

Karyological and molecular analysis of *Leucanthemum* (Compositae, Anthemideae) in Corsica

Authors: Oberprieler, Christoph, Schinhärl, Lena, Wagner, Florian, Hugot, Laetitia, and Vogt, Robert

Source: Willdenowia, 49(3) : 411-420

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: <https://doi.org/10.3372/wi.49.49311>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

CHRISTOPH OBERPRIELER^{1*}, LENA SCHINHÄRL¹, FLORIAN WAGNER¹, LAETITIA HUGOT² & ROBERT VOGT³

Karyological and molecular analysis of *Leucanthemum* (*Compositae*, *Anthemideae*) in Corsica

Version of record first published online on 4 December 2019 ahead of inclusion in December 2019 issue.

Abstract: Karyological, flow-cytometric and molecular analyses indicate that the genus *Leucanthemum* Mill. (*Compositae*, *Anthemideae*) is represented in Corsica (Corse) by two species: the tetraploid *L. ircutianum* DC. and the hexaploid *L. corsicum* (Less.) DC. The indication of the occurrence of the diploid *L. vulgare* Lam. on the island and of a tetraploid chromosome number for *L. corsicum*, given in former treatments of the genus for Corsica, could not be corroborated. AFLP fingerprinting further suggests that the infraspecific taxonomy of *L. corsicum* with two subspecies (*L. corsicum* subsp. *corsicum* and subsp. *fenzlii*) and three forms (*L. corsicum* f. *corsicum*, f. *pinnatifidum* and f. *eschenlohrianum*), which is mainly based on differences in the degree of leaf dissection, is not backed by genetic discontinuities. Owing to the observed little variation in leaf dissection within populations in the wild and the constancy of these features under cultivation, we propose the rank of varieties to taxonomically acknowledge these different stages in the broad spectrum of leaf-dissection grades exhibited by *L. corsicum*. As a consequence, the two new combinations *L. corsicum* var. *eschenlohrianum* (Gamisans) Vogt, Hugot & Oberpr. and *L. corsicum* var. *fenzlii* (Gamisans) Vogt, Hugot & Oberpr. are proposed.

Key words: AFLP fingerprinting, *Anthemideae*, *Asteraceae*, chromosome numbers, *Compositae*, Corse, Corsica, cytology, *Leucanthemum*, morphology, taxonomy

Article history: Received 14 June 2019; peer-review completed 26 September 2019; received in revised form 15 October 2019; accepted for publication 18 October 2019.

Citation: Oberprieler C., Schinhärl L., Wagner F., Hugot L. & Vogt R. 2019: Karyological and molecular analysis of *Leucanthemum* (*Compositae*, *Anthemideae*) in Corsica. – Willdenowia 49: 411–420. doi: <https://doi.org/10.3372/wi.49.49311>

Introduction

The genus *Leucanthemum* Mill. (marguerites, ox-eye daisies; *Compositae*, *Anthemideae*) comprises 42 flowering plant species (Euro+Med, 2006+) distributed all over the European continent and represents an attractive system for studying reticulate evolution on the diploid (Oberprieler & al. 2014; Konowalik & al. 2015; Wagner & al. 2017) and polyploid level (Oberprieler & al. 2011, 2014, 2018; Greiner & al. 2012, 2013; Vogt & al. 2018). Following the treatments of the genus in the *Compléments au Prodrome de la Flore Corse* (Gamisans 1998) and

Flora Corsica (Jeanmonod & Gamisans 2013), this genus is represented in Corsica by two species: *L. vulgare* Lam. and *L. corsicum* (Less.) DC. The former is widely distributed throughout Europe and even introduced and naturalised on other continents, while the latter is an endemic species to the Corsican flora. According to Vogt (cited in Gamisans 1998: 287) and *Flora Gallica* (Tison & de Foucault 2014), it is not the diploid *L. vulgare* that is introduced to Corsica, but the equally widespread tetraploid *L. ircutianum* DC.

Owing to the paramount importance of karyological information for a well-informed taxonomic treatment

1 Evolutionary and Systematic Botany Group, Institute of Plant Sciences, University of Regensburg, Universitätsstr. 31, D-93053 Regensburg, Germany; *e-mail: christoph.oberprieler@ur.de (author for correspondence).

2 Conservatoire Botanique National de Corse, Avenue Jean Nicoli, F-20250 Corte, France.

3 Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin, Königin-Luise-Str. 6–8, 14195 Berlin, Germany.

of the polyploid complex of *Leucanthemum*, the mentioned treatment of Gamisans (1998) has added no additional facts to the chromosome counts given in the author's former revision of *L. corsicum* (Gamisans 1972). Here, the extremely high morphological variability of this species – especially in terms of leaf dissection – has been countered by the description/acknowledgement of two subspecies and four forms. However, chromosome number reports were limited to two of them (for *L. corsicum* f. *corsicum* and f. *eschenlohrianum*) in addition to an even older report by Contrandrioploulos (1964), attributed to *L. corsicum* subsp. *fenzlii* Gamisans. This lack of karyological information for infraspecific taxa of *L. corsicum* and the complete ignorance about the chromosome number of the other species of *Leucanthemum* in Corsica (i.e. *L. vulgare* s.l.) motivated us to subject the Corsican populations of this genus to a more comprehensive sampling of populations for genome-size analyses by flow-cytometry, complemented and validated by chromosome counts.

In addition to these cytological investigations, we were interested in the genetic background of infraspecific taxa of *Leucanthemum corsicum*. For this purpose we conducted AFLP fingerprinting based on silica-gel dried leaf material sampled during an excursion to Corsica in August 2018. This molecular method has proven being an important technique for solving taxonomic problems in the genus in other parts of its distributional range (Greiner & al. 2013; Konowalik & al. 2015; Wagner & al. 2017; Oberprieler & al. 2018).

Material and methods

Plant material — The plant material for the present study was collected during an excursion to Corsica in August 2018. The permit for these collections was issued by the Conseil National de la Protection de la Nature (CNPN) under the project number 2018-08-17-00916) to one of the authors (L. H.). We sampled 12 populations of *Leucanthemum* representing the wide-spread European species *L. vulgare* Lam. s.l. and the Corsican endemic *L. corsicum* (Less.) DC. with its morphologically defined infraspecific taxa *L. corsicum* f. *corsicum*, f. *pinnatifidum*, f. *eschenlohrianum* and subsp. *fenzlii* (Fig. 1, Table 1). For flow-cytometric and molecular analyses, leaf material was collected and dried in silica-gel. Additionally, for most populations voucher specimens were prepared and are housed at the herbarium of the Botanical Museum Berlin.

