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Karyological analysis reveals two new polyploid marguerite taxa (*Leucanthemum*, *Compositae*–*Anthemideae*) in S France and NW Italy

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Abstract: New chromosome counts suggest that the former *Leucanthemum vulgare* var. *esterellense* Briq. & Cavill. deserves acknowledgement as an independent species. The new combination *L. esterellense* (Briq. & Cavill) Vogt, Konowalik & Oberpr., proposed here, denotes a marguerite species endemic to the Massif de l'Esterel (S France) with an octoploid chromosome number of $2n = 72$. It is also demonstrated that recently formed hybrids between *L. glaucophyllum* and *L. pallens* at Mt Bignone (Liguria, NW Italy) exhibit hepta- and octoploid chromosome numbers. These hybrids are described as *L. ×marchii* Konowalik, Vogt & Oberpr., a nothospecies new to science. Additionally, the names *L. subglaucum* de Laramb., *L. vulgare* var. *esterellense* Briq. & Cavill. and *L. vulgare* subsp. *glaucophyllum* Briq. & Cavill. are lectotypified and new chromosome counts for all presently surveyed *Leucanthemum* taxa are provided.

Key words: *Anthemideae*, *Asteraceae*, chromosome numbers, *Compositae*, France, hybridization, Italy, lectotypification, *Leucanthemum*, new combination, new nothospecies, polyploidy

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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 (Oberprieler & al. 2011, 2014; Greiner & al. 2012, 2013) level. While working towards an evolution-based taxonomy of ox-eye daisies throughout their natural distribution range in C and S Eu-

rope, we found a new, octoploid chromosome number for populations of a taxon described as *L. vulgare* [subsp. *glaucophyllum*] var. *esterellense* by Briquet & Cavillier (1916) in their treatment of *Compositae* in Émile Burnat's *Flore des Alpes maritimes*. This taxon was putatively assigned to *L. adustum* (W. D. J. Koch) Greml. by Tison & de Foucault (2014). *Leucanthemum adustum* is a species occurring in the W and E parts of the Alps, from which *L. vulgare* var. *esterellense* deviates karyologically (hexaploid vs octoploid), morphologically (leaves somewhat glaucous, involucre bracts less dark margined) and ecologically (growing on acidic instead of calcifer-

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ous soils). Accordingly, a clarification of its evolutionary relationships to other co-distributed *Leucanthemum* species was initiated using karyological, morphological, and molecular sources of evidence. The results of this study are on the way to being published (C. Oberprieler and collaborators, submitted) and support the taxonomic independence of *L. vulgare* var. *esterellense*. The present contribution, however, is intended to communicate chromosome numbers of the study group and to draw the necessary nomenclatural consequences.

Material and methods

The present study comprises chromosome counts for the three varieties of *Leucanthemum vulgare* subsp. *glaucophyllum* (Briquet & Cavillier 1916) (i.e. *L. vulgare* var. *esterellense*, *L. vulgare* var. *glaucophyllum* and *L. vulgare* var. *subglaucum*), along with the co-distributed *L. pallens*, with which mixed stands are formed in some regions. Chromosome numbers were obtained from somatic mitoses of root tips of plants collected in the field and cultivated in the Berlin Botanic Garden or raised from seed. Root tips were pre-treated with hydroxyquinoline (0.002 molar aqueous solution) for 2 hours, fixed in 96% ethanol/glacial acetic acid (3:1) and refrigerated. Hydrolysis was carried out with 1–2N hydrochloric acid for 10–15 minutes at 60°C. For chromosome staining root tips were squashed in aceto-orcin. Chromosome counts were made for several plants of a common seed origin. Five to ten metaphase stages were examined for every plant.

Voucher specimens of the original collections and of plants cultivated in the Berlin Botanic Garden are deposited in B.

Results

Results indicate that the three putative varieties of *Leucanthemum vulgare* subsp. *glaucophyllum* exhibit three different ploidy levels: While the typical *L. vulgare* var. *glaucophyllum* is characterized by a decaploid chromosome number, *L. vulgare* var. *esterellense* was found to be an octoploid, and *L. vulgare* var. *subglaucum* to be a hexaploid. In one of the sampled localities (Monte Bignone near Alassio, Italy), where populations of *L. pallens* (hexaploid) and *L. vulgare* var. *glaucophyllum* were found growing in close proximity, individuals with intermediate ploidy levels were found (heptaploids, octoploids).

