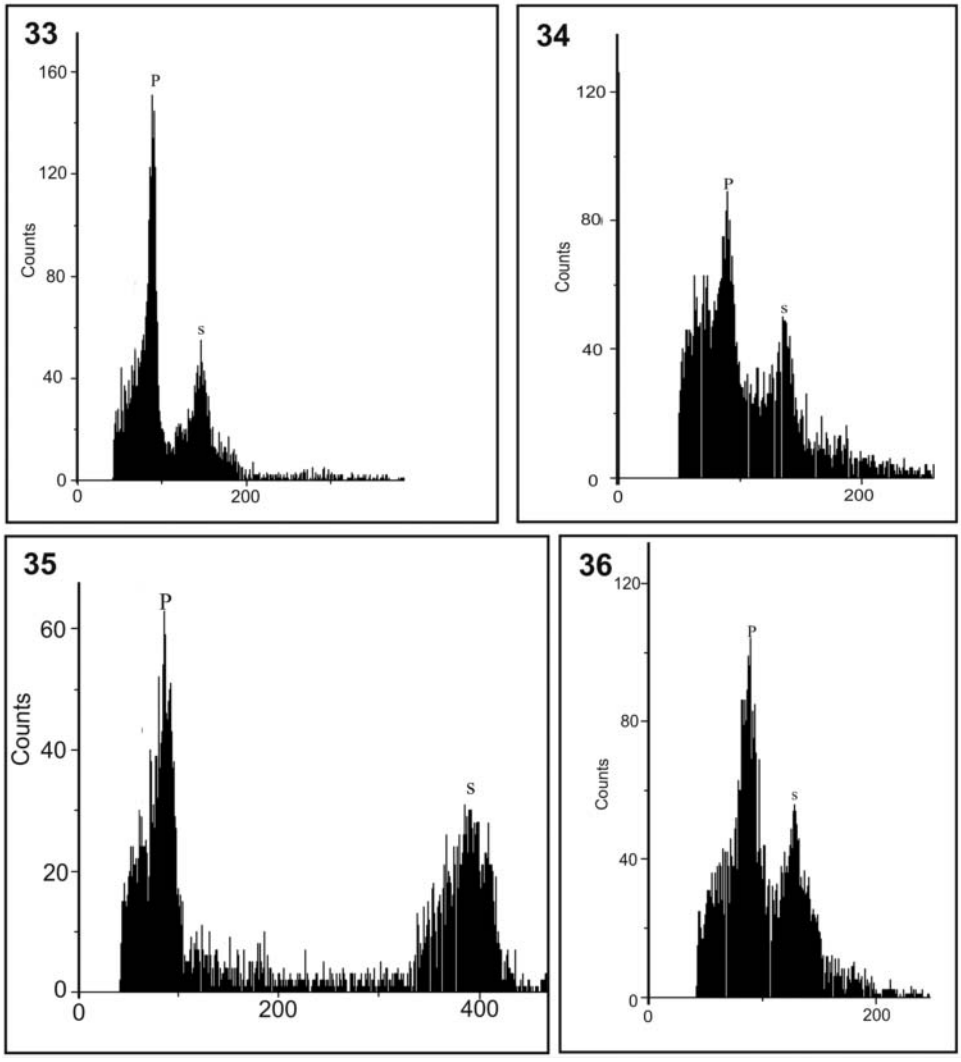


**Fig. 33–36.** Flow cytometry plots. The x axis represents the propidium iodide (PI) fluorescence (i.e. relative DNA amount) and the y axis represents the number of nuclei measured (× 100). S, sample measured in each histogram; P, reference standard used. The histograms belong to: (33) *B. incisifolia* Novoa *without number*; (34) *G. dissecta* No. 2968; (35) *G. cristata* No. 2930; (36) *G. trinervis* No. 3039.



intron comprised 529 pb, of which 278 pb were constant and 73 pb were potentially parsimony-informative. The comparisons of pairwise distances of *trn*L-F sequences between *Guindilia* and *Bridgesia* indicated a divergence of 3.1%; however, between *Guindilia* species these were only 0.1%.

The *trn*L sequences obtained of the *Guindilia* species ranged from 512 to 533 bp, and the sequences studied ranged from 418 bp in *B. incisifolia* to 534 bp in *Koelreuteria paniculata.* The matrix aligned with 650 pb contained 357 pb constant sites and 98 pb were potentially parsimony-informative. The comparisons between *Guindilia* and *Bridgesia* indicated a divergence of 2.2–2.4%, and less than 0.2% between *Guindilia* species.

