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Research Article

Genome Size Study in the Valerianaceae: First Results and New Hypotheses

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The purpose of this study is to provide a new focus to contribute, from the perspective of genomic evolution, towards a better understanding of the Valerianaceae evolutionary history. Chromosome numbers were determined by Feulgen staining in 24 populations of 18 species (first count for *Valerianella multidentata*, $2n = 2x = 14$–16), and DNA contents were assessed by flow cytometry in 74 populations of 35 species (first assessments in all taxa but *Centranthus ruber*). A molecular phylogeny based on the *trn*L-*trn*F and including 41 new sequences was established, with the first DNA sequence for *Centranthus nevadensis*, *Valeriana rotundifolia*, *V. saxatilis*, *Valerianella multidentata*, and *V. turgida*. This work is the first large genome size study devoted to the Valerianaceae, showing a range of DNA amounts from 2C = 0.39 pg (*Valerianella turgida*) to 2C = 8.32 pg (*Valeriana officinalis*). At the family level, changes in basic chromosome number and genome size coincide with or precede major shifts in the evolutionary history of the group, such as those concerning stamen number and floral symmetry.

1. Introduction

The family Valerianaceae (currently considered within the Caprifoliaceae s.l.; [1]) comprises ca. 400 species of which approximately 200 are included in *Valeriana* L., the biggest genus of the order Dipsacales. The Valerianaceae are cosmopolitan in their natural distribution, with the exceptions of Australia and the Pacific islands, where they were introduced, and can be nowadays considered as naturalized. Several of their representatives have economic interest as medicinal (e.g., *V. officinalis* L.), edible (e.g., *Valerianella* Mill., corn salad or lamb's lettuce), or ornamental plants (e.g., *Centranthus* DC.). The family always roused important scientific interest, and its study has broadly benefited from the new molecular techniques, especially the phylogenetic reconstructions. Recent phylogenies based on DNA sequencing [2–7] have considerably modified the traditional classification of the family [8–12]. Molecular results recognize only six genera: *Centranthus*, *Fedia* Gaertn., *Nardostachys* DC., *Patrinia* Juss., *Valeriana* (including *Aretiastrum* DC., *Astrephia* Dufr., *Belonanthus* Graebn., *Phyllactis* Pers., *Porteria* Hook., and *Stangea* Graebn.), and *Valerianella*. *Plectritis* (Lindl.) DC. is nested amongst South American *Valeriana* [5, 6]. Moreover, Bell [7] suggested a possible further taxonomic treatment of the family considering *Fedia* as a synonym of *Valerianella*. New genera may also be described for some of the *Valeriana* species that do not group with their congenerics in the phylogenetic reconstructions and make the genus *Valeriana* paraphyletic in its current circumscription. These are *Valeriana longiflora* Willk., which appears closely related

to the genus *Centranthus*, and also *Valeriana celtica* L. and *V. hardwickii* Wall., branched somewhere between *Nardostachys* and *Valerianella* plus *Fedia* clades [4, 5].

Morphology in Valerianaceae is of great interest for its impressive diversity of forms, mainly resulting in adaptations to a wide range of ecological conditions (from the sea board to Andean páramos at 4000 m), and concerning both vegetative and reproductive parts. This diversity has been especially well studied from the inflorescential structure perspective, with a common basic type, the thyrse, and very different forms and levels of complexity [13, 14]. Family Valerianaceae stands out in Dipsacales by presenting four different stamen numbers in a series (mainly four stamens in *Patrinia* and *Nardostachys*, three in *Plectritis*, *Valerianella* and *Valeriana*, two in *Fedia*, and one in *Centranthus*), while the other families show one or two distinct stamen numbers [15, 16]. These features, along with some cases of strong corolla zygomorphy (in *Centranthus*, with 4 : 1 petals orientation; in *Fedia*, with 2 : 3 petals orientation) make the family very attractive for studying the genetic base of floral characters, which has been initiated through an evolutionary-developmental approach by [16, 17]. Evo-devo studies highlight the crucial importance of duplication events in the evolution of genes involved in developmental processes (e.g., [18, 19]). These events often correspond to whole duplication genome ([20], and references therein), making the karyological and cytogenetic data essential for understanding many evolutionary processes, as, for example, the floral morphological changes.

Nevertheless, karyological and cytogenetic data in Valerianaceae are basically limited to chromosome counts. The Valerianaceae exhibit a dysploid series of five basic chromosome numbers [21]: $x = 15$ in American *Valerianella*, $x = 13$ in *Nardostachys*, $x = 11$ in *Patrinia* and *Valeriana celtica*, $x = 8$ in *Centranthus*, *Fedia*, *Valeriana* and *Valerianella*, and $x = 7$ in *Valeriana* and *Valerianella*. Polyploidization events are common, some genera being exclusively polyploid, such as *Centranthus* (tetraploid) or *Fedia* (tetraploid and hexaploid), while species of *Patrinia* and *Valeriana* can exhibit various ploidy levels, from diploid to octoploid. Hence, both polyploidy and dysploidy seem to have played a significant role in the differentiation and evolution of these plants. Cytogenetic data (such as banding, fluorescent *in situ* hybridization and genome size assessment) would certainly be of a great interest for understanding the evolution of the family. Nuclear DNA amount assessments constitute a fundamental complement to chromosome counts and, in addition, a powerful tool in order to establish the relationships between closely related taxonomical groups (e.g., in *Echinops* L.; [22], *Orobanche* L.; [23]), as well as to understand the evolution within related genera from a broader point of view (e.g., Liliaceae; [24], Orchidaceae; [25]). Furthermore, genome size is a useful tool to detect possible hybrid and polyploid origins of taxa (e.g., in *Carthamus* L.; [26], *Nicotiana* L.; [27], *Artemisia* L.; [28]), and intraspecific variation can reveal incipient speciation [29].

The aims of the present work are to: (a) enlarge the knowledge concerning nuclear DNA amounts in the Valerianaceae, to date limited to only one report ("*Kentranthus ruber* Druce" 1C = 0.42 pg; [30]), (b) integrate these results with those of chromosome number and molecular phylogeny, and (c) provide a new focus that could contribute, from the perspective of genome evolution, to verify the hypotheses of previous works on the relationships among the above-mentioned genera.

2. Material and Methods

2.1. Plant Material. Table 1 shows the provenance of the species investigated. The sampling includes representatives of all the six genera considered in the family in regard to the results of molecular phylogenies [4–6]. Studied plants come from germinated cypselas, collected in the field or obtained from Botanic Gardens. Due to difficulties in germinating seeds of *Valeriana* representatives, whole individuals were collected in the field and cultivated in the Institut Botànic de Barcelona.

2.2. Karyological and Cytogenetic Analyses

2.2.1. Chromosome Counts and/or Determination of the Ploidy Level. Root tip meristems were obtained either by germinating seeds on wet filter paper in Petri dishes at room temperature, or from plants cultivated in pots in the greenhouse. They were pretreated with 0.002 M 8-hydroxyquinoline for 3 h at 16°C. Material was fixed in absolute ethanol, trichloromethane, and glacial acetic acid (6 : 3 : 1) and stored in the fixative at 4°C during at least two days before processing. Samples were hydrolysed in 1 N HCl for 5 min at 60°C, stained with 1% aqueous aceto-orcein for 1 h minimum, and squashed on slides into a drop of 45% acetic acid-glycerol (9 : 1). Metaphase plates were photographed with a digital camera (Zeiss AxioCam HRm) mounted on a Zeiss Axioplan microscope, and images were analysed with Axio Vision Ac version 4.2.