Discussion

Following the presently provided karyological evidence, we consider it well supported that the three varieties of *Leucanthemum vulgare* subsp. *glaucophyllum* constitute three different evolutionary lineages that merit acknowl-

edgement as three independent species. While these taxonomic consequences have been drawn already in the cases of the hexaploid *L. vulgare* var. *subglaucum* (through its original description at specific rank as *L. subglaucum* de Laramb.) and the decaploid *L. vulgare* var. *glaucophyllum* (by Jahandiez 1922), raising of the octoploid *L. vulgare* var. *esterellense* to specific rank is proposed here.

Additionally, owing to the observation of recently formed hepta- and octoploid hybrids between the hexaploid *Leucanthemum pallens* and the decaploid *L. glaucophyllum* in areas of joint occurrence (as in the mixed stand on Monte Bignone studied in the present contribution), we propose the name *L. xmarcii* for these karyologically and morphologically intermediate plants. This nothospecies epithet acknowledges P. Marchi, who in Marchi & al. (1983) first reported on hybridization between *L. pallens* and *L. glaucophyllum* and the occurrence of octoploid hybrid individuals in these stands.

1. *Leucanthemum esterellense* (Briq. & Cavill.) Vogt, Konowalik & Oberpr., **comb. & stat. nov.** = *Leucanthemum vulgare* var. *esterellense* Briq. & Cavill. in Burnat, Fl. Alpes Marit. 6: 103. 1916 = *Leucanthemum glaucophyllum* var. *esterellense* (Briq. & Cavill.) Jahand. in Ann. Soc. Hist. Nat. Toulon 7: 40. 1922. – Ind. loc.: “Massif de l’Esterel*: Théoule!! au-dessous de la Sainte-Baume!!, aux Suvières! (Saint-Yves) et ravin de Mourrefrey!!”. – **Lectotype (designated here)**: Deuxième voyage botanique de l’année 1899 dans les Alpes maritimes par Emile Burnat et François Cavillier, Vallon de Mourrefrey, entre le Gratadis et la S^{te} Baume (Esterel), 8 Jun 1899 (G-BU! [G00848001, two-sheet specimen, cf. Turland & al. 2018: Art. 8 Ex. 9]). – Remaining syntypes: Voyage botanique dans les Alpes maritimes de Emile Burnat et François Cavillier, au-dessous de la S^{te} Baume d’Agay (Esterel), 130–170 m, bords du chemin, silice, 1 Jun 1901 (G-BU! [G00848002]); Théoule, près de Cannes, l’Esterel, Alpes Maritimes, 29 May 1884, É. Burnat (G-BU! [G00848003]); Théoule, au pied de l’Esterel, vallon près du tunnel, Alpes Maritimes, 13 Jun 1875, É. Burnat (G-BU! [G00848004, two-sheet specimen]).

Chromosome number — $2n = 72$ (octoploid).

Distribution — S France, where the species is endemic to the Massif de l’Esterel and adjacent areas (Briquet & Cavillier 1916).

New chromosome counts

$2n = 72$: France, Provence-Alpes-Côte d’Azur, Département Var, Fréjus, Domaniale de l’Esterel, rue du Gratadis N of Agay, 43°26.859’N, 06°51.138’E, 18 m, slopes along road, edge of *Quercus ilex* / *Q. suber* woodland, 11 Jun 2011, Vogt 16897 & Oberprieler 10807 (B [B 10 0350137, B 10 0411749]).