The maximum likelihood analyses with ITS (log L −8788.788), trnL (log L −2561.474) and trnL-F (log L −2229.481) were congruent (ITS/trnL: $I_{cong} = 1.878$, $P = 7.31^{-07}$; ITS/trnL-F: $I_{cong} = 1.760$, $P = 5.13^{-06}$; trnL/trnL-F: $I_{cong} = 1.878$, $P = 7.31^{-07}$). The combined matrix of ITS, trnL and trnL-F regions comprised 2065 aligned nucleotides and released a ML tree with log L −14146.899. The ML tree (Fig. 37) shows five clades; clade A groups species belonging to the tribes Paullinieae and Thouinieae, including *B. incisifolia*; clade B comprises species of the *Guindilia* genus; and the other tree clades groups species that were not analysed in detail in this work. The results of the Bayesian analyses were congruent with the ML analyses, and posterior probability values for many nodes were high (≥ 0.95; Fig. 37).

**Table 5.** Species analysed and sequence ID, extracted from GenBank.

| Species | Sequence ID | | |
|---|---|---|---|
| | ITS | trnL | trnL-F |
| *Acer saccharum* Marshall | EU720502.1 | EU721272.1 | EU721460.1 |
| *Aesculus wangii* Hu | AF406968.1 | AF411085.1 | EU721461.1 |
| *Alectryon connatum* Radlk. | EU720415.1 | EU721169.1 | EU721357.1 |
| *Allophylus africanus* P. Beauv. | JN190965.1 | JN191056.1 | JN191015.1 |
| *Allophylus welwitschii* Gilg | JN190974.1 | JN191062.1 | JN191020.1 |
| *Athyana weinmanniifolia* (Griseb.) Radlk. | EU720487.1 | EU721257.1 | EU721445.1 |
| *Blomia prisca* (Standl.) Lundell | EU720444.1 | EU721208.1 | EU721396.1 |
| *Bridgesia incisifolia* Bertero ex Cambess. | This Paper | EU721247.1 | This Paper |
| *Cardiospermum halicacabum* L. | HE586146.1 | JN681469.1 | JN681535.1 |
| *Cupania dentata* DC. | EU720523.1 | EU721289.1 | EU721477.1 |
| *Dictyoneura obtusa* Blume | EU720428.1 | EU721187.1 | EU721375.1 |
| *Dodonaea viscosa* (L.)Jacq. | FJ546953.1 | JN191076.1 | DQ978578.1 |
| *Doratoxylon chouxii* Capuron | EU720513.1 | EU721282.1 | EU721333.1 |
| *Guindilia cristata* (Radlk.) Hunz. | This Paper | This Paper | This Paper |
| *Guindilia dissecta* (Covas & Burkart) Hunz. | This Paper | This Paper | This Paper |
| *Guindilia trinervis* Gillies ex Hook. & Arn. | This Paper | This Paper | This Paper |
| *Haplocoelum foliolosum* (Hiern) Bullock | EU720479.1 | EU721164.1 | EU721352.1 |
| *Harpullia arborea* Radlk. | EU720448.1 | EU721215.1 | EU721403.1 |
| *Harrisonia abyssinica* Oliv. | GU178980.1 | EU721202.1 | EU721390.1 |
| *Koelreuteria paniculata* Laxm. | EU720548.1 | EU721317.1 | EU721505.1 |
| *Lepisanthes senegalensis* (Poir.) Leenh. | EU720492.1 | JN191085.1 | EU721451.1 |
| *Litchi chinensis* Sonn. | EU720400.1 | JN191088.1 | EU721341.1 |
| *Macphersonia chapelieri* (Baill.) Capuron | EU720459.1 | EU721227.1 | EU721415.1 |
| *Melicoccus lepidopetalus* Radlk. | EU720443.1 | EU721206.1 | EU721394.1 |
| *Paullinia pinnata* L. | This Paper | JN191092.1 | EU721355.1 |
| *Paullinia subauriculata* Radlk. | EU720494.1 | EU721266.1 | EU721454.1 |
| *Serjania communis* Cambess. | EU720472.1 | EU721241.1 | EU721429.1 |
| *Serjania glabrata* Kunth | EU720557.1 | EU721327.1 | EU721515.1 |
| *Talisia angustifolia* Radlk. | EU720558.1 | EU721328.1 | EU721516.1 |
| *Talisia nervosa* Radlk. | EU720474.1 | EU721244.1 | EU721432.1 |
| *Thouinia acuminata* S. Watson | EU720478.1 | EU721249.1 | EU721437.1 |
| *Tristiropsis acutangula* Radlk. | EU720453.1 | EU721220.1 | EU721408.1 |
| *Urvillea ulmacea* Kunth | This Paper | EU721270.1 | EU721458.1 |