2.2.2. Nuclear DNA Assessments. Leaf tissue of five individuals for each studied population was chopped in 600 μl of LB01 isolation buffer [33] with a razor blade, together with the chosen internal standard. The species *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg) and *Pisum sativum* L. 'Express long' (2C = 8.37 pg) were used as internal standards [34] to cover the range of 2C-values found. Seeds of the standards were provided by the Institut des Sciences du Végétal (CNRS), Gif-sur-Yvette (France). For each individual, two independent samples were extracted and measured the same day. Samples were supplemented with 100 μg/ml of ribonuclease A (RNase A, Boehringer) and subsequently stained with 36 μl of propidium iodide (1 mg/ml) to a final concentration of 60 μg/ml (Sigma-Aldrich Química), kept on ice for 20 min and measured in an Epics XL flow cytometer (Coulter Corporation). The instrument was set up with the standard configuration. Excitation of the sample was performed using a standard 488-nm air-cooled argon-ion laser at 15 mW power. Forward scatter (FSC), side scatter (SSC), and red (620 nm) fluorescence for propidium iodide were acquired. Optical alignment was based on optimized

Table 1: Origin of the populations studied with indications of the herbaria where voucher specimens are deposited, chromosome counts, genome size data, and GenBank accession numbers (only for the new sequences).

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Centranthus angustifolius (Mill.) DC. (1)	France: Vaucluse, Mt. Ventoux, Hidalgo 212 & Hidalgo (BC)	P	1.30 ± 0.03	32	4x	0.33	1271.4	3.83 ± 0.63	1.25 ± 0.59	HM162366
Centranthus angustifolius (Mill.) DC. (2)	France: Hautes-Alpes, VI-2005, Hidalgo & Romashchenko (MPU)	P	1.35 ± 0.02	32	4x	0.34	1320.3	5.57 ± 0.86	3.77 ± 0.65	HM162367
Centranthus angustifolius (Mill.) DC. (3)	France: Hautes-Alpes, Les Infournas, Hidalgo 201 & Martin (BC)	P	1.41 ± 0.07	32	4x	0.35	1379	3.35 ± 0.55	1.50 ± 0.43	
Centranthus calcitrapae (L.) Dufr. (1)	Spain, Catalonia, Port d'Àger, Garnatje 229 & Vallès (BCN)	A	1.56 ± 0.04	32	4x	0.39	1525.7	5.22 ± 1.14	4.12 ± 0.95	
Centranthus calcitrapae (L.) Dufr. (2)	France: Gard, Saint André de Majencoules, 4-VI-2005, Hidalgo, Romashchenko & Romo (BC)	A	1.58 ± 0.02	32	4x	0.40	1545.2	3.28 ± 0.70	1.99 ± 0.62	HM162368
Centranthus calcitrapae (L.) Dufr. (3)	Greece, Crete: Elafonissi, Bot. Gard. MNHN 1996-08	A	1.61 ± 0.01	32	4x	0.40	1574.6	3.62 ± 0.96	1.06 ± 0.87	
Centranthus calcitrapae (L.) Dufr. (4)	Greece, Crete: Hania, Bot. Gard. MNHN 1996-15	A	1.62 ± 0.03	32	4x	0.4	1584.4	3.16 ± 1.45	1.51 ± 0.56	
Centranthus cf. *calcitrapae* (L.) Dufr. (5)	Morocco: Hidalgo, Romo 13128 & Soriano (BC)	A	1.80 ± 0.03	**32**	**4*x***	0.45	1760.4	3.46 ± 0.60	2.20 ± 0.70	HM162369
Centranthus lecoqii Jord. (1)	France: Lozère, Les Vignes, Bot. Gard. MNHN 2002-39	P	1.22 ± 0.05	32	4x	0.31	1193.2	3.62 ± 1.58	2.09 ± 0.48	
Centranthus lecoqii Jord. (2)	France: Cons. Bot. Porquerolles Ly815	P		**32**	**4*x***					
Centranthus lecoqii Jord. (3)	France: Hérault, St. Guilhem le Désert, Mathez 1076 (MPU)	P		32	4x					HM162370

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Centranthus longiflorus Steven var. *latifolius* Boiss.	Lebanon: Les Cèdres, Bou Dagher-Kharrat, Hidalgo & Romashchenko 409 (BC)	P	1.42 ± 0.03	32	4x	0.36	1388.8	7.90 ± 0.68	7.71 ± 2.41	
Centranthus macrosiphon Boiss.	Bot. Gard. Hauniensis 2000-2315	A	1.97 ± 0.06	**32**	**4*x***	0.49	1926.7	2.58 ± 1.19	1.64 ± 1.26	HM162371
Centranthus nevadensis Boiss. (1)	Morocco: Akchar, sur Tizi Ouzli, Hidalgo, Romo 13035 & Soriano (BC)	P	1.16 ± 0.02	32	4x	0.29	1134.5	4.23 ± 0.98	2.27 ± 0.77	
Centranthus nevadensis Boiss. (2)	Morocco: Djebel Lechâab, Hidalgo, Romo 13147 & Soriano (BC)	P	1.19 ± 0.01	32	4x	0.3	1163.8	3.80 ± 0.37	1.87 ± 0.78	HM162372
Centranthus ruber (L.) DC. (1)	France: Bouches-du-Rhône, la Sainte Baume, VI-2005, Hidalgo, Romashchenko & Romo (BC)	P	1.14 ± 0.04	32	4x	0.29	1114.9	3.77 ± 1.59	0.81 ± 0.41	
Centranthus ruber (L.) DC. (2)	Rumania: Valea Moril, Distr. Cluj, Bot. Gard. Cluj-Napoca 2006-1820	P	1.14 ± 0.02	32	4x	0.29	1114.9	4.56 ± 0.49	2.36 ± 0.22	HM162373
Centranthus ruber (L.) DC. (3)	Italy, Sicily: Siracusa, Buccheri Mt., Bot. Gard. Palermo 2006-750	P	1.16 ± 0.04	**32**	**4*x***	0.29	1134.5	3.67 ± 0.71	0.88 ± 0.44	
Fedia cf. *cornucopiae* (L.) Gaertn. (1) (as *F. caput-bovis*)	Italy, Sicily: Siracusa, Lauro-Buccheri Mt., Bot. Gard. Palermo 2006-751	A	1.70 ± 0.02	32	4x	0.43	1662.6	2.68 ± 0.31	0.18 ± 0.08	HM162374
Fedia cornucopiae (L.) Gaertn. (2)	Willd coll., Bot. Gard. Coimbra 2005-1321	A	1.79 ± 0.04	32	4x	0.45	1750.6	5.28 ± 0.96	3.28 ± 1.16	
Fedia graciliflora Fisch. & C.A.Mey.	Bot. Gard. Montpellier (MPU)	A	1.53 ± 0.04	32	4x	0.38	1496.3	4.48 ± 0.96	2.53 ± 1.17	HM162375