- 2n = 72: France, Provence-Alpes-Côte d'Azur, Département Var, Fréjus, Domaniale de l'Esterel, Sainte Baume, near source and along road, 43°27.442'N, 06°53.867'E, 160 m, 11 Jun 2011, *Vogt 16902 & Oberprieler 10812* (B [B 10 0350142]).
- 2n = 72: France, Provence-Alpes-Côte d'Azur, Département Var, Théoule-sur-Mer, E city limits, 43°30.354'N, 06°56.688'E, 50 m, steep slope along road, 11 Jun 2011, *Vogt 16903 & Oberprieler 10813* (B [B 10 0350143]).
- 2n = 72: France, Provence-Alpes-Côte d'Azur, Département Var, Fréjus, Domaniale de l'Esterel, road between Agay and Sainte Baume, near chapelle d'Honarar, 43°27.313'N, 06°53.360'E, 195 m, slopes along road and along source creek, 11 Jun 2011, *Vogt 16898 & Oberprieler 10808* (B [B 10 0350138]).
- 2n = 72: France, Provence-Alpes-Côte d'Azur, Département Var, Fréjus, Domaniale de l'Esterel, road between Sainte Baume and Pic de l'Ours, 43°28.704'N, 06°54.752'E, 315 m, slopes along road, 11 Jun 2011, *Vogt 16901 & Oberprieler 10811* (B [B 10 0350141]).

2. *Leucanthemum glaucophyllum* (Briq. & Cavill.) Jahand. in Ann. Soc. Hist. Nat. Toulon 7: 39. 1922 = *Leucanthemum vulgare* subsp. *glaucophyllum* Briq. & Cavill. in Burnat, Fl. Alpes Marit. 6: 102. 1916 = *Chrysanthemum leucanthemum* var. ["o"] *glaucophyllum* (Briq. & Cavill.) Fiori, Nouv. Fl. Italia 2: 626. 1927 = *Leucanthemum glaucophyllum* (Briq. & Cavill.) Marchi & Illuminati in Ann. Bot. (Roma) 33: 174. 1975, isonym. – Ind. loc.: “Collines, coteaux, rocaillies, des régions littorale et montagnaise inférieure, d'Albenga à Agay.” and “Env. D'Albenga*: Mont Piscivino!, 500 m., mont Bignone!, 500 m., entre Albenga et Garlenda!; versant S. du monte Nero!*** près Zuccarello, 700–800 m.; versant S. du mont Galè!***; vallée de l'Arroscia*: mont Castellerno sur Onzo!, 900–1000 m., mont Sprandega sur Vessalico!, 750 m., mont Riondo, entre Casanova-Lerrone et Vessalico!, 500 m.” – **Lectotype (designated here)**: Voyage botanique de MM. Emile Burnat, J. Briquet, Paul Burnat, A. Saint-Yves, Fr. Cavillier, E. Abrezol et J. Lascaud, dans les Alpes maritimes italiennes et françaises (1^{er}–26 juin 1914), Vallée de l'Arroscia, Mont Castellerno sur Onzo, 900–1000 m, rocaillies, grès-calcaire, 8 Jun 1914 (G-BU! [G00848005]). – Remaining syntypes: Mont Riondo, entre Casanova-Lerrone et Vessalico (Ligurie occid.), 500 m, grès, 13 Jun 1914, *É. Burnat & al.* (G-BU! [G00848007]); Mont Sprandega sur Vessalico, Vallée de l'Arroscia, Ligurie occid., 750 m, rocaillies herbeuses, grès, 12 Jun 1914, *É. Burnat & al.* (G-BU! [G00848006]); Crêtes du monte Nero sur le revers sud, env. de Zuccarello, 700–800 m, 27 Jun 1897, *J. Briquet & F. Cavillier* (G-BU! [G00848008]);

Versant oriental du Mont Galero, extrem. or. des Alpes maritimes, aux env. de Garessio, 24 Jul 1880, *J. J. Vetter & al.* (G-BU! [G00848008; G00848009, four-sheet specimen]); Entre Albenga et Garlenda, Ligurie occid., 11 Jun 1879, *É. Burnat* (G-BU! [G00848010]); Mont Bignone près Albenga, 500 m, rocaillies à l'ubac, silice, 5 Jun 1914, *É. Burnat & al.* (G-BU! [G00848011, two-sheet specimen]); Mont Piscivino près Albenga, 500 m, pentes herbeuses à l'ubac, grès, 5 Jun 1914, *É. Burnat & al.* (G-BU! [G00848012, three-sheet specimen]).

