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DCDB: an updated online database of chromosome numbers of tribe *Delphinieae* (*Ranunculaceae*)

Abstract

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A new version of the earlier chromosome database of tribe *Delphinieae* is presented (v. 2.0, updated 23/4/2016 and available online at http://hdl.handle.net/2445/98702), after an accurate extensive literature and Internet survey, by adding the chromosome counts for the genera *Aconitum* L. [including *Gymnaconitum* (Stapf) Wei Wang & Z. D. Chen], *Delphinium* L. (including *Staphisagria* Spach), *Consolida* (DC.) S. F. Gray and *Aconitella* Spach, accumulated in the last 17 years.

A total number of 2598 reports are presented, belonging to 389 species and to a total of 467 taxa, and representing 44.5% of the total species number of the tribe (an increase of c. 137% compared with the 1097 reports gathered in the 1999 version). This increase is due both to chromosome research progress (analysed as counts/year) and an improved information capture system (including checking of populations location through Cyrillic alphabet, and Japanese and Chinese writing systems). Additionally, recent taxonomic advances, synonimization and new phylogenetic criteria have also been taken into account.

The main basic number $x = 8$ is found at $2x$, 3x, $4x$, $5x$, $6x$, and $8x$ ploidy levels, whereas x = 9 is much rarer. Polyploidy is more frequent in perennial taxa (*Aconitum* and *Delphinium* s. str.) whereas in annuals, disploidy (both increasing and decreasing) takes more importance and should be considered as a source of new evolutionary opportunities. The most frequent counts are $2n = 16$ and 32, but counts of $2n = 12$, 14, 17, 18, 20, 24, 26, 28, 30, 34, 40, 46, 48, 52, and 64 have also been recorded. In 20 species more than one different chromosome number has been reported and 81 species showed different ploidy levels.

The *Delphinieae* Chromosome Database (DCDB) provides the most complete current available information on chromosome numbers of *Delphinieae*, yearly updated, and aimed to be useful for general building of cytotaxonomical databases and for specific research ongoing projects of systematics of *Ranunculaceae*. It is based in MsAccess/MsExcel software, and includes three levels of taxonomic resolution (published name, database accepted name, and Plant List standard name), and the geographic origin of each count (country, and population when provided in the original source).

Key words: karyology, *Aconitum*, *Delphinium*, *Consolida*.

* Extended and enriched version of the poster presentation given at the XV Optima meeting in Montpellier, 6-11 June 2016.

Introduction

The tribe *Delphinieae* Warming is a large group within the *Ranunculaceae*, traditionally including four genera: *Aconitum* L., *Delphinium* L., *Consolida* (DC.) S. F. Gray, and *Aconitella* Spach. Recent advances in phylogeny (including nuclear and chloroplast DNA sequencing) proposed a better arrangement of previously recognized groups: *Aconitella* forms a clade embedded in *Consolida* whereas the wide *Consolida* is nested within *Delphinium* (Jabbour & Jenner 2011a, 2012), and *Staphisagria* J. Hill is restated as an independent genus (Jabbour & Jenner 2011b). More recently, the whole tribe *Delphinieae* has been confirmed as monophyletic within the subfamily *Ranunculoideae* after an analysis with multiple molecular markers (Cossard & al. 2016). However, as new major/medium taxonomic rearrangements seem still necessary/possible [i.e., recognizing a new subgenus for the *Delphinium anthriscifolium* group, looking for a new placement for *Aconitum fletcherianum* G. Taylor—its recent accommodation in a new sect. *Fletcherum* by Tamura (1996) or in a new subgenus *Tangutica* by Kadota (2001) are considered not definitive (see Hong & al. 2016)—or a clarified status of *Aconitum* subg. *Gymnaconitum* (Stapf) Rapaics, as well as some ongoing verification of the *D. staphisagria* group position, among others], a complete taxonomic framework is not fully established.

The tribe *Delphinieae* contains about 875 species primarily distributed in cold and temperate areas of the Northern Hemisphere (Greuter & al. 1989; Tamura 1995; the estimated total species number is reduced to 700 following Jabbour & Jenner 2012). Representatives are found in Africa, America, Asia, and Europe. The genus *Aconitum* and most species of *Delphinium* are perennials and geophytes. The remaining species of *Delphinium* and *Staphisagria* are annuals or biennials (including sect. *Delphinium*, sect. *Anthriscifolium* W. T. Wang and the entire formerly considered genera *Consolida* and *Aconitella*, which form a relatively small group of taxa mainly restricted to the Mediterranean and Irano-Turanian regions; Simon & al. 1999; Jabbour & Jenner 2011a). *Aconitum* (s. l.) traditionally included a single annual species, the Tibetan endemic *A. gymnandrum* Maxim.; in order to preserve the monophyly of *Aconitum*, Wang & al. (2013) removed this species from the genus and placed it within its own genus, *Gymnaconitum* (Stapf) Wei Wang & Z. D. Chen. In the present state of knowledge, thus, the tribe is composed by two large genera (*Aconitum*, *Delphinium*) and two minor ones (*Staphisagria* and *Gymnaconitum*) although new approaches are not unexpected in the near future. To be useful in the current situation, a three column system is provided to make easier the finding of a given searched name under the present version of the DCDB database.

Material and methods

Chromosome data have been gathered after an accurate review of published reports both on paper and electronic support. As far as possible, the original source has been directly checked out and is currently kept at our department, available upon request.