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	2n[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Fedia pallescens (Maire) Mathez	Morocco: Mehdyia, El-Oualidi s.n.-1998 (MPU)	A	1.14 ± 0.02	32	4x	0.29	1114.9	5.85 ± 1.54	2.71 ± 1.14	HM162376
Nardostachys jatamansi (D. Don) DC.	Bot. Gard. Lautaret, France	P	3.49 ± 0.04					2.06 ± 0.72	1.25 ± 0.82	
Patrinia rupestris Dufr.	Russia: Siberia, Bot. Gard. Lautaret, France	P	2.56 ± 0.01					2.81 ± 1.01	1.87 ± 1.1	
Patrinia scabiosifolia Link (1)	Japan: Bot. Gard. Ofuna 314-2006	P	1.50 ± 0.03					5.09 ± 0.71	4.08 ± 0.37	
Patrinia scabiosifolia Link (2)	Japan: Tsukiono-machi, Tone-gun, Bot. Gard. Chiba University 2006-193	P	2.57 ± 0.07	**ca. 44**	**4*x***	0.64	2513.5	3.7 ± 0.68	1.91 ± 0.58	HM162377
Valeriana apula Pourr.	Spain, Aragon: Huesca, Anglios, 2005, Vallès (BCN)	P	0.97 ± 0.02	**16**	**2*x***	0.49	948.66	5.58 ± 0.87	2.16 ± 0.54	HM162378
Valeriana celtica L. (1)	France: Savoie, Evettes circus, Bot. Gard. Lautaret, France	P	2.11 ± 0.04					1.81 ± 1.37	1.29 ± 1.10	
Valeriana celtica L. (2)	Italy: Aosta, Cogne, Hidalgo 503 (MPU)	P								HM162379
Valeriana dioica L. (1)	France: Hautes-Alpes, Lautaret pass, VI-2005 Douzet, Hidalgo & Romashchenko (BC)	P	2.87 ± 0.05					3.25 ± 0.31	0.72 ± 0.70	
Valeriana dioica L. (2)	France: Gard, Bonheur valley, 5-VI-2005, Hidalgo, Romashchenko & Romo (BC)	P	3.01 ± 0.01 (♀) 3.08 ± 0.01 (♂)					3.11 ± 0.21 (♀) 3.22 ± 0.14 (♂)	0.72 ± 0.49 (♀) 1.78 ± 0.51 (♂)	HM162380
Valeriana longiflora Willk. (1)	Spain, Aragon: Santa Anna dam, Garnatje 172 & Vallès (BCN)	P	0.96 ± 0.02					5.84 ± 0.99	2.91 ± 0.30	

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx[4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valeriana longiflora Willk. (2)	Spain, Aragon, Santa Anna dam, Garnatje, Hidalgo 500 & Luque (MPU)	P								HM162381
Valeriana montana L. (1)	France: Hautes-Alpes, Les Infournas, Hidalgo 202 & Martin (BC)	P	1.23 ± 0.01					3.92 ± 1.32	1.59 ± 1.05	
Valeriana montana L. (2)	Spain, Catalonia: Girona, Camprodon, Garnatje 179 (BC)	P	2.24 ± 0.04					5.98 ± 2.41	0.88 ± 0.62	
Valeriana montana L. (3)	Italy: Maritime Alps, Cuneo, 6-VIII-2005, Peccenini & Vallès (BCN)	P	2.40 ± 0.05					2.79 ± 0.36	2.21 ± 0.83	
Valeriana montana L. (4)	France: Hautes-Alpes, between the Galibier and Lautaret passes, Garnatje & Hidalgo 220 (BC)	P	2.42 ± 0.03					2.79 ± 0.49	2.17 ± 0.73	HM162382
Valeriana montana L. (5)	Spain, Catalonia: Lleida, Vall d'Aran, on the way to the Restanca from Arties, Garnatje 234 & Vallès (BCN)	P	2.50 ± 0.05	**32**	***4x***	0.63	2445	2.08 ± 0.28	1.32 ± 0.80	HM162383
Valeriana montana L. (6)	France: Hautes-Alpes, spontaneous in the Lautaret Bot. Gard., Garnatje & Hidalgo 221 (BC)	P	2.51 ± 0.03					2.81 ± 0.69	2.35 ± 0.84	
Valeriana montana L. (7)	Spain, Catalonia: Lleida, Vall d'Aran, on the way to the Restanca from Arties, Garnatje 235 & Vallès (BCN)	P	2.54 ± 0.03					2.52 ± 0.98	0.87 ± 0.77	HM162384
Valeriana montana L. (8)	France: Hautes-Alpes, between Le Roy and Pise pass, Hidalgo 210 & Martin (BC)	P	2.60 ± 0.03	**32**	***4x***	0.65	2542.8	2.38 ± 0.83	1.36 ± 0.54	

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valeriana montana L. (9)	France: Hautes-Alpes, Gleize pass, Hidalgo 208 & Martin (BC)	P	2.64 ± 0.05					2.48 ± 1.00	1.82 ± 0.70	HM162385
Valeriana montana L. (10)	France: Hautes-Alpes, Galibier pass, 4-VIII-2001, Hidalgo & Hidalgo (BC)	P								HM162386
Valeriana cf. *montana* L. (11)	Spain, Catalonia: Barcelona, Montserrat, 2005, Casanova (BC)	P	2.46 ± 0.03					3.65 ± 2.06	2.06 ± 0.67	
Valeriana cf. *montana* L. (12)	France: Hautes-Alpes, Gleize pass, VI-2005, Hidalgo & Romashchenko (BC)	P	2.54 ± 0.02					3.3 ± 0.24	1.98 ± 0.60	
Valeriana officinalis L. (1)	Croatia: Dalmatia, Biokovo Mt., 2006, Siljak-Yakovlev (BC)	P	2.97 ± 0.04	**14**	***2x***	1.49	2904.7	4.07 ± 0.29	2.22 ± 0.49	HM162387
Valeriana officinalis L. (2)	France: Gard, Aigoual Mt., 3-VI-2005, Hidalgo, Romashchenko & Romo (BC)	P	3.16 ± 0.03					3.19 ± 0.24	1.44 ± 0.42	HM162388
Valeriana officinalis L. (3)	Spain, Catalonia: Girona, Llanars, Garnatje 169, D. Roca & J. Roca (BC)	P	4.62 ± 0.08					2.76 ± 0.48	3.60 ± 0.66	
Valeriana officinalis L. (4)	Spain, Catalonia: Lleida, Vielha, 2005, Vallès (BCN)	P	6.51 ± 0.11					1.49 ± 1.01	2.89 ± 1.24	
Valeriana officinalis L. (5)	Spain, Catalonia: Lleida, Vall d'Aran, Garnatje 175 & Vallès (BCN)	P	8.05 ± 0.08					1.78 ± 0.76	2.91 ± 0.64	

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valeriana officinalis L. (6)	Norway: Trs, Storfjord, Rovijok, Bot. Gard. Oulu 2004/5-425	P	8.15 ± 0.25	**ca. 56**	**8*x***	1.02	7970.7	1.40 ± 0.92	2.52 ± 1.67	HM162389
Valeriana officinalis L. (7)	France: Gironde, Gradignan, V-2005, Hidalgo & Revel-Hidalgo (BC)	P	8.32 ± 0.10 (leaf) 8.09 ± 0.08 (root)					3.14 ± 1.26 (leaf) 1.40 ± 1.11 (root)	4.99 ± 0.49 (leaf) 5.83 ± 0.47 (root)	
Valeriana pyrenaica L.	Spain, Catalonia: Lleida, Vall d'Aran, Garnatje 176 & Vallès (BCN)	P	1.26 ± 0.01	**16**	**2*x***	0,6	1232.3	5.37 ± 0.64	2.81 ± 0.26	HM162390
Valeriana rotundifolia Vill.	France: Hautes-Alpes, Devoluy, La Cluse, Hidalgo 207 & Martin (BC)	P	2.52 ± 0.05					2.16 ± 0.30	1.41 ± 0.51	HM162391
Valeriana saliunca All.	France: Savoie, Galibier pass, Garnatje 166 & Vallès (BC)	P	1.46 ± 0.03	**16**	**2*x***	0.73	1427.9	4.21 ± 0.36	3.21 ± 0.25	HM162392
Valeriana saxatilis L. (1)	Italy: Apuan Alps, summit of Sagro Mt., Hidalgo 213 (BC)	P	1.05 ± 0.00					3.17 ± 0.28	2.21 ± 0.65	
Valeriana saxatilis L. (2)	Italy: Apuan Alps, Hidalgo 217 (BC)	P	1.02 ± 0.01					3.62 ± 0.38	2.36 ± 0.24	HM162393
Valeriana tripteris L. (1)	France: Hautes-Alpes, Lautaret pass, Hidalgo 203 & Martin (BC)	P	1.46 ± 0.03	**16**	**2*x***	0.73	1427.9	3.28 ± 0.71	1.79 ± 0.69	
Valeriana tripteris L. (2)	France: Gard, Mt. Aigoual, 3-VI-2005, Hidalgo, Romashchenko & Romo (BC)	P	1.51 ± 0.01 (♀♂) 1.52 ± 0.02 (♀)					4.01 ± 0.38 (♀♂) 3.75 ± 0.24 (♀)	2.24 ± 0.48 (♀♂) 2.12 ± 0.53 (♀)	
Valeriana tripteris L. (3)	France: Vaucluse, Mt. Ventoux, Hidalgo 211 & Hidalgo (BC)	P	1.51 ± 0.01	**18**	**2*x***	0.76	1476.8	2.75 ± 0.35	1.34 ± 0.40	HM162394