# Discussion

## Pollen morphology and statistical analysis

The pollen morphology of four species belonging to the genera *Bridgesia* and *Guindilia* is presented here, all of which are analysed for the first time. Although the literature about pollen morphology in the family is quite extensive, undoubtedly the most relevant contribution at family level is that of Muller and Leenhouts (1976). These authors evaluated the different types of pollen in relation to the systematics proposed by Radlkofer (1931–1934), based on macromorphological characters and pollen morphology. The results reported by Muller and Leenhouts (1976), with emphasis on the Thouinieae tribe, are now evaluated together with cytogenetic and molecular data among the species studied here. *Bridgesia* and *Guindilia* share the basic pollen type within the family, i.e., tricolporate grains. As to pollen morphology, an update of the family reveals that the basic pollen type is present in all the tribes.

Regarding ornamentation, *Guindilia* species have tectum striate. On the other hand, in *Bridgesia incisifolia* the ornamentation varies within the species, even within a sample: striate-perforate or rugulate. This intraspecific variation in ornamentation has also been observed by van der Ham (1990) in the tribe Nephelieae. This author found

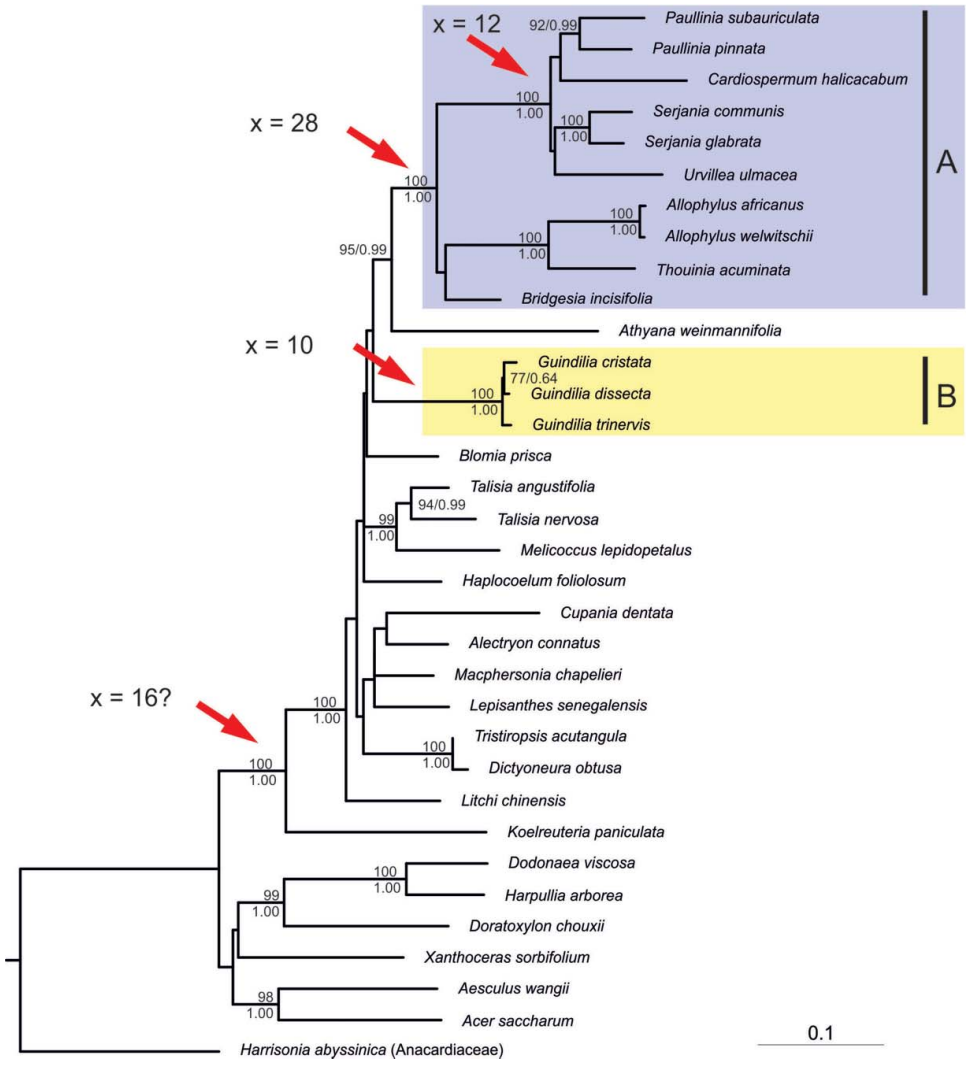


**Fig. 37.** Maximum likelihood phylogram of *Bridgesia* and *Guindilia* with other genera of Sapindaceae based on the combined analysis of ITS, trnL and trnL-F. Numbers above/left each branch are ML bootstrap values and below/right are posterior probabilities from the Bayesian analysis. The support values < 90% not shown. The boxes indicate the clades *Paullinia* (Clade A) and *Guindilia* group (Clade B). Chromosome basic numbers (x) are specified in some clades.