Karyological and flow-cytometric analyses — Chromosome numbers were obtained from somatic mitoses of root tips of plants raised from seed in the Botanic Garden Berlin and the Botanical Garden of the University of Regensburg. Root tips were pre-treated with hydroxyquinoline (0.002 molar aqueous solution) for 2 hours, fixed

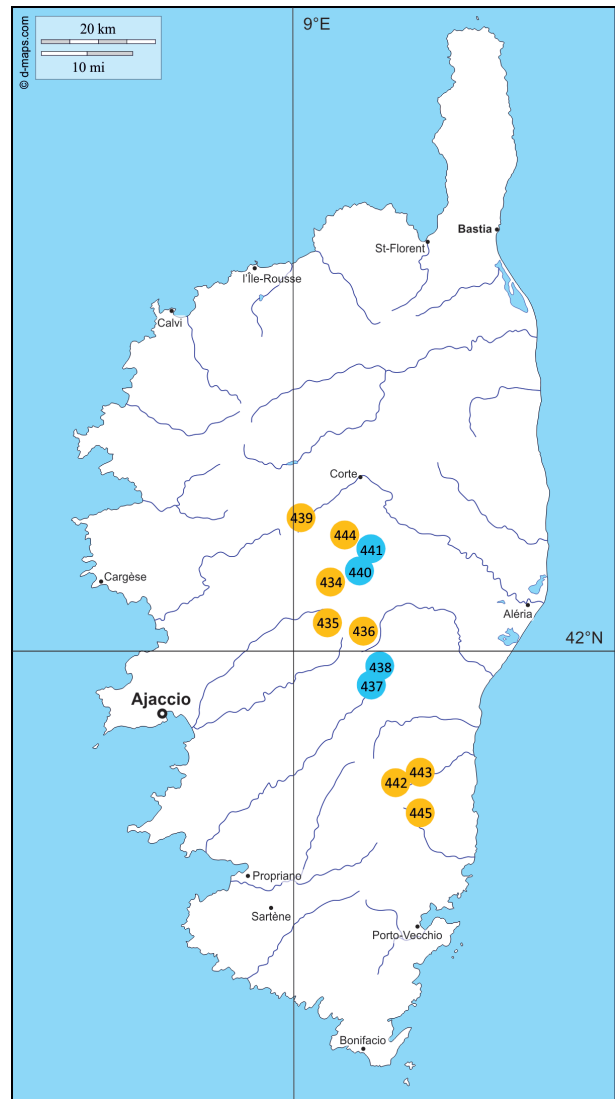


Fig. 1. Map of Corsica (source and © d-maps.com: https://d-maps.com/carte.php?num_car=14334&lang=de; modified) with localities of sampled populations of *Leucanthemum ircutianum* (blue) and *L. corsicum* (orange) surveyed for the present study. Population numbers refer to further information on infraspecific taxa, localities, voucher specimens and results of flow-cytometric analyses in Table 1.

in 96% ethanol/glacial acetic acid (3:1) and refrigerated. Hydrolysis was carried out with 1–2 N hydrochloric acid for 10–15 minutes at 60°C. For chromosome staining root tips were squashed in aceto-orcein. Voucher specimens of the original collections and of plants cultivated in the Botanic Garden Berlin are deposited in B.

For flow cytometry, a two step protocol was used (Doležal & al. 2007) with leaf material of *Pisum sativum* L. 'Citrad' (2C = 9.09 pg) as an internal standard. The amount of a leaf sample (c. 100–200 mm²) was approximately threefold compared to the material of the internal standard. Leaf fragments of 96 individuals from 12 populations (Table 1) were chopped with a razor blade in citric-acid-Triton isolation buffer (0.2 M citric acid, 0.5% Triton X), the suspension of nuclei was filtered

Table 1. Corsican *Leucanthemum* populations sampled for the present study with information on localities, voucher specimens and indication of mean values for DNA content from flow-cytometric analyses and numbers of surveyed individuals in cytometric and AFLP-fingerprinting analyses.

Population	Taxon	Locality	Coordinates	Collectors	Voucher specimens	2C DNA content (pg) ± SD (n)	AFLP (n)
<i>Silica-gel-dried leaf material from Corsican wild populations</i>							
Leu434	<i>L. corsicum</i> subsp. <i>fenzlii</i>	Co, Haute-Corse, Monte d'Oro, 1666 m	42°07'53.4"N, 09°05'09.8"E	Vogt 17862 & al.	B 10 1003362, B 10 1003364	28.39 ± 0.64 (10)	5+1
Leu435	<i>L. corsicum</i> subsp. <i>fenzlii</i>	Co, Corse-du-Sud, La Gravona, 1200 m	42°04'40.8"N, 09°06'27.9"E	Vogt 17863 & al.	B 10 1003360, B 10 1003361	29.07 ± 0.49 (10)	5
Leu436	<i>L. corsicum</i> subsp. <i>fenzlii</i>	Co, Haute-Corse, Monte Renoso, 1760 m	42°04'24.8"N, 09°08'49.4"E	Vogt 17864 & al.	B 10 1003340, B 10 1003358, B 10 1003359	28.86 ± 0.66 (9)	5+1
Leu437	<i>L. ircutianum</i>	Co, Corse-du-Sud, Col de Verde, 920 m	41°58'21.6"N, 09°11'03.4"E	Vogt 17865 & al.	B 10 1003356	23.77 ± 0.48 (2)	2
Leu438	<i>L. ircutianum</i>	Co, Corse-du-Sud, Col de Verde, 1058 m	41°59'29.6"N, 09°11'14.8"E	Vogt 17867 & al.	B 10 1003354, B 10 1003355	22.41 ± 0.40 (9)	4+1
Leu439	<i>L. corsicum</i> f. <i>pinnatifidum</i>	Co, Haute-Corse, Restonica, 1430 m	42°14'08.8"N, 09°02'08.9"E	Vogt 17868 & Oberprieler	B 10 1003352, B 10 1003353	28.24 ± 1.07 (10)	5
Leu440	<i>L. ircutianum</i>	Co, Haute-Corse, Canaglia, 740 m	42°09'38.1"N, 09°08'19.6"E	Vogt 17874 & Oberprieler	B 10 1003345, B 10 1003346	21.29 ± 0.52 (9)	4+1
Leu441	<i>L. ircutianum</i>	Co, Haute-Corse, Canaglia, 697 m	42°09'26.5"N, 09°08'26.8"E	Vogt 17875 & Oberprieler	B 10 1003344	20.98 ± 0.81 (10)	4+1
Leu442	<i>L. corsicum</i> f. <i>corsicum</i>	Co, Corse-du-Sud, Bavelia, 1439 m	41°48'06.8"N, 09°12'54.3"E	Vogt 17876 & al.	B 10 1003341	29.38 (1)	2
Leu443	<i>L. corsicum</i> f. <i>corsicum</i>	Co, Corse-du-Sud, Bavelia, 1400 m	41°48'02.5"N, 09°13'00.2"E	Vogt 17877 & al.	B 10 1003342	27.68 ± 0.06 (2)	3
Leu444	<i>L. corsicum</i> f. <i>eschenlohrianum</i>	Co, Haute-Corse, Paratella, 1621 m	42°13'08"N, 09°06'48"E	Hugot s.n. & al.	not collected	29.26 ± 0.48 (10)	5+2
Leu445	<i>L. corsicum</i> f. <i>corsicum</i>	Co, Corse-du-Sud, Punta Velaco, 1372 m	41°46'35"N, 09°14'01"E	Hugot s.n. & al.	not collected	29.33 ± 0.67 (10)	4
<i>Fresh leaf material of plants cultivated in the Botanical Garden of the University of Regensburg</i>							
Leu434	<i>L. corsicum</i> subsp. <i>fenzlii</i>	Co, Haute-Corse, Monte d'Oro, 1666 m	09°05'09.8"E, 42°07'53.4"N	Vogt 17862 & al.		29.70 ± 0.04 (2)	
Leu436	<i>L. corsicum</i> subsp. <i>fenzlii</i>	Co, Haute-Corse, Monte Renoso, 1760 m	09°08'49.4"E, 42°04'24.8"N	Vogt 17864 & al.		29.60 (1)	
Leu439	<i>L. corsicum</i> f. <i>pinnatifidum</i>	Co, Haute-Corse, Restonica, 1430 m	09°02'08.9"E, 42°14'08.8"N	Vogt 17868 & Oberprieler		29.98 (1)	