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valeriana tripteris L. (4)	Spain, Catalonia: Lleida, Espot, road to Espot Ski, 17-VI-2007, Garnatje 233 & Vallès (BCN)	P	1.54 ± 0.03					5.63 ± 1.00	4.86 ± 1.03	HM162395
Valeriana tripteris L. (5)	Spain, Catalonia: Girona, Setcases, Garnatje 170, D. Roca & J. Roca (BC)	P	1.58 ± 0.06					3.88 ± 0.74	2.01 ± 0.81	HM162396
Valeriana tripteris L. (6)	France: Gard, Mt. Aigoual, 14-VII-00, Hidalgo (BC)	P								HM162397
Valeriana cf. *tripteris* L. (7)	Italy: Apuan Alps, Hidalgo 218 (BC)	P	1.23 ± 0.06	**32**	**4*x***	0.31	1202.9	3.93 ± 0.66	2.34 ± 0.98	HM162398
Valeriana tuberosa L.	France: Les Sièges, Mathez 1088 (MPU)	P	3.74 ± 0.03					2.96 ± 0.99	3.66 ± 0.42	HM162399
Valerianella coronata (L.) DC. (1)	Italy: Aymavilles, Cerignan, Bot. Gard. Cogne 2006/7-838	A	0.48 ± 0.00	**14**	**2*x***	0.24	469.44	0.59 ± 0.23	0.99 ± 0.53	
Valerianella coronata (L.) DC. (2) (as *V. pumila*)	France: Vaucluse, "la Bastide des Jourdans", Cons. Bot. de Porquerolles 1998-CO 2688	A	0.53 ± 0.01	**14**	**2*x***	0.27	518.34	8.90 ± 0.70	3.26 ± 0.65	
Valerianella dentata (L.) Pollich (1) (as *V. rimosa*)	France: Maine-et-Loire, Montreuil-Bellay, "Méron", Bot. Gard. Nantes 2004-306	A	0.42 ± 0.01	**16**	**2*x***	0.21	410.76	11.88 ± 2.54	0.55 ± 0.37	

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valerianella dentata (L.) Pollich (2)	Bot. Gard. Bonn 2004/5-23687	A	0.49 ± 0.01 (leaf) 0.47 ± 0.02 (root)	**16**	**2*x***	0.25	479.02	9.64 ± 0.85 (leaf) 8.69 ± 0.83 (root)	1.09 ± 0.85 (leaf) 2.29 ± 0.74 (root)	
Valerianella dentata (L.) Pollich (3)	Italy, Sicily: Palermo, Rocca Busambra-Ficuzza, Bot. Gard. Palermo 2006-756	A		16	2*x*					HM162400
Valerianella discoidea Loisel	Greece, Crete: Hania, Bot. Gard. MNHN 2005-96/14	A		**14**	**2*x***					
Valerianella echinata DC.	France: Aveyron, near Brunas de Creissels (Larzac N.), Bernard	A	0.60 ± 0.01	16	2*x*	0.3	586.8	5.54 ± 2.24	2.16 ± 0.71	
Valerianella eriocarpa Desv. (1)	Italy, Sicily: Palermo, Rocca Busambra-Ficuzza, Bot. Gard. Palermo 2006-757	A	0.47 ± 0.02	**16**	**2*x***	0.24	459.66	6.31 ± 0.50	0.92 ± 0.86	HM162401
Valerianella eriocarpa Desv. (2)	Bot. Gard. Nantes 2004-302	A	0.54 ± 0.01	16	2*x*	0.29	528.12	10.07 ± 2.83	1.86 ± 0.61	
Valerianella locusta L. (1)	France: Loire Atlantique, cheméré "Le Moulin", Bot. Gard. Nantes 2004-303	A	0.44 ± 0.02	**16**	**2*x***	0.22	430.32	11.37 ± 1.69	1.18 ± 0.84	HM162402
Valerianella locusta L. (2) (as *V. carinata*)	France: Loire Atlantique, Gard. Nantes 2004-301	A	0.49 ± 0.02	16	2*x*	0.25	479.22	8.50 ± 1.57	0.20 ± 0.19	HM162403

Table 1: Continued.

Species (population number)	Collection data	Life cycle[1]	2C (pg)[2]	$2n$[3]	Ploidy level	1Cx [4]	2C (Mbp)[5]	Mean HPCV of sample (%)	Mean HPCV of standard (%)	*trn*L-*trn*F GenBank accession number
Valerianella microcarpa Loisel	Spain, Catalonia: Tarragona, Ebre delta, 2004, Pyke (BC)	A	0.46 ± 0.02	16	2x	0.23	449.88	8.43 ± 1.29	0.22 ± 0.20	
Valerianella multidentata Loscos & Pardo	Spain, Aragon: Zaragoza, 2004, Pyke (BC)	A	0.52 ± 0.02	**14–16**	**2*x***	0.26	508.56	7.78 ± 0.77	0.97 ± 0.92	HM162404
Valerianella pontica Velen.	Ukraine: Crimea, Boratinsky, Didukh, Romashchenko, Romo 1029bis & Susanna (BC)	A	0.51 ± 0.02		2x	0.26	498.78	11.47 ± 1.33	0.18 ± 0.10	
Valerianella turgida Betcke (1)	Greece: Oxia, Bot. Gard. MNHN 2002-190	A	0.39 ± 0.01	**16**	**2*x***	0.2	381.42	12.15 ± 1.54	0.65 ± 0.67	HM162405
Valerianella turgida Betcke (2)	Ukraine: Crimea, Boratinsky, Didukh, Romashchenko, Romo 10171 & Susanna (BC)	A	0.47 ± 0.02	16	2x	0.24	459.66	7.49 ± 2.66	1.97 ± 0.52	HM162406
Valerianella vesicaria Moench	Israel: Judean Mts. Bot. Gard. Tel Aviv 2001	A	0.61 ± 0.05		2x	0.31	596.58	9.58 ± 2.98	0.36 ± 0.33	

[1] Life cycle: A annual, P perennial. [2] 2C nuclear DNA content (means ± SD of 10 measurements—two replicates for five individuals each). [3] $2n$ somatic chromosome number. $2n$ and ploidy levels are estimated through bibliographical record (using the database http://www.tropicos.org/), except those in bold, which were verified in this study. [4] 1Cx monoploid genome size (DNA content per basic chromosome set [31]). [5] 2C [Mbp]: 1 pg = 978 Mbp [32].

signal from 10-nm fluorescent beads (Immunocheck, Epics Division, Coulter Corporation). Time was used as a control for the stability of the instrument. Red fluorescence was projected onto a 1,024 monoparametric histogram. Gating single cells by their area versus peak fluorescence signal excluded aggregates. Acquisition was automatically stopped at 8,000 nuclei. Measurements were made at the Serveis Cientificotècnics (Universitat de Barcelona).