– “*Leucanthemum vulgare* var. *eu-glaucophyllum*” (Briquet & Cavillier in Burnat, Fl. Alpes Marit. 6: 104. 1916, nom inval., Turland & al. 2018: Art. 24.3).

Chromosome number — 2n = 90 (decaploid) (Favarger & Villard 1965, 1966).

Distribution — NW Italy (Liguria), where the species is endemic to the Ligurian Alps (Lo Presti & al. 2018).

New chromosome counts

2n = 90: Italy, Liguria, Province Imperia, Castell'Ermo N of Onzo, 44°05.804'N, 08°03.316'E, 1045 m, meadows between limestone rocks, 13 Jun 2011, *Vogt 16935 & Oberprieler 10842* (B [B 10 0350175]).

2n = 85–90: Italy, Liguria, Province Imperia, Alassio, summit of Monte Bignone above Vegliasco, 44°01.662'N, 08°09.766'E, 580 m, meadows and clearings in forest, 13 Jun 2011, *Vogt 16942 & Oberprieler 10849*, cult. Hort. Bot. Berlin (202-43-11-10), 26 Jun 2014 (B [B 10 0537917, B 10 0537921]).

3. *Leucanthemum x marchii* Konowalik, Vogt & Oberpr., **nothosp. nov.** [= *Leucanthemum glaucophyllum* (Briq. & Cavill.) Jahand. × *L. pallens* (Perreym.) DC.].

Holotype: Italy, Liguria, Province Imperia, Alassio, summit of Monte Bignone above Vegliasco, 44°01.662'N, 08°09.766'E, 580 m, meadows and clearings in forest, 13 Jun 2011, *R. Vogt 16942 & C. Oberprieler 10849*, cult. Hort. Bot. Berlin, 8 Jul 2014 (B [B 10 0543018]).

Diagnosis — In terms of morphological characters with light brown bordered involuclal bracts and ploidy levels of 7x / 8x (chromosome counts) and 9x (flow cytometry, C. Oberprieler and collaborators, submitted) intermediate between *Leucanthemum glaucophyllum* (Briq. & Cavill.) Jahand. (involuclal bracts with dark brown margins; 10x) and *L. pallens* (Perreym.) DC. (involuclal bracts pale; 6x).

Chromosome number — 2n = 63, 72 (heptaploid, octoploid) (Marchi & Illuminati 1974).

Distribution — NW Italy; hitherto observed only on the summit of Monte Bignone (Alassio).

Eponymy — This new nothospecies is named in honour of Dr. Palmer Marchi (Rome) in acknowledgement of his contributions to the knowledge of the genus *Leucanthemum* in Italy. He ascertained the chromosome number of this taxon for the first time and recognized its hybrid origin.

New chromosome counts

$2n = c. 63$: Italy, Liguria, Province Imperia, Alassio, summit of Monte Bignone above Vegliasco, 44°01.662'N, 08°09.766'E, 580 m, meadows and clearings in forest, 13 Jun 2011, *Vogt 16942 & Oberprieler 10849* (B [B 10 0350182, B 10 0350183]).

$2n = 72$: Italy, Liguria, Province Imperia, Alassio, summit of Monte Bignone above Vegliasco, 44°01.662'N, 08°09.766'E, 580 m, meadows and clearings in forest, 13 Jun 2011, *Vogt 16942 & Oberprieler 10849*, cult. Hort. Bot. Berlin (202-43-11-10), 8 Jul 2014 (B [B 10 0543018]), 26 Jun 2014 (B [B 10 0537918]).

4. *Leucanthemum subglaucum* de Laramb. in Soc. Litt. Sci. Castres 4: 446. 1861 $\equiv$ *Leucanthemum maximum* subsp. *subglaucum* (de Laramb.) Nyman, Consp. Fl. Eur. Suppl. 2: 169. 1889 $\equiv$ *Leucanthemum vulgare* var. *subglaucum* (de Laramb.) Rouy, Fl. France 8: 273. 1903 $\equiv$ *Leucanthemum vulgare* subsp. *subglaucum* (de Laramb.) Bonnier, Fl. Ill. France 5: 95. 1922 $\equiv$ *Chrysanthemum subglaucum* (de Laramb.) Braun-Blanq. in Commun. Stat. Int. Géobot. Médit. Montpellier 20: 312. 1933. – Ind. loc.: “Il habite les rochers les plus élevés au sommet de la montagne boisé, qui domine les ruines de l’ancien ermitage de Burlats, près Castres (Tarn), [...] Nous l’avons observé pour la première fois en compagnie de M. Contié, en juin 1859.” – **Lectotype (designated here)**: Burlas [Burlats] près Castres (Tarn), rochers granitiques, 14 Jun 1860, *de Larambergue* (P! [P00729951]).