through a mesh with a pore size of 50 µm and kept on ice. After centrifugation for 5 min at 150 g and 4°C, the isolation buffer was removed aside from a rest of c. 50 µl, and this pellet was dissolved in ice-cold LB01 buffer (Doležel & al. 1989) containing 4 mg/l of DAPI (Carl Roth, Karlsruhe, Germany). Excitation of the sample was done using a UV-LED (365 nm; 3 W) and a sensitive blue photo-multiplier tube detecting fluorescent light between 435 nm and 560 nm on a CyFlow Ploidy Analyser (Sysmex, Norderstedt, Germany). Acquisition was automatically stopped at 8000 measured nuclei of the standard peak. The DNA content of *Leucanthemum* probes was calculated by referencing to the internal standard peak of *Pisum sativum*.

DNA extraction and AFLP fingerprinting — Extraction of total genomic DNA was done with a CTAB extraction protocol (Doyle & Doyle 1987; Doyle & Dickson 1987) including RNA digestion. DNA concentration of all extracts used in the AFLP fingerprinting procedure was measured with a Qubit® fluorometer (Invitrogen, Carlsbad, CA, U.S.A.) and then dilutions for a final DNA concentration of 12.5 ng/µl were prepared. The AFLP protocol followed the original description of Vos & al. (1995) with modifications described in Oberprieler & al. (2011) and Greiner & al. (2013). In the first step, *MseI* and *EcoRI* restriction enzymes were used together with T4 DNA ligase and adaptors compatible with either of the two restriction sites. Restriction-ligation was carried out at 37°C for 2 h, after which the ligase was heat-inactivated. The Pre-selective Amplification (PA) step involved primers with one and two selective nucleotides (A for the *EcoRI* primer and CT for the *MseI* primer), while Selective Amplification (SA) used primers with additional two selective nucleotides (CTAG for the *MseI* primer, and the three fluorescently labelled *EcoRI* primers EcoRI-ACC, EcoRI-AGG and EcoRI-ACA). The PCR products were united, precipitated and subsequently dissolved in a mixture of GenomeLab Sample Loading Solution and CEQ Size Standard 400 (Beckman Coulter, Germany). The fragment detection was performed on a CEQ8000 capillary sequencer (Beckman Coulter, Germany). To quantify AFLP genotyping errors, replicates were generated for seven randomly selected samples, representing 12.7% of the total sample number (Table 1).

A 0/1 matrix was generated by automatic band scoring using GELCOMPAR II v.5.10 (Applied Maths NV, Sint-Martens-Latem, Belgium) and a screening through 112 parameter combinations comprising different combinations of values for *peak minimal profiling* (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0), *peak minimal area* (0.1, 0.2, 0.3 and 0.4) and *band matching tolerance* (0.1, 0.2, 0.3 and 0.4) was carried out using the seven replicate samples. In order to choose the most reliable combination, the Euclidean error, the Jaccard distance, the number of correctly paired individuals, and the phylogenetic resolution score were calculated using a Python script developed by

Holland & al. (2008). The parameter combination with the highest scores was then chosen for band-scoring in all remaining individuals.

To visualize the genetic similarity among individuals, a Neighbor-Net diagram was constructed based on Nei-Li distances (Nei & Li 1979) using the software SPLITSTREE v.4.14.6 (Huson & Bryant 2017). Additionally, a Bayesian clustering of individuals was done with the software STRUCTURE v.2.3 (Pritchard & al. 2000). For estimation of the optimal cluster number *k* the method of Evanno & al. (2005) was used. Allele frequencies were set to correlated, all individuals were assigned to haploid level to account for the dominant marker system and the mixed ploidy levels in the dataset. The burn-in was set to 50 000 generations and chain length to 500 000 generations. The analysis was run 10 times and the results were averaged using CLUMPP v.1.1.2 (Jakobsson & Rosenberg 2007). For visualization of the results, the software POPHELPER v.1.0.10 (Francis 2017) was used.

Results

Karyological and flow-cytometric analyses — As summarised in Table 1, all 30 accessions from Corsican populations of *Leucanthemum vulgare* s.l. revealed DNA contents between 19.7 pg and 24.3 pg (mean: 21.7 pg; SD: 1.0 pg) and therefore showed values typical for the tetraploid *L. ircutianum* DC. Unexpectedly, all 66 accessions of *L. corsicum*, irrespective of their infraspecific classification, revealed considerably higher DNA contents ranging between 26.2 pg and 30.5 pg (mean: 28.9 pg; SD: 0.9 pg) arguing for a hexaploid ploidy level realised in this species (Table 1). This interpretation was confirmed by mitotic chromosome counts on individuals raised from seed originating from populations 434 and 436 of *L. corsicum* subsp. *fenzlii* of the present study and cultivated plants from the same subspecies collected in 2011 close to our present population 434 (Table 2, Fig. 2).

AFLP fingerprinting — The automatic band scoring procedure with the Python scripts of Holland & al. (2008) revealed as the best combination of parameter values a *peak minimal profiling* of 2.5, a *peak minimal area* of 0.3 and a *band matching tolerance* of 0.4. With these parameter values, automatic band scoring yielded 425 bands with an Euclidean error rate of 13%, a Jaccard error rate of 47%, a resolution score of 46% and six out of the seven replicates were consistently paired. As expectable from the flow-cytometrically determined differences in genome size between representatives of *Leucanthemum ircutianum* (tetraploid) and *L. corsicum* (hexaploid), representatives of the former species produced on average less bands than the latter ones (88.9 vs 95.5, respectively). Five individuals (three from population 444 and two from population 445) showed comparably faint AFLP

Table 2. Mitotic chromosome number records based on plant material of *Leucanthemum corsicum* subsp. *fenzlii* cultivated in the Botanical Garden of the University of Regensburg (first two records) or in the Botanic Garden Berlin (third record).