2.2.3. Effect of Valepotriates on Measurements. Valerianaceae contain, *inter alia*, valepotriates, a family of chemical compounds of great medicinal interest, thanks to which some representatives are considerably exploited by the pharmaceutical industry. Valepotriates are known DNA intercalators (they do have severe effects on DNA in e.g., PCR amplifications) and might thus possibly influence genome size measurements by altering the hydrodynamic diameter of the DNA. Furthermore, they could constitute endogenous staining inhibitors, which affect results by causing stoichiometric errors [35, and references therein]. Valepotriates are mainly stored in oil vesicles in the roots and rhizomes [36]. Therefore, we performed measurements on some roots and compared them with DNA C-values obtained for leaves of the same individuals to detect a potential effect of these chemical compounds on genome size assessments if any.

2.2.4. Statistical Analyses. ANOVA and LSD test were performed with the Statgraphics Plus 5.1 program (Statistical Graphics Corp., USA).

2.3. Phylogenetic Analyses

2.3.1. DNA Extraction, Amplification and Sequencing. Total genomic DNA was extracted from silica-dried, herbarium voucher or fresh leaves using the Nucleospin Plant extraction kit (Macherey-Nagel, GmbH & Co., Duren, Germany). PCRs were carried out with PTC100 (MJ Research, Inc.) research thermal cyclers in 25 μl volume. The whole plastid *trn*L-*trn*F region [including the *trn*L intron, the 3′*trn*L (UAA) exon, and the intergenic spacer between *trn*L (UAA) and *trn*F (GAA)] was amplified and sequenced with the universal primers trnL-c, forward, and trnL-f, reverse, and, in some cases, trnL-d, reverse, and trnL-e, forward [37]. The PCR amplification conditions used were 94°C, 1 min 35 sec; 34x (93°C, 1 min; 58°C, 1 min; 72°C, 1 min); 72°C, 10 min, and storage at 4°C. PCR products were purified with QIAquick PCR Purification Kit (Qiagen, Valencia, California, USA) or with DNA Clean & Concentrator-5 D4003 (Zymo Research, Orange, California, USA). Direct sequencing of the amplified fragments was performed using the Big Dye Terminator Cycle Sequencing v3.1 (PE Biosystems, Foster City, California, USA). Nucleotide sequencing was carried out at the Serveis Cientificotècnics (Universitat de Barcelona) with an ABI PRISM 3700 DNA Analyzer (PE Biosystems, Foster City, California, USA).

2.3.2. Sequence Assembly, Alignment, and Analyses. Nucleotide sequences were assembled and edited using MacClade 4.08 [38]. Bayesian inference (BI) was carried out with MrBayes version 3.1.2 [39]. The most appropriate nucleotide substitution models were chosen with MrModeltest version 2.3 [40]. Four Markov chains were run simultaneously for 1×10^6 generations, and these were sampled every 100 generations. Data from the first 1,000 generations were discarded as the *burn-in* period, after confirming that likelihood values were stabilized prior to the 1,000th generation. The 50% majority rule consensus trees and posterior probability (PP) of nodes were calculated from the pooled samples. We proceeded to an additional analysis, adding to the matrix the gap data codified with the Barriel method, as previously used for Valerianaceae in [3]. Partitioned dataset analysis was carried out for this dataset including the codified gaps. A gamma-shaped rate variation was stated for the codified data following the manufacturer's protocol, applying the model selected with MrModeltest for the DNA data.

3. Results

3.1. Chromosome Counts. Chromosome numbers of the populations studied are indicated in Table 1, and metaphase plates are presented in Figure 1. According to our data, we provide the first count for *Valerianella multidentata* Loscos & Pardo. Ploidy levels ranging from diploid to octoploid were detected although diploid and tetraploid were predominantly reported. Good metaphase plates are quite difficult to obtain for Valerianaceae. In fact, this is certainly the main reason why only few chromosome studies have been carried out in the family, and that especially few chromosome pictures have been published to present.

3.2. Genome Size Assessments. Data on nuclear DNA content are presented in Figure 2 and Table 1. The present data are the first reports on nuclear DNA content in all the genera studied excepting *Centranthus* and in all the species studied of this genus except for *C. ruber* (L.) DC., which was to date the only member of the family with a known genome size [30]. Genome size in Valerianaceae varies about 21.3-fold, from 2C = 0.39 pg (*Valerianella turgida* Betcke, 2*x*) to 2C = 8.32 pg (*Valeriana officinalis*, 8*x*). Although the half peak coefficients of variation (HPCV) are rather high for some species (in all cases but one of the genus *Valerianella*), their mean value is 4.62% for the target plants and 2.04% for the standards. More in-deep research is suitable in *Valerianella* to try and obtain measurements with better HPCV.

The difference in genome size between leaves and roots measured in the same population has been addressed for *Valeriana officinalis* (7) (leaves: 2C of 7.67–8.97 pg and roots: 2C of 8.04–8.14 pg, at 95% confidence interval) and *Valerianella dentata* (L.) Pollich (2) (leaves: 2C of 0.485–0.495 pg and roots: 2C of 0.464–0.476 pg, at 95% confidence interval). A significant difference has been detected for *Valerianella dentata* (ANOVA, $P = 0.0036$), which may indicate a possible effect of valepotriates on genome size assessments in this species, also suggested by the especially high HPCV values found in this annual genus (Table 1).