– “*Leucanthemum candolleianum*” (Martrin-Donos, Pl. Crit. Tarn: 29. 1862, nom. inval., pro syn., Turland & al. 2018: Art. 36.1(b)).

– “*Chrysanthemum leucanthemum* subsp. *subglaucum*” (Guinochet & Vilmorin, Fl. France 4: 1451. 1982, nom. inval., Turland & al. 2018: Art. 41.5).

Chromosome number — $2n = 54$ (hexaploid) (Favarger 1975).

Distribution — S France (Massif Central), where the species is endemic to the Cevennes and the Montagne noire (Tison & de Foucault 2014).

Remarks — The sole available specimen collected prior to publication of the name *Leucanthemum subglaucum* is selected as as the lectotype.

New chromosome counts

$2n = 54$: France, Languedoc-Roussillon, Département Hérault: Bédarieux – Lodève, road between Laval de Nize and road D 35, 43°42'44.8"N, 03°14'07.5"E, 430 m, slopes along road and roadside, 31 May 2010, *Vogt 16690, Oberprieler 10645 & Konowalik* (B [B 10 0464681, B 10 0464682]).

$2n = 54$: France, Languedoc-Roussillon, Département Hérault: Lodève, Roqueredonde – Le Clapier, road D 142E c. 500 m NW of bridge over river Orb, 43°48'56.8"N, 03°10'28.4"E, 535 m, rocks and roadside, 1 Jun 2010, *Vogt 16695, Oberprieler 10650 & Konowalik* (B [B 10 0464630]).

$2n = 54$: France, Languedoc-Roussillon, Département Gard: Cévennes, Alès, Anduze, D 907 c. 2 km N of Anduze, 44°04'24.4"N, 03°58'00.5"E, 170 m, slopes along road, 2 Jun 2010, *Vogt 16721, Oberprieler 10676 & Konowalik* (B [B 10 0464613]).

5. *Leucanthemum pallens* (Perreym.) DC., Prodr. 6: 47. 1838 $\equiv$ *Chrysanthemum pallens* Perreym. in Arch. Bot. (Paris) 2: 545. 1833; Pl. Phan. Fréjus: [91]. 1833 $\equiv$ *Chrysanthemum montanum* var. *pallens* (Perreym.) Mutel, Fl. Franç. 2: 154. 1835 $\equiv$ *Tanacetum pallens* (Perreym.) Sch. Bip., Tanacetee: 35. 1844 $\equiv$ *Leucanthemum vulgare* subsp. *pallens* (Perreym.) Bonnier & Layens, Tabl. Syn. Pl. Vasc. France: 166. 1894 $\equiv$ *Pontia pallens* (Perreym.) Bubani, Fl. Pyren. 2: 223. 1900 $\equiv$ *Leucanthemum atratum* subsp. *pallens* (Perreym.) Rouy, Fl. France 8: 269. 1903 $\equiv$ *Chrysanthemum leucanthemum* var. [“*δ*”] *pallens* (Perreym.) Fiori in Fiori & Béguinot, Fl. Italia 3: 240. 1903 $\equiv$ *Leucanthemum vulgare* “*raç.*” *pallens* (Perreym.) Samp., Lista Esp. Herb. Port.: 132. 1913 $\equiv$ *Chrysanthemum leucanthemum* subsp. *pallens* (Perreym.) Braun-Blanq. in Bull. Acad. Int. Géogr. Bot. 28: 44. 1918. – Ind. loc.: “Esterel, le long de la route; juin”. – Holotype: Esterel, Perreymond misit Octobr. 1832, Herb. J. Gay (K! [K000929449]).