Chromosome count	Population (voucher information)	Figure
$2n = 54$	Leu434: France, Corsica, Dep. Haute-Corse, Vizzavona, Monte d'Oro, valley of l'Agnone SE of Pointe Muratello, 42°07'53.4"N, 09°05'09.8"E, 1666 m, limestone rocks, 13 Aug 2018, <i>Vogt 17862</i> , <i>Oberprieler 11211</i> & <i>Hugot</i> (B [B101003362, B101003364]).	Fig. 2A
$2n = 54$	Leu436: France, Dep. Haute-Corse, Ghisoni, Monte Renoso, Bergeries de Capannelle, along track to Lac de Bastani, 42°04'24.8"N, 09°08'49.4"E, 1760 m, Krummholz and stony slopes, 14 Aug 2018, <i>Vogt 17864</i> , <i>Oberprieler 11213</i> & <i>Hugot</i> (B [B101003358, B 10 1003359, B 10 1003340]).	Fig. 2B
$2n = 54$	France, Corsica, Salendo il sentiero del GR20 che da "Colle di Vizzavona" porta al Monte d'Oro, "Vallone Agnone", 42°07'53"N, 09°05'10"E, 1700 m, Wegrand, 9 Aug 2011, <i>Tomasello 411</i> (B [B100464097, B100464098]); Cult. Hort. Bot. Berlin (059-01-12-20), 14 Sep 2012, <i>Vogt s.n.</i> (B [B 10 0458571]).	Fig. 2C

banding patterns and were omitted from the subsequent analyses.

Following the method of Evanno & al. (2005), the optimal number of clusters in the Bayesian clustering of STRUCTURE was inferred to be $k = 2$, corresponding to the two species involved, *Leucanthemum corsicum* and *L. ircutianum* (Fig. 3). There was only a single individual found that was not assigned to one of the two clusters with a posterior probability of $PP > 0.9$; i.e. an individual from population 438 (438-01) being clustered to *L. ircutianum* with only $PP = 0.873$. The clear bipartition of the dataset was also revealed in the Neighbor-Net network reconstruction based on Nei-Li distances (Fig. 4). These two analyses also clearly showed that the two subspecies (and three forms) of *L. corsicum* do not represent genetically distinct lineages.

Discussion

Ploidy of Corsican Leucanthemum — Our present finding of DNA contents characteristic for hexaploid *Leucan-*

themum species in 67 accessions of *L. corsicum* from eight populations and the corroboration of a hexaploid number through counting of chromosomes in mitotic cells of root tips of plants from two different populations are in unexpected contrast to all previously published chromosome numbers for this species. Contandriopoulos (1964) has been the first publishing a chromosome number for *L. corsicum* and indicated and pictured a tetraploid number for plant material collected at Monte d'Oro by "Mme Conrad et étudié par nous [Planche 1, fig. 12]" (l.c.: 378). It is not clear whether any voucher specimen exists for this chromosome count and has been seen by J. Gamisans, but due to the locality indicated (Monte d'Oro), this author assigned this count to his *L. corsicum* subsp. *fenzlii* (Gamisans 1972: 195, 1998: 284).

Our interpretation for the discrepant chromosome count by J. Contandriopoulos from the same locality (*locus classicus* of *Leucanthemum corsicum* subsp. *fenzlii*) is that either there was some confusion with the labelling at the Neuchâtel Botanical Garden or that its collector, Mme Conrad, misidentified the plant as *L. corsicum*. In

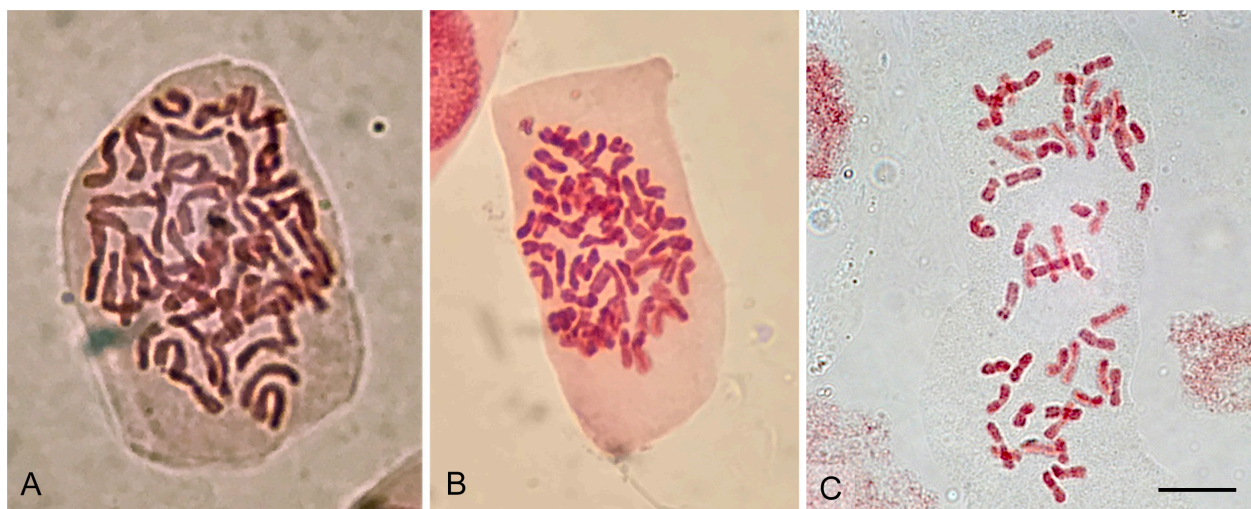


Fig. 2. Micrographs of metaphases of root-tip mitoses. – A: *Leucanthemum corsicum* subsp. *fenzlii* (population 434), $2n = 54$. – B: *L. corsicum* subsp. *fenzlii* (population 436), $2n = 54$. – C: *L. corsicum* subsp. *fenzlii* (Tomasello 411), $2n = 54$.