a complete series from rugulate to striate in the genera *Alectryon* Gaertn., *Nephelium* L. and *Stadmannia* Lam. which is restricted to a minor part of the samples of a species, or to a part of the grains in a sample. The sculptural characters of the exine appeared to be more variable, and of secondary importance at family level; this is consistent with findings reported by Muller and Leenhouts (1976), who observed that the aperture type is more conservative than exine sculpture.

The ANOVA showed that of the four species studied, *G. cristata* reached the highest values for pore length, pore width, polar axis, nexine thickness and colpus length. These five morphological variables allowed us to differentiate *G. cristata* from the remaining species. The results were reflected in the biplot (Fig. 24), where the trait vectors of these variables exhibited the highest inertia in the first component (explaining 95.2% of the total variability of the data) and were strongly and positively correlated with *G. cristata*.

On the basis of the material studied, pollen morphology has limited taxonomic value as a diagnostic character to separate both genera.

***Guindilia* and *Bridgesia* karyotype evolution.** The results of this study revealed a detailed description of *Bridgesia* and *Guindilia* chromosomes and their pattern of variation. The chromosome number in *Bridgesia* ($2n = 28$, $x = 14$) is very frequent in Thouinieae; however, the genus *Guindilia* shows a basic chromosome number $x = 10$. This feature is a whole new basic number for the tribe

Thounieae and represents 4.6% of the total basic chromosome number of the family (Ferrucci, 2000), which is present in distant clades as *Cardiospermum* (Ferrucci, 1981; Urdampilleta et al., 2013), *Aesculus* (Mehra, 1976) and *Handeliodendron* (Cao, Xia, & Xiong, 2005). The distribution of chromosome numbers suggests that the x = 10 originates from at least three independent events in chromosomal evolution of Sapindaceae.

The karyotypes recorded in both genera show a common pattern, with all species having metacentric (m) chromosomes; in *Guindilia* the subtelocentrics (st) are always present in the karyotypic formulae, whereas the submetacentric ones are absent in *G. dissecta.* Moreover, *G. cristata* is hexaploid, with an interesting DNA content which will be discussed below. The karyotype formula and their quantitative data show a conservative behaviour among *Guindilia* species, except for total length due to the polyploidy status of *G. cristata.* The constancy in the asymmetry indexes are evidence of this fact (King, 1970; Shaw, Wilkinson, & Coates, 1983).

Nuclear DNA content is characteristic of the species, and comparisons of nuclear DNA amounts have proved to be useful in many cytotaxonomic, phylogenetic, and evolutionary studies (Bennett & Leitch, 1995). The analysis of nuclear DNA variation within a genus provides a useful approach to investigating ancestry and genome composition of species included in any genus. According to the available genome size data, Sapindaceae *s.l.* is characterized by mainly possessing very small genomes, i.e., 1C < 1.4 pg (Coulleri, Urdampilleta, & Ferrucci, 2014; Soltis, Soltis, Endress, & Chase, 2005) and similar to *Bridgesia* and *Guindilia* species analysed, indicating that the variation of the basic numbers would be related to chromosome rearrangements. Moreover, genome size in Sapindaceae has a conservative behaviour, tending to maintain a very small or small genome size among all the component tribes (Coulleri et al., 2014).

Polyploidy is an important driving force in plant evolution and especially frequent among angiosperms (Otto & Whitton, 2000; Wendel, 2000), but is uncommon in Sapindaceae. Some records of polyploids were cited in *Aesculus*, *Allophylus*, *Cardiospermum*, *Melicoccus*, *Paullinia* and *Urvillea* (Ferrucci & Solís Neffa, 1997; Soltis et al., 2005; Urdampilleta, 2009; Urdampilleta, Ferrucci, Torezan, & Vanzela, 2006, 2013). The new report of hexaploid (2n = 60) for *G. cristata* suggests that polyploidy would arise independently in Sapindaceae. The case of *G. cristata*, with 2C-value 7.08 pg, belonging to a intermediate genome size (Leitch, Soltis, Soltis, & Bennett, 2005), is three-fold the DNA content of the mean of the 2C-values of *G. dissecta* and *G. trinervis*, suggesting that *G. cristata* is a recent polyploidy.