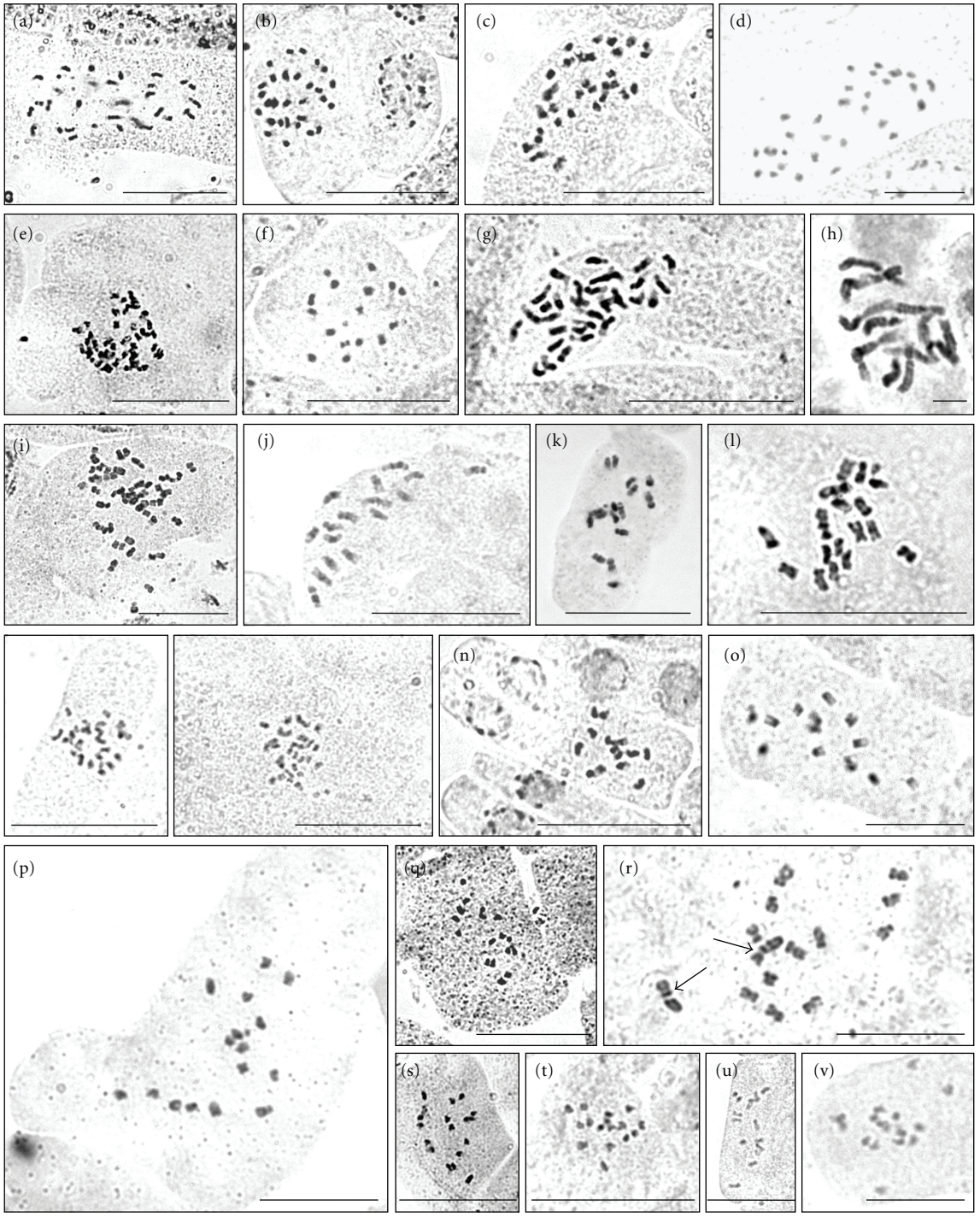

Figure 1: (a)–(v). Somatic metaphases. (a) *Centranthus* cf. *calcitrapae* (5), $2n = 32$. (b) *C. lecoqii* (2), $2n = 32$. (c) *C. macrosiphon*, $2n = 32$. (d) *C. ruber* (3), $2n = 32$. (e) *Patrinia scabiosifolia*, $2n$= ca. 44. (f) *Valeriana apula* $2n = 16$. (g) *V. montana* (5), $2n = 32$. (h) *V. officinalis* (1), $2n = 14$. (i) *V. officinalis* (6), $2n$ = ca. 56. (j) *V. pyrenaica*, $2n = 16$. (k) *V. saliunca*, $2n = 16$. (l) *V. tripteris* (3), $2n = 18$. (ma-b) *V.* cf. *tripteris* (7), $2n = 32$. (n) *Valerianella coronata* (1), $2n = 14$. (o) *V. coronata* (2), $2n = 14$. (p) *V. dentata* (1), $2n = 16$. (q) *V. dentata* (2), $2n = 16$. (r) *V. discoidea*, $2n = 14$. Arrows indicate a chromosome pair much larger than the rest. (s) *V. eriocarpa* (1), $2n = 16$. (t) *V. locusta*, $2n = 16$. (u) *V. multidentata*, $2n = 14$–16. (v) *V. turgida* (1), $2n = 16$. Scale bars = 10 μm.

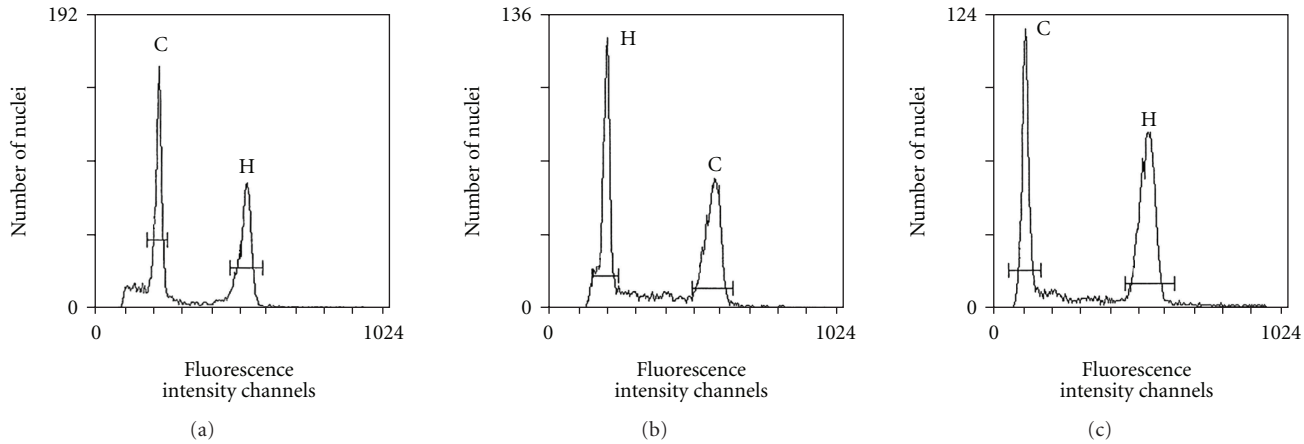

Figure 2: (a)–(c). Histograms of nuclear DNA content obtained for some representatives of Valerianaceae. (a) *Centranthus nevadensis* (2) (2C = 1.19 ± 0.01). (b) *Valeriana officinalis* (7) (2C = 8.32 ± 0.10). (c) *Valerianella coronata* (2) (2C = 0.53 ± 0.01). Peak C: sample species nuclei. Peak H: standard species nuclei.

3.3. Molecular Phylogeny. Both AIC (Akaike Information Criterion) and hLRT (hierarchical Likelihood Ratio Tests) selected GTR+G (General Time Reversible model with gamma distribution) as the best-fit model. The results of the Bayesian analyses are presented in Figure 3. Phylogenetic trees obtained through the analyses of the two different datasets basically led to the same tree topology and supports. Nevertheless, three clades are only significantly supported when codified gap information is taken into account. These are (a) *Valeriana* clade I (PP = 0.95, Figure 3), (b) *Valeriana longiflora* Willk. plus *Centranthus* clade (PP = 1.00, Figure 3), and (c) the grouping of *Fedia cornucopiae* (L.) Gaertn. AF446986 with *Fedia graciliflora* Fisch. & C.A.Mey. (PP = 0.96, Figure 3).

4. Discussion

We address here the analysis of genome size variation and its phylogenetic and taxonomical implications in the taxa considered.

4.1. The Basal Grades. They consist of the taxa showing the most ancestral characters within the Valerianaceae, namely, the genera *Nardostachys* and *Patrinia*, along with the clade grouping *Valeriana celtica* and *Valeriana saxatilis* L. (*Valeriana* clade III, Figure 3). Shifts in biogeography, flower morphology, and basic chromosome number occurred at this point of the evolutionary history of the family. From this moment on, the distribution area of the Valerianaceae, until that time restricted to Asia (*Nardostachys* and *Patrinia*), was enlarged to the other continents. At the same time the number of stamens decreased from four to three. These changes just preceded the change in basic chromosome numbers from $x = 11$ to $x = 8$, occurring at the arising of Valerianeae tribe (Figure 3).