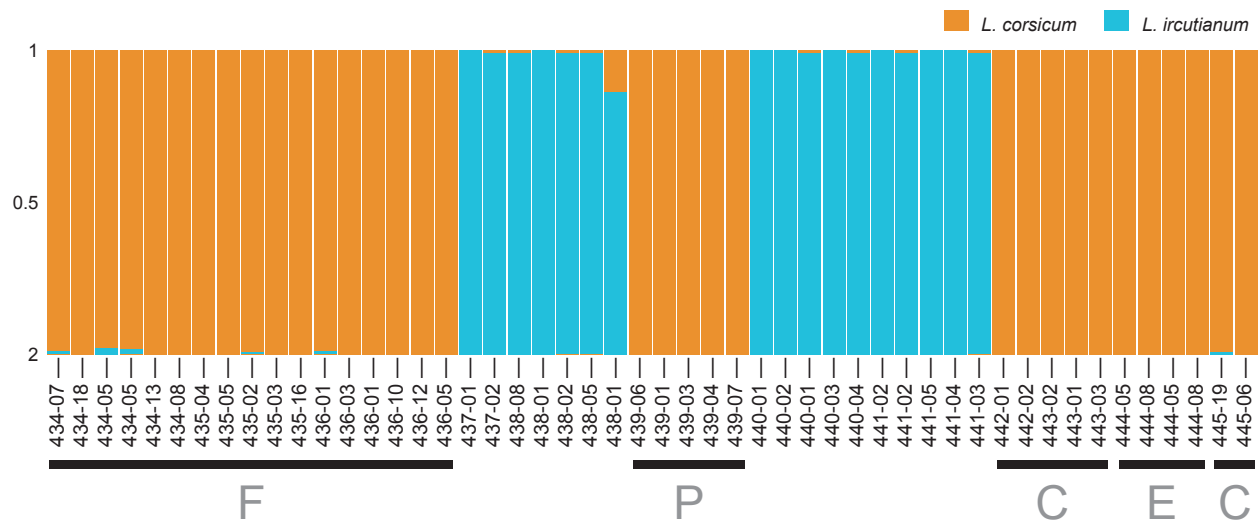


Fig. 3. Results of a Bayesian cluster analysis (STRUCTURE) of AFLP fingerprint data, with $k = 2$ found as the optimal cluster number. The histograms illustrate posterior probabilities of membership in the two clusters for each *Leucanthemum* accession under study. Letters below accession numbers refer to infraspecific taxa of *L. corsicum* (i.e. C: *L. corsicum* f. *corsicum*; P: f. *pinnatifidum*; E: f. *eschenlohrianum*; F: subsp. *fenzlii*).

favour of the latter interpretation is the fact that tetraploids (the typical chromosome number of *L. ircutianum*) are found close to Monte d'Oro, for example at Vivario (populations 440 and 441) and Vizzavona (indicated by Gamisans 1998: 287 under the wrong name *L. vulgare*). In contrast to this interpretation, however, is the fact that Contandriopoulos (1964: 378) stated strong morphological affinities of her cultivated *L. corsicum* plants to *L. monspeliense* L. [sub *L. cebennense* (L.) H. J. Coste] from the Massif Central ("gorges de l'Héric"); therefore, indicating that the plants had strongly dissected leaves, typical for *L. corsicum* subsp. *fenzlii*, but not for *L. ircutianum*.

The deviating counts of tetraploid chromosome numbers for *Leucanthemum corsicum* subsp. *corsicum* published by Gamisans (1972) are even harder to explain, especially because two populations from two different forms (i.e. f. *corsicum* and f. *eschenlohrianum*) were concerned and the author explicitly focussed on the taxonomy of *Leucanthemum* in Corsica in this contribution. In the former case (f. *corsicum*), a meiotic chromosome number of $n = 18$ is given for a pollen mother cell observed in flower bud fixations of a plant collected in the Massif de Bavella ("SSW la Bocca del Marro") close to our hexaploid populations 442 and 443 (both also f. *corsicum*); in the latter, a tetraploid mitotic ($2n = 36$) and meiotic count ($n = 18$) is given for the Monte Rotondo area ("vallée du Manico") close to our hexaploid population 444 (also f. *eschenlohrianum*).

Genetic differentiation of Leucanthemum corsicum — In contrast to their genetic distinctness from the tetraploid *Leucanthemum ircutianum* in all analyses based on AFLP fingerprint data, the infraspecific taxa of the hexaploid *L. corsicum* involved in the present study

(i.e. *L. corsicum* f. *corsicum*, f. *pinnatifidum*, f. *eschenlohrianum* and *L. corsicum* subsp. *fenzlii*) were found lacking genetic differentiation paralleling their morphological separation. When considering the diagnostic features described by Gamisans (1972, 1998), it becomes obvious, however, that these are solely based on different intensities of leaf-lobe incision, ranging from *L. corsicum* subsp. *corsicum* f. *corsicum* (and the later-on synonymised f. *dentatum*) showing dentate to pinnatifid middle cauline leaves and a rachis broader than 5 mm, over f. *pinnatifidum* (pinnatifid to pinnatipartite, rachis 3.5–5 mm broad), f. *eschenlohrianum* (pinnatipartite, rachis 2.5–3.5 mm broad) to the other extreme *L. corsicum* subsp. *fenzlii*, showing pinnatisect leaves having a rachis 1–2(–2.5) mm broad. It appears reasonable, therefore, that these infraspecific taxa are representing more or less artificially demarcated entities in an obviously continuous spectrum of morphological variation. Additionally, there seems to be only a quite weak geographical pattern in this morphological gradient: while *L. corsicum* subsp. *fenzlii* is limited to the very central part of the Corsican mountain backbone (Monte d'Oro-Migliarellu, Punta di u Fornellu), the forms of *L. corsicum* subsp. *corsicum* are indicated (often sympatrically and without elevational tendencies) for the further massifs of this chain between Monte Cintu in the NW and the Massif de Bavella in the SE (Gamisans 1972, 1998). Nevertheless, during the sampling excursion to Corsica in summer 2018, we have observed that local stands of *L. corsicum* are very homogenous in morphological respects and exhibit only little variation in leaf dissection. Additionally, the retention of leaf-dissection characteristics of plants grown from seed from populations 434 and 436 (subsp. *fenzlii*) and population 439 (f. *pinnatifidum*) under common-garden conditions in the Botanical Gar-