Chromosome number — $2n = 54$ (hexaploid) (Favarger & Villard 1965, 1966; Marchi 1972; Marchi & Illuminati 1974; Ritter 1974; Favarger 1975; Marchi & al. 1983; D’Ovidio 1986; Vogt 1991).

Distribution — NE Spain, S France, N Italy (Vogt 1991; Tison & de Foucault 2014; Lo Presti & al. 2018).

Remarks — This species displays a considerable resemblance to the tetraploid taxon *Leucanthemum ircuitianum* subsp. *leucolepis* (Briq. & Cavill.) Vogt & Greuter, which is distributed in C and N Italy and in the Balkan Peninsula (Slovenia, Croatia, Bosnia and Herzegovina, Serbia). For a final decision on the delimitation of these two taxa, a more detailed study based on a

denser sampling on the Apennine and Balkan Peninsulas is necessary.

The name and description of *Chrysanthemum pallens* Perreym. were provided (in litt.) by Jacques Étienne Gay, who examined herbarium specimens sent to him by Jean Honoré Perreymond. Gay's handwritten description is attached to the holotype specimen in his personal herbarium, which is kept in K ([K000929449]).

New chromosome counts

- 2n = 54: France, Provence-Alpes-Côte d'Azur, Département Alpes-Maritimes, valley of river Roya W of La Giandola on road to Col de Brouis, 43°57.206'N, 07°30.932'E, 365 m, road embankment and olive grove, 10 Jun 2011, Vogt 16882 & Oberprieler 10792 (B [B 10 0411733, B 10 0411734]).
- 2n = 54: France, Provence-Alpes-Côte d'Azur, Département Alpes Maritimes, Nizza – Menton, highway toll station near Carpre, 43°44.684'N, 06°22.820'E, 310 m, slopes, 11 Jun 2011, Vogt 16904 & Oberprieler 10814 (B [B 10 0350144]).
- 2n = 54: Italy, Liguria, Province Imperia, Molini di Triora – Pigna, E of Colla di Langan, 43°58.980'N, 07°45.602'E, 690 m, along tracks in *Castanea sativa* grove, 12 Jun 2011, Vogt 16910 & Oberprieler 10817 (B [B 10 0350147]).
- 2n = 54: Italy, Liguria, Province Imperia, Molini di Triora – Rezzo, 44°00.744'N, 07°50.524'E, 895 m, roadsides in forest, 13 Jun 2011, Vogt 16922 & Oberprieler 10829 (B [B 10 0350160]).
- 2n = 54: Italy, Liguria, Province Imperia, road between Onzo and Castell'Ermo, 44°04.481'N, 08°02.946'E, 530 m, roadside, 13 Jun 2011, Vogt 16939 & Oberprieler 10846 (B [B 10 0350179]).
- 2n = 54: Italy, Liguria, Province Imperia, Alassio, summit of Monte Bignone above Vegliasco, 44°01.662'N, 08°09.766'E, 580 m, meadows and clearings in forest, 13 Jun 2011, Vogt 16941 & Oberprieler 10848 (B [B 10 0350181]).
- 2n = 54: France, Languedoc-Roussillon, Département Aude, Carcassonne, Montagne d'Alaric, track (GR77) between Moux and Roc de L'Aigle, c. 500 m above ruin of St. Pierre d'Aleric, 43°09'35.0"N, 02°37'49.5"E, 250 m, *Quercus* woodland, 30 May 2010, Vogt 16644, Oberprieler 10595 & Konowalik (B [B 10 0464664, B 10 0464665, B 10 0464666, B 10 0464667, B 10 0464668, B 10 0464669, B 10 0464670, B 10 0464671, B 10 0464672, B 10 0464673]).
- 2n = 54: France, Languedoc-Roussillon, Département Aude, Carcassonne, Montagne d'Alaric, track (GR77) between Moux and Roc de L'Aigle, below ruin of St. Pierre d'Aleric, 43°09'55.2"N, 02°38'21.0"E, 235 m, *Quercus* woodland, 30 May 2010, Vogt 16666, Oberprieler 10617 & Konowalik (B [B 10 0464662]).

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