The *Valeriana* clade III (P = 1.00, Figure 3) constituted by two species from the Alps, *V. celtica* and *V. saxatilis*, is branched between Patrinieae and Valerianeae, a position that was previously found for *V. celtica* [4, 5], and stated for the first time here for *V. saxatilis*. Although their genome sizes fall within the range of the remaining *Valeriana*, these two valerian species present $x = 11$ as basic chromosome number [41, 42], which had only been detected in *Patrinia*. Furthermore, *V. celtica* has a yellow corolla, also like species of *Patrinia*. Nevertheless, species of clade III differ from *Patrinia* by a pappus-like *Centranthus* and *Valeriana*, and the number of stamens reduced to three as in *Valeriana* and *Valerianella* (whereas *Nardostachys* presents four and occasionally five stamens and *Patrinia* presents five or less stamens; [43, 44]). Therefore, species of clade III are from morphological, cytogenetic, and phylogenetic points of view in between Patrinieae and Valerianeae. These results raise numerous questions. Should this clade III form a new genus? Could any other *Valeriana* species be susceptible to join this group? Then, should this group be classified within Valerianeae, or as a new tribe (their inclusion in the Patrinieae being impossible under the monophyly criterion)?

4.2. Fedia *Plus* Valerianella *Clade.* These species have the smallest genome sizes known in the family (Table 1) to present. *Fedia* and *Valerianella* are exclusively composed of annual herbaceous plants. All the species belonging to these two genera, excepted *F. pallescens* (Maire) Mathez, are common weeds in waste ground and cultivated land. *Valerianella*, which comprises around 50 species that are mainly diploid and with three stamens, is widely distributed in Eurasia, Africa and America. The genus *Fedia* includes only three tetraploid species and one hexaploid subspecies [45], with two stamens, and is restricted to the Mediterranean region. Molecular inferences suggest that *Fedia* species should have originated from *Valerianella* (Figure 3; [7]), in other words that autogamous strongly associate to anthropogenic environment (*Valerianella*) would have given rise to allogamous, polyploid (*Fedia*).

Genome size in those genera shows that *Fedia* ($1Cx$ = 0.29 to 0.45 pg) has significantly higher DNA values than

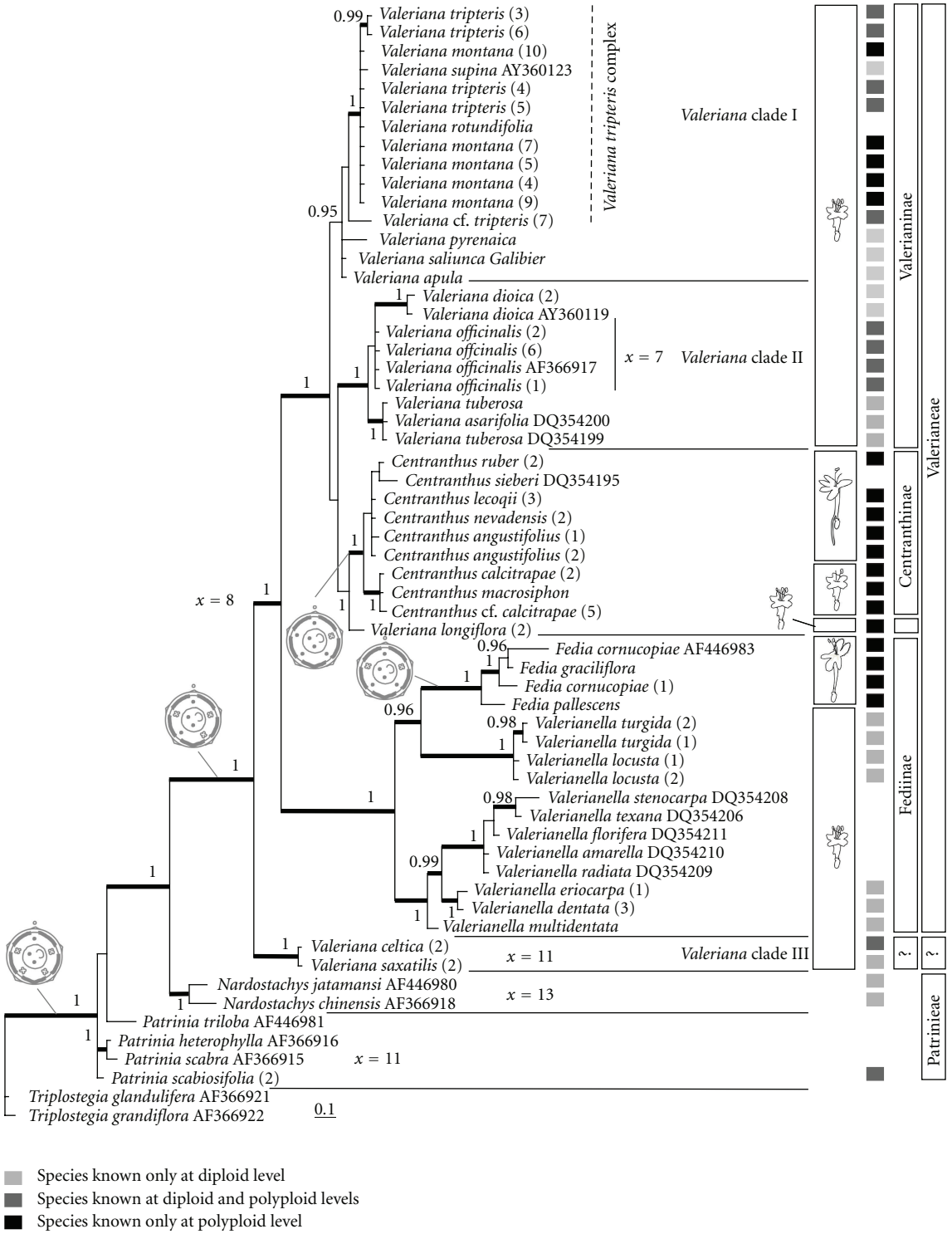

Figure 3: Molecular phylogeny based on the Bayesian analysis of the *trn*L-*trn*F region and the codified gap information, with posterior probability values ≥0.95 indicated on the branches. Wider branches are those significantly supported in the Bayesian analysis of the *trn*L-*trn*F region without including the information from the gaps. Taxonomic information, relevant floral characters, basic chromosome number, and data on ploidy levels are indicated. GenBank accession numbers are provided for the previously published sequences. Numbers in brackets differentiate populations of a same species (see Table 1).

Valerianella (from 1Cx = 0.20 to 0.31 pg) (P = 0.0000), which corroborates the conclusions of Albach & Greilhuber [46] that allogamy is related to higher DNA values than autogamy. Furthermore, *Fedia* presents the "allogamy syndrome," morphological adaptations linked to the breeding system (e.g., zygomorphic corolla, development of a nectar gibbosity, tube elongation, and occurrence of polychroic corolla). The intensity of this syndrome varies between the species and the subspecies of *Fedia* [47]. The tendency within the genus is toward an increasing genome size and degree of allogamy syndrome, from *Fedia pallescens* (1Cx = 0.29 pg) to *F. graciliflora* (1Cx = 0.38 pg) and *F. cornucopiae* (1Cx = 0.43–0.45 pg).

The *Valerianella* group splits in two major clades (Figure 3), one including *V. turgida*, *V. locusta* (L.) Laterr., and the *Fedia* species. The genus *Valerianella* is revealed in this study as a relatively homogeneous group respecting genome size within Valerianaceae, with 2C values that range from 0.39 pg (*V. turgida*) to 0.61 pg (*V. vesicaria* Moench). Furthermore, some of *Valerianella* species, such as *V. turgida*, account for very small genomes, which fall into the range of the smallest genome size records in angiosperms [48], around the same scale than *Arabidopsis thaliana* (L.) Heynh. (1C = 0.16 pg; [49]), for long considered as the lowest angiosperms C-DNA value, nowadays reported in the genus *Genlisea* (*G. margaretae* 1C = 0.064 pg; [50]).