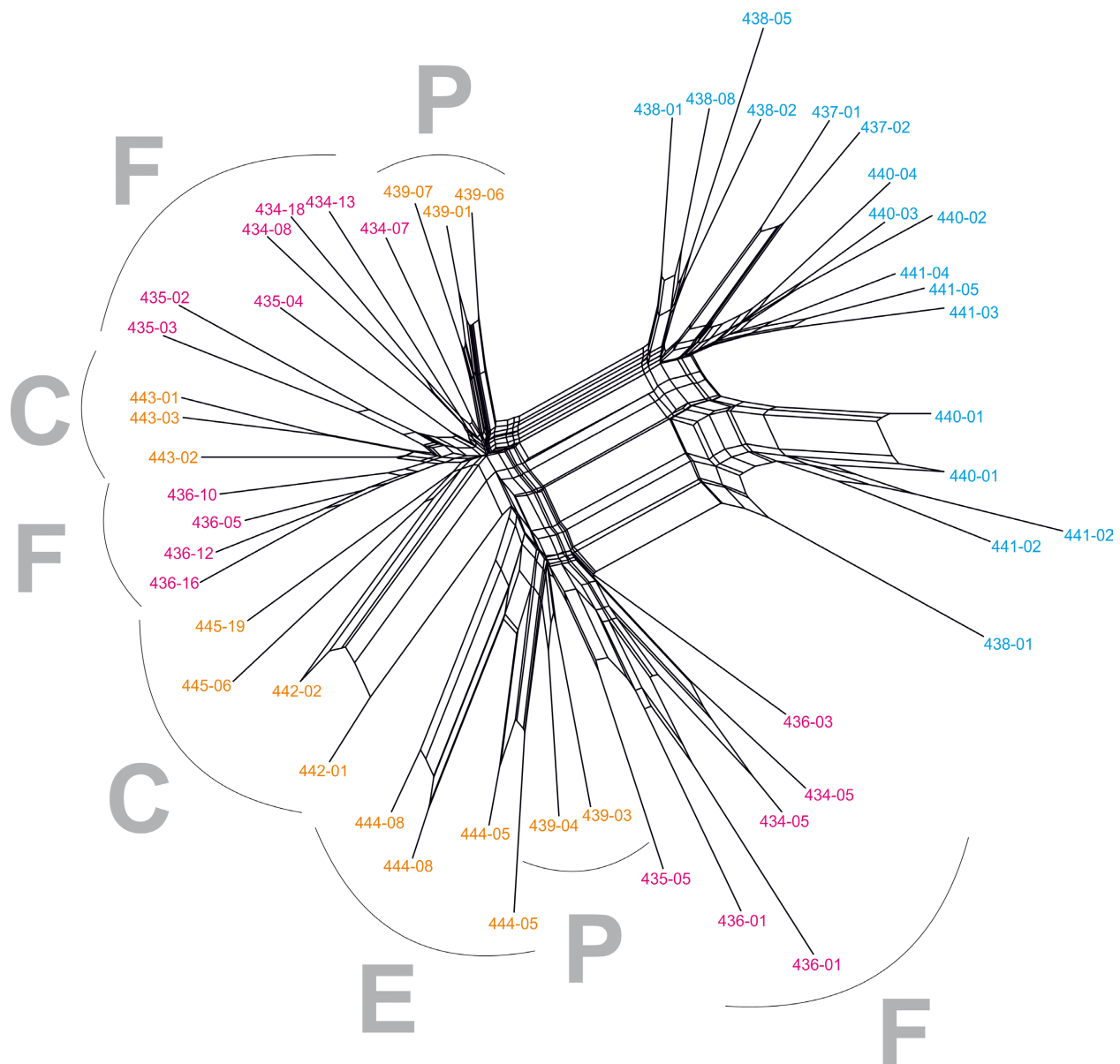


Fig. 4. Results of a Neighbor-Net network analysis based on AFLP fingerprint data, with accessions of *Leucanthemum ircutianum* in blue, of *L. corsicum* subsp. *corsicum* in orange and of subsp. *fenzi* in red. Letters refer to infraspecific taxa of *L. corsicum* (i.e. C: *L. corsicum* f. *corsicum*; P: f. *pinnatifidum*; E: f. *eschenlohrianum*; F: subsp. *fenzi*).

den of the University of Regensburg argue for a genetic background of these taxonomically important features (see Fig. 5 vs Fig. 6).

Leaf shape and leaf dissection are, besides the colour of margins of involucral bracts and fruit characteristics, very important characters in the taxonomy of *Leucanthemum* (Vogt 1991). In no other species of this genus leaf dissection varies such tremendously as in *L. corsicum*, ranging from nearly entire to finely dissected middle cauline leaves with nearly capillary lobes. The evolutionary adaptive value of leaf-division intensity is difficult to test, but there are observations that leaf dissection could increase photosynthesis and/or could be involved in thermoregulation (Maugarny-Calès & Laufs 2018). Therefore, leaf dissection has even been used in palaeobotany to reconstruct past climates based on fos-

sil records (Greenwood 2005). On the other hand, and in support of the adaptation argument, it has also been demonstrated that leaf-blade dissection may be under oligogenic control (Sicard & al. 2014; Maugarny-Calès & Laufs 2018). This may explain our present observation that variation in ecologically adaptive and taxonomically decisive morphological features is not paralleled by genetic patterns based on molecular markers scattered throughout the whole genome like AFLP fingerprint loci.

Taxonomic consequences — The consistent finding of the hexaploid level in all sampled populations of *Leucanthemum corsicum*, together with the continuous morphological variation found in leaf dissection and the little genetic structure exhibited in the AFLP analysis,

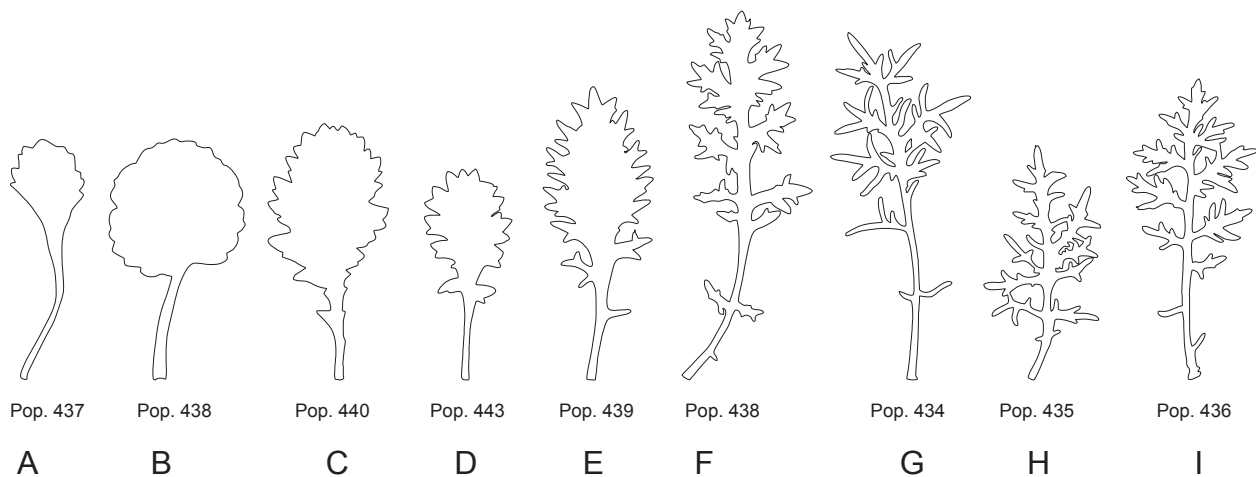


Fig. 5. Leaves of plants collected in the wild. – A–C: *Leucanthemum ircutianum*; D: *L. corsicum* f. *corsicum*; E: *L. corsicum* f. *pinnatifidum*; F: *L. corsicum* f. *eschenlohrianum*; G–I: *L. corsicum* subsp. *fenzi*.

indicates that all populations belong to a single biological species. When deciding on a suitable taxonomic treatment for the considerable infraspecific morphological variation observed in a sexually reproducing species, Stuessy (2009: 156) suggested consideration of “morphological distinctness, geographical cohesiveness, and where known, genetic divergence, natural reproductive isolation, and degrees of fertility or sterility of natural hybrids”. While information on natural reproductive isolation between morphologically divergent populations of *L. corsicum* and the fertility of crossing products are missing, the other criteria could be included into a discussion on taxonomic consequences of the present findings.