Polyploids have been postulated to be evolutionary dead ends because of the inefficiency of selection when genes are masked by multiple copies (Stebbins, 1971). However, Soltis, Visger, and Soltis (2014) raised in line with Wood et al. (2009), Jiao et al. (2011) among other investigations, suggest that polyploids show better performance and lower extinction rates in new environments or extreme environments, as was proposed by Hagerup (1932); Stebbins (1950), and Brochmann et al. (2004). In fact, the environmental conditions under which *Guindilia cristata* occurs also support the assumption that this species would be a recent polyploidy. We base this assumption on the hypothesis that species with a large genome are progressively excluded from extreme environments with short growing seasons (Bennett, 1987; Bennett, Smith, & Lewis Smith, 1982; Knight & Ackerly, 2002; Knight, Molinari, & Petrov, 2005; Levin & Funderburg, 1979; Rayburn, 1990; Suda, Kyncl, & Freiova, 2003); accordingly, San Juan Province is mainly characterized by a hot desert climate (Bwh) *sensu* Köppen climate classification (Poblete & Minetti, 1989). The autopoloploidy from a common ancestor with *G. dissecta*, due to karyotype similarities, could be the most probable scenario for the origin of *G. cristata*.

## Circumscription of Guindilia and phylogeny of Paullinia group

At the family level, we are in accordance with Buerki et al. (2010) who resurrect the temperate families Aceraceae and Hippocastanaceae, and restrict Sapindaceae *s.s.* nearly identical to that used for over a century by excluding *Xanthoceras*. On the other hand, we take into account the subfamily classification proposed by Buerki et al. (2009) that recognizes four subfamilies of which Dodonaeoideae and Sapindoideae belong to Sapindaceae *s.s.* The latter was defined by Radlkofer (1931–1934) and is characterized by a single apotropous ovule per locule, upright or ascending. However, the inclusion of several genera with two ovules per locule, such as *Conchopetalum* Radlk., *Delavaya* Franch., *Koelreuteria* Laxm., and *Ungnadia* Endl., by Harrington et al. (2005), Thorne (2007), and Buerki et al. (2009), renders this key-character obsolete. Sapindoideae comprises *sensu* Buerki et al. (2009) 10 informal tribal groupings. Our results show the polyphyly of all the tribes studied, with the exception of Paullinieae and Thouinieae, as described by Buerki et al. (2009, 2010). The phylogenetic information also reveals the validity of the *Paullinia* group defined by Buerki et al. (2009), which comprises Paullinieae and Thouinieae tribes, and the closest relationship between this group with *Melicoccus* and *Blomia* groups.

Although the groups proposed by Buerki et al. (2009) are represented exactly as was described by the authors, we introduce a new genus in the analysis, the *Guindilia*

genus, which belongs to the Thouinieae tribe *sensu* Radlkofer (1931−1934, as *Valenzuelia*). However, in all the phylogenetic analyses performed in this work *Guindilia* was grouped in a clade different from the expected group. This fact encourages us to define a new informal tribal grouping, the *Guindilia* Group that includes all three species belonging to this genus: *G. cristata*, *G. dissecta*, and *G. trinervis.* This group, as the *Paullinia* Group, is closely related to the *Melicoccus* and *Blomia* groups, although no phenotypic synapomorphies exist to relate all the groups cited above. The *Guindilia* group is well supported and is also a monophyletic clade.

Morphologically, the *Guindilia* group is characterized by the decussate leaf arrangement, which would be the only apomorphic character of the genus. In the family only three species of the genus *Matayba* (*c*. 50 spp.), belonging to the tribe Cupanieae, show leaves that may be subopposite or alternate. In turn, *Bridgesia* is recognized by alternate simple leaves and fruits with comparatively well-developed wings in relation to *G. cristata* and *G. dissecta*.

The phylogenetic analysis of *Guindilia* showed that the genus is not grouped in the expected clade (Paullinieae−Thouinieae clade); instead, the genus forms a single clade that is not related to the *Paullinia* group. Based on these results, we established a new informal tribal group, named the *Guindilia* group, in which the species share the basic number x = 10; *Guindilia* is the only genus in the entire family presenting opposite leaves as an apomorphic trait.



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## Disclosure statement

No potential conflict of interest was reported by the authors.

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