The chromosome number of *Valerianella multidentata*, $2n = 2x$ = 14–16, is reported here for the first time. This endemic restricted to a small area of Catalonia, Spain, is very close to *V. discoidea* Loisel (see [51]; $2n = 2x$ = 14–16), which has been in turn related to *V. coronata* (L.) DC. ([52]; $2n = 2x = 14$). All these species belong to section *Coronatae*. The chromosome number of $2n = 14$ is the smaller within the genus. Metaphase plates of *V. discoidea* (Figure 1(r)) show a chromosome pair much larger than others that may result from a chromosome fusion sparking off the descending diploidy observed in the section.

4.3. Centranthus *Plus* Valeriana longiflora *Clade.* *Centranthus* is a circum-Mediterranean genus of nine species, characterized by flowers with one stamen, related to *Valeriana longiflora* (PP = 1.00, Figure 3; [5]). All the species show the same chromosome number, $2n = 4x = 32$, both annuals (*C. calcitrapae* (L.) Dufr., *C. macrosiphon* Boiss.) and perennials (the other species), and both widespread (*C. calcitrapae*, *C. ruber*) and narrowly distributed species (the remaining). *Centranthus* is organized in three sections: Section *Calcitrapae* (*C. calcitrapae* and *C. macrosiphon*), sect. *Centranthus* (represented in our study by *C. angustifolius* (Mill.) DC., *C. lecoqii* Jord., *C. longiflorus* Stev. and *C. ruber*), and sect. *Nervosa* (not represented in our study). In this case, section *Calcitrapae*, annual (1Cx = 0.39–0.49 pg), has higher genome size than section *Centranthus*, perennial (1Cx = 0.29–0.36 pg). Annual plants have been usually reported to account for smaller genome sizes than perennials [53], as it is comprehensible that the transcriptional machinery would be more efficient in smaller genomes, in order to quickly complete the life-cycle. Otherwise, exceptions to this rule have been found in different plant groups [22, 54], and this seems to be the trend in the genus *Centranthus*.

The common occurrence of hybridization events in the overlapping areas of *Centranthus* species [55], and the fact that some species or infraspecific taxa show intermediate morphological characters, can indicate that several of these taxa could have a hybrid origin. As it has been reported in different plant groups, nuclear DNA content of hybrids corresponds to approximately the mean of both parental genome sizes, or is slightly smaller/larger than expected [26, 27, 56–64]. Within the section *Calcitrapae*, representatives of southern Spain and Morocco have a doubtful taxonomic assignation, somewhere between *C. calcitrapae* and *C. macrosiphon* ([55] for southern Spain; e.g., J Mathez, Université Montpellier II, France, "unpubl. res." for Morocco). One population of Morocco was assessed, *C.* cf. *calcitrapae* (5), and the 1Cx value obtained of 0.45 pg turns out to be the average between the means of typical *C. calcitrapae* (1Cx = 0.40 pg) and *C. macrosiphon* (1Cx = 0.49 pg), this being consistent with the hypothesis of a hybrid origin for this Moroccan population. Also, *C. lecoqii* is morphologically intermediate between *C. angustifolius* and *C. ruber* [55]. The 1Cx amount of *C. lecoqii* (0.31 pg), the mean of those of *C. angustifolius* (0.34 pg) and *C. ruber* (0.29 pg), is compatible with the hypothesis of a hybrid origin for the former taxon.

4.4. Valeriana montana *Clade.* This group of alpine plants is constituted by species with hermaphrodite flowers and of restricted area (*V. saliunca* All. and *V. supina* Ard. from the Alps, *V. pyrenaica* L. and *V. apula* Pourr. from the Pyrenees), two gynodioecious species widely distributed, *V. tripteris* L. and *V. montana* L. (from the mountains of Southern Europe). *Valeriana rotundifolia* Vill. is a gynodioecious taxon of obscure status, present in the Alps and Corsica, and morphologically close to *V. tripteris* and *V. montana* [65]. The molecular phylogeny shows a *Valeriana tripteris* complex constituted by the populations of *V. montana*, *V. rotundifolia*, *V. supina* and *V. tripteris*, who are forming a robust clade (PP = 1.00, Figure 3), with the exception of *V.* cf. *tripteris* (7) that is sister to the remaining ones. This result raises the question of the monophyly of *V. tripteris*, but also shows a possible interesting biogeographical pattern as the population *V.* cf. *tripteris* (7) is the only sequenced that grows at the east of the Alps. Furthermore, this population is not only divergent in terms of DNA sequence, but also for its cytogenetic characters. Although *V.* cf. *tripteris* (7) is a tetraploid (Figure 1 ma-b), its genome size 2C = 1.23 pg ranks low if compared with tetraploid *V. montana* (2C = 2.50–2.60 pg), and even with diploid *V. tripteris* (2C = 1.46–1.51 pg).

Intermediate forms between *V. tripteris* and *V. montana*, such as V. cf. *montana* (11-12), *V.* cf. *tripteris* (7) or *V. rotundifolia*, occur throughout the range of distribution, which makes their taxonomic delimitation difficult. This is the reason why these taxa have been sometimes considered to represent a single species with various subspecific entities, and also as two closely related species with their intermediate

forms as subspecies (see [65], and references therein). Cryptic hybrid and/or polyploid taxa may largely account for the taxonomic heterogeneity of *V. tripteris* complex and the genome size variation observed in the group, especially at intraspecific level. However, Briquet & Cavillier [65] pointed out that intermediate forms are found in isolated populations, without *V. montana*, which makes their hybrid origin improbable and their polyploid origin from *V. tripteris* maybe more likely. Should this polyploid hypothesis be sustained, the question remains as to why tetraploid *V. tripteris* are morphologically similar to *V. montana*. One explanation could be that the speciation of *V. montana* could have also been induced by a polyploidization event of *V. tripteris*, which would have arisen previously in evolutionary time. This would explain both the more notable morphological and ecological differentiation between the two species, and in some cases the more important decrease in monoploid genome size with respect to that of the intermediate forms [23].

4.5. Valeriana officinalis *Clade (*Valeriana *Clade II, Figure 3).* *Valeriana officinalis* and relatives constitute a difficult complex of numerous taxa of specific and infraspecific ranges, with doubtful morphological delimitation. *Valeriana officinalis* presents an unusual basic chromosome number for the genus of $x = 7$. This basic number is also found in *V. wallrothii* Kreyer, a species closely related to *Valeriana officinalis* in the molecular phylogeny [6], but not in other representatives of the group as, for example, *V. dioica* or *V. tuberosa*. The populations of *V. officinalis* measured exhibit different DNA amounts, suggesting four different DNA ploidy levels of 2*x*, 4*x*, 6*x*, and 8*x* (Table 1). Even so, it would be necessary to determine whether those differences really correspond to different ploidy levels, or if they are partially due to a high intraspecific variability within *V. officinalis*. This doubt concerns particularly the ploidy levels that are uncommon in the *V. officinalis* complex, like the hexaploid level (corresponding to 42 chromosomes), known only from *V. coreana* Briq. [66] and *V. transjenisensis* Kreyer [67]. The results compiled in the present study allow us to conclude that the complexity within *V. officinalis*, in which polyploidization events are largely implicated, is much more than previously considered.

5. Concluding Remarks

At the family level, changes in basic chromosome number and genome size coincide with or precede major shifts in the evolutionary history of Valerianaceae. One interesting example is the arising of strong zygomorphic flower in *Centranthus* and *Fedia*, which is in both cases consecutive to a polyploidization event. Therefore, cytogenetic studies are essential for understanding the family, and, in this sense, we will follow our effort for providing new data of this type, especially in those genera poorly studied or unknown at different levels (i.e., genome size) such as *Plectritis*.

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