The lack of a clear-cut morphological discontinuity between *Leucanthemum corsicum* subsp. *corsicum* and subsp. *fenzi* caused by the intermediate taxa *L.*

corsicum f. *dentatum* (already sunk into synonymy of f. *corsicum* by Gamisans 1998), f. *pinnatifidum* and f. *eschenlohrianum* is paralleled by the lack of a clear genetic structure. This, together with the absence of a geographical cohesiveness of morphologically and genetically similar populations argue in our opinion against acknowledgement of the two subspecies proposed. On the other hand, the morphological constancy within populations and its (albeit presumably oligogenic) genetic control shown by common-garden cultivation is an argument against a taxonomic treatment of these morphotypes as mere forms appearing interspersed in populations together with the typical form. Therefore, in accordance with Stuessy’s (2009) suggestions and in line with the treatment of comparable cases in Oberprieler (1998) in NW African taxa of *Anthemis* L., we propose the rank of varieties as the most suitable one for casting



Fig. 6. Young plants cultivated in the Botanical Garden of the University of Regensburg from seed material collected in the wild. – Left: *Leucanthemum corsicum* subsp. *fenzi* (population 434). – Middle: *L. corsicum* subsp. *fenzi* (population 436). – Right: *L. corsicum* f. *pinnatifidum* (population 439).

the observed morphological, genetic and geographical patterns into a formal taxonomic system. As a consequence, the following taxa based on Gamisans's (1998) latest treatment can be discriminated in *L. corsicum*:

1. *Leucanthemum corsicum* (Sieber ex Less.) DC., Prodr. 6: 47. 1838 var. ***corsicum*** \equiv *Phalacrodiscus corsicus* Sieber ex Less., Syn. Gen. Compos.: 254. 1832 \equiv *Chrysanthemum montanum* var. *corsicum* (Sieber ex Less.) Mutel, Fl. Franç. 2: 154. 1835 \equiv *Leucanthemum coronopifolium* subvar. *corsicum* (Sieber ex Less.) Nyman, Consp. Fl. Eur.: 371. 1879 \equiv *Chrysanthemum atratum* var. [" δ "] *corsicum* (Sieber ex Less.) Fiori, Nuov. Fl. Italia 2(4): 627. 1927 \equiv *Leucanthemum atratum* subsp. *corsicum* (Sieber ex Less.) Horvatić in Acta Bot. Inst. Bot. Univ. Zagreb 10: 75. 1935. – Lectotype (designated by Gamisans in Candollea 27: 192. 1972): Monte d'Oro, Corsicae (W! [W0010623A]).

= *Leucanthemum corsicum* f. *dentatum* Gamisans in Candollea 27: 192. 1972. – Holotype: Corse, Massif de Bavella, couloir rocaillieux dominant vers le N le ravin de Polischello [41°50'N, 09°13'E], 1670 m, siliceux, 23 Jul 1969, *Gamisans* 21 (G! [G00220670]).
– *Chrysanthemum corsicum* Sieber ex Less., Syn. Gen. Compos.: 254. 1832, nom. inval., pro syn.

2. *Leucanthemum corsicum* var. *pinnatifidum* (Fenzl) Briq. & Cavill. in Burnat, Fl. Alpes. Marit. 6: 117. 1916 \equiv *Tanacetum monspeliense* var. *pinnatifidum* Fenzl in Verh. Zool.-Bot. Vereins Wien 3: 346. 1853 \equiv *Chrysanthemum atratum* f. *pinnatifidum* (Fenzl) Fiori, Nuov. Fl. Italia 2(4): 627. 1927 \equiv *Leucanthemum corsicum* f. *pinnatifidum* (Fenzl) Gamisans in Candollea 27: 193. 1972. – Lectotype (designated by Gamisans in Candollea 27: 193. 1972): Monte d'oro, Corsica (W! [W0010623B]).

= *Leucanthemum vulgare* var. *cyrneum* Litard. in Bull. Soc. Sci. Hist. Nat. Corse 42: 239. 1922. – Holotype: Corse, Massif de Rotondo, rochers de la base de crête de San Cypriano, rive g. de la Restonica, en aval des bergeries de Grotello [42°14'N, 09°02'E], 1300 m, 20 Aug 1919, *Litardière* (G! [G100100134]).

3. *Leucanthemum corsicum* var. *eschenlohrianum* (Gamisans) Vogt, Hugot & Oberpr., **stat. nov.** \equiv *Leucanthemum corsicum* f. *eschenlohrianum* Gamisans in Candollea 27: 194. 1972. – Holotype: Massif de Rotondo, Monte Cardo, vers. S, ravin de Paratello [42°13'N, 09°07'E], 1600 m, paroi rocheuse dominant le torrent, siliceux, 26 Jul 1968, *Gamisans* 3 (Herb. Gamisans; isotype: G! [G00220680]).

4. *Leucanthemum corsicum* var. *fenzlii* (Gamisans) Vogt, Hugot & Oberpr., **stat. nov.** \equiv *Leucanthemum corsicum* subsp. *fenzlii* Gamisans in Candollea 27: 194. 1972. – Holotype: Monte d'Oro, vers. ESE [42°08'N, 09°06'E], 1600 m, rochers, 30 Jun 1967, *Gamisans* 17 (G! [G00220681]; isotype: G! [G00220682]).

Acknowledgements

This work was funded by the grant of the German Research Foundation (DFG) in the frame of the SPP 1991 "Taxon-omics – New Approaches for Discovering and Naming Biodiversity" to C.O. (OB 155/13-1) and R.V. (VO 1595/3-1). We would like to thank Anja Heuschneider for her most valuable technical help in the molecular laboratory of the Evolutionary and Systematic Botany Group at Regensburg University. The technical help of the late Monika Lüchow from the Botanical Museum Berlin is gratefully acknowledged as well as the supportive work of Jenny M. Köppe and Richard Pauwels (both Berlin) with the determination of chromosome numbers. Salvatore Tomasello (Göttingen) is thankfully acknowledged for the collection of plant material in 2011 used for karyological studies. We thankfully appreciate the issuing of a permit for collection of leaf material of *Leucanthemum corsicum* by the Conseil National de la Protection de la Nature (project number 2018-08-17-00916). Additionally, Lorenzo Peruzzi (Pisa) and an anonymous reviewer are acknowledged for useful comments on a former version of this article.

Conflict of interest statement

The authors declare no conflict of interests.

References

- Contandriopoulos J. 1964: Recherches sur la flore endémique de la Corse et sur ses origines (II). – Rev. Gén. Bot. **71**: 361–384.
- Doležel J., Binarová P. & Lucretti S. 1989: Analysis of nuclear DNA content in plant cells by flow cytometry. – Biol. Pl. **31**: 113–120.
- Doležel J., Greilhuber J. & Suda J. 2007: Estimation of nuclear DNA content in plants using flow cytometry. – Nature Protocols **2**: 2233–2244.
- Doyle J. J. & Dickson E. E. 1987: Preservation of plant samples for DNA restriction endonuclease analysis. – Taxon **36**: 715–722.
- Doyle J. J. & Doyle J. L. 1987: A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochemical Bulletin **19**: 11–15.
- Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. – Published at <http://ww2.bgbm.org/EuroPlusMed/> [accessed 6 Jun 2019].
- Evanno G., Regnaut S. & Goudet J. 2005: Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. – Molec. Ecol. **14**: 2611–2620.
- Francis R. M. 2017: POPHELPER: an R package and web app to analyse and visualize population structure.

- Molec. Ecol. Resources **17**: 27–32. – Version 2.3.0 at <https://github.com/royfrancis/pophelper>
- Gamisans J. 1972: Contribution à l'étude de la flore de la Corse. IV.: *Leucanthemum corsicum* (Less.) DC.: une espèce mal connue. – *Candollea* **27**: 189–209.
- Gamisans J. 1998: *Leucanthemum* Miller, Gard. Dict. Abr. ed. 4. 1754. – Pp. 278–287 in: Gamisans J. & Jeanmonod D. (ed.), Compléments au prodrome de la Flore Corse. *Asteraceae* I. – Genève: Conservatoire et Jardin botaniques de la ville de Genève.
- Greenwood D. R. 2005: Leaf form and the reconstruction of the past. – *New Phytol.* **166**: 355–357.
- Greiner R., Vogt R. & Oberprieler C. 2012: Phylogenetic studies in the polyploid complex of the genus *Leucanthemum* Mill. (*Compositae*, *Anthemideae*) based on cpDNA sequence variation. – *Pl. Syst. Evol.* **298**: 1407–1414.
- Greiner R., Vogt R. & Oberprieler C. 2013: Evolution of the polyploid north-west Iberian *Leucanthemum pluriflorum* clan (*Compositae*, *Anthemideae*) based on plastid DNA sequence variation and AFLP fingerprinting. – *Ann. Bot. (Oxford)* **111**: 1109–1123.
- Holland B. R., Clarke A. C. & Meudt H. M. 2008: Optimizing automated AFLP scoring parameters to improve phylogenetic reconstruction. – *Syst. Biol.* **57**: 347–366.
- Huson D. & Bryant D. 2017: SPLITSTREE4 (Version 4.14.6). – Version 4.15.1 at <https://software-ab.informatik.uni-tuebingen.de/download/splitstree4/welcome.html>
- Jakobsson M. & Rosenberg N. A. 2007: CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. – *Bioinformatics* **23**: 1801–1806.
- Jeanmonod J. & Gamisans J. 2013: Flora Corsica, ed. 2. – *Bull. Soc. Bot. Centre-Ouest*, num. spéc. **39**: 1–1072.
- Konowalik K., Wagner F., Tomasello S., Vogt R. & Oberprieler C. 2015: Detecting reticulate relationships among diploid *Leucanthemum* Mill. (*Compositae*, *Anthemideae*) taxa using multilocus species tree reconstruction methods and AFLP fingerprinting. – *Molec. Phylogen. Evol.* **92**: 308–328.
- Maugarny-Calès A. & Laufs P. 2018: Getting leaves into shape: a molecular, cellular, environmental and evolutionary view. – *Development* **145**: dev161646.
- Nei M. & Li W. H. 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. – *Proc. Natl. Acad. Sci. U.S.A.* **76**: 5269–5273.
- Oberprieler C. 1998: The systematics of *Anthemis* L. (*Compositae*, *Anthemideae*) in W and C North Africa. – *Bocconeia* **9**: 1–328.
- Oberprieler C., Eder C., Meister J. & Vogt R. 2011: AFLP fingerprinting suggests the allopolyploid origin of two members of the *Leucanthemum vulgare* aggregate (*Compositae*, *Anthemideae*) in Central Europe. – *Nordic J. Bot.* **29**: 370–77.
- Oberprieler C., Greiner R., Konowalik K. & Vogt R. 2014: The reticulate evolutionary history of the polyploid NW Iberian *Leucanthemum pluriflorum* clan (*Compositae*, *Anthemideae*) as inferred from nrDNA ETS sequence diversity and eco-climatological niche-modelling. – *Molec. Phylogen. Evol.* **70**: 478–91.
- Oberprieler C., Konowalik K., Fackelmann A. & Vogt R. 2018: Polyploid speciation across a suture zone: phylogeography and species delimitation in S French *Leucanthemum* Mill. representatives (*Compositae*–*Anthemideae*). – *Pl. Syst. Evol.* **304**: 1141–1155.
- Pritchard J. K., Stephens M. & Donnelly P. 2000: Inference of population structure using multilocus genotype data. – *Genetics* **155**: 945–959.
- Sicard A., Thamm A., Marona C., Lee Y. W., Wahl V., Stinchcombe J. R., Wright S. I., Kappel C. & Lenhard M. 2014: Repeated evolutionary changes of leaf morphology caused by mutations to a homeobox gene. – *Curr. Biol.* **24**: 1880–1886.
- Stuessy T. F. 2009: Plant taxonomy: the systematic evaluation of comparative data, ed. 2. – New York: Columbia University Press.
- Tison J.-M. & de Foucault B. (coord.) 2014: Flora gallica. Flore de France. – Mèze: Biotope.
- Vogt R. 1991: Die Gattung *Leucanthemum* Mill. (*Compositae*–*Anthemideae*) auf der Iberischen Halbinsel. – *Ruizia* **10**: 1–261.
- Vogt R., Konowalik K. & Oberprieler C. 2018: Karyological analysis reveals two new polyploid marguerite taxa (*Leucanthemum*, *Compositae*–*Anthemideae*) in S France and NW Italy. – *Willdenowia* **48**: 221–226.
- Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J. & Kuiper M. 1995: AFLP: a new technique for DNA fingerprinting. – *Nucl. Acids Res.* **23**: 4407–4414.
- Wagner F., Härtl S., Vogt R. & Oberprieler C. 2017: “Fix Me Another Marguerite!”: Species delimitation in a group of intensively hybridizing lineages of ox-eye daisies (*Leucanthemum* Mill., *Compositae*–*Anthemideae*). – *Molec. Ecol.* **26**: 4260–4283.

Willdenowia

Open-access online edition bioone.org/journals/willdenowia



Online ISSN 1868-6397 · Print ISSN 0511-9618 · Impact factor 1.156

Published by the Botanic Garden and Botanical Museum Berlin, Freie Universität Berlin

© 2019 The Authors · This open-access article is distributed under the CC BY 4.0 licence