The collected data include the previous information presented in Simon & al. (1999) plus the results of the extensive search of reports published since 1999 to date, as well as the newly verified counts given in old publications. The current version of DCDB is updat-

ed up to April 23, 2016, just the day after the online publication of the significant paper of Hong & al. (2016) that comprised 60 new chromosome reports from 20 species of *Aconitum* subg. *Lycoctonum*, including a new basic number ($x = 6$; $2n = 12$). These very recent contributions have also been incorporated to the *Delphinieae* database.

DCDB is expected to be updated with a c. 1-year frequency.

The structure and composition of fields in the DCDB follows the system presented in Simon & al. (1999), with some modifications, as summarized below:

Published name – The scientific name under which the report has been published in the referenced source.

The Plant List name – Taxonomic standard given by *The Plant List* online platform (www.theplantlist.org), which is increasingly accepted by the botanical community as reference system since its launching in 2014. In the case of tribe *Delphinieae*, significant deviations from the published name have been detected, both by disagreements at low taxonomic ranks (i.e., subspecies and varieties recognized or not) and by changes at genus level (e.g., *Aconitella* or *Consolida* as separate genera or within a wide genus *Delphinium* s. l. resulting in different nomenclatural combinations). The conversion to the Plant List system will help browsing of *Delphinieae* chromosome database to non-specialist users but must be considered merely as an auxiliar indexation tool, and not a true consensus treatment.

Consensus name – For most taxa, the Plant List names are coincident with those adopted by the current compilation of chromosome databases called *Chromosome Counts Database* (CCDB, Rice & al. 2015), but not in all cases due to taxonomic different conceptions. In addition, a number of recently new proposed taxa are not recognized under CCDB standards [e.g., *Staphisagria macrocarpa* Spach—the name adopted by Jabbour & Jenner (2011b)—is listed under its classic basyonym *Delphinium staphisagria* L.]. Moreover, in certain groups our criteria on taxa delimitation are not fully coincident with those of the Jabbour & Jenner (2012) system nor with those of the Plant List, particularly regarding W Mediterranean or E Asian (Chinese, Japanese, Korean) species, for which treatments suggested by authors such as Kadota, Warnock, Hong or Wang (see full electronic version of DCDB) merit to be retained. In summary, while a stable and complete taxonomic system is not still available, a provisional working consensus list is needed and it is a third alternative to find a given report; following this DCDB provisional checklist, the number of recognized species is 875, and the total number of listed taxa is 1005. A supplementary and separate list includes published reports for *Delphinieae* of uncertain identity (mainly garden forms, induced mutants, artificial hybrids) and will be published in further updated versions.

Reports – The chromosome numbers are presented as published (n or $2n$). Very few reports are estimations of chromosome numbers from DNA values; when well established and fully documented, they have been also listed in DCDB (and noted in the "Observations" column).

Population, Country – In this 2016 version of our database, the effort of obtaining primary sources helped us to check the geographic origin of counts, otherwise in other compilations it can be only estimated from article titles (and only if the country or location is included in the title!). Now the origin has been established in terms of (i) population identification and (ii) country (state and, if possible substate or main geographic units).

This makes possible to identify the number of populations truly studied (in some cases, a single report in a given table of a standard database means, in fact, up to 60 analysed populations, which is informative of significant chromosome knowledge, for instance on variation levels of karyotype structure); when possible, the number of populations studied is provided (an independent line for each population) and the geographic origin (at country level and, when needed, substate entity) is given in the column.

Bibliographic reference – In abbreviated form. The complete citation is given in the "References" section.

Observations – A last column of open content is devoted to notes, remarks or observations of very different nature. They include, among others, precisions on the followed author for taxonomic criteria (if discrepancies concerning the consensus name exist), details on studied population identification or location, and comments on confidence of a given count (mainly coming from hybrids or garden forms).

Technically, the *Delphinieae* database is working in a Microsoft© environment (MsAccess and MsExcel). In further updates, a 3.0 version running over Dreamweaver© software will allow an easier browsing experience and will replace the current static interface.

Chromosome data provided by this DCDB database are available directly from the *Dipòsit Digital* (Digital Repository) of the Universitat de Barcelona in a complete format (http://hdl.handle.net/2445/98702). Simultaneously, DCDB will contribute to the resources of CCDB and will be searchable through the amount of data offered by this platform (http://ccdb.tau.ac.il/).

Results and discussion

A total number of 2598 reports have been gathered and included in the 2016 version of DCDB (listed in Appendix 1), belonging to 389 species (representing about 44.5% of the species of the tribe) and to a total of 467 taxa (46.7% of taxa). These figures mean an increase of c. 137% compared with the 1097 reports captured in the 1999 version.

The coverage degree of chromosome number knowledge varies from 39% (genus *Delphinium*) to 100% (genus *Gymnaconitum*) (Fig. 1).

Historical evolution of chromosomal knowledge in Delphinieae – The first chromosome reports recorded from *Delphinieae* are as old as the last quarter of the 19th century and belong to *Delphinium ajacis* L. [≡ *Consolida ajacis* (L.) Schur], an annual species widely used in gardening since long time ago, from which Guignard (1889) counted $n = 12$. This report was followed by the studies devoted to *Aconitum napellus* L. by Overton (1893, $2n = 24$ counts, trying to verify if the chromosome number was the same in all tissues) and, some years later, by Osterwalder (1898, $2n = 24$). In all three cases, the articles belong to the early days of cytology, and were oriented to the field of reproductive biology/embryology, at that time a part of an emerging plant physiology, although published in botanical journals from France, England or Germany (*Bulletin de la Societé Botanique de France*, *Annals of Botany*, or *Flora*). These counts date to even earlier than the commonly accepted putative discovery of a constant species-specific chromosome number by Strasburger (1910; *cf.* Peruzzi & Altinordu 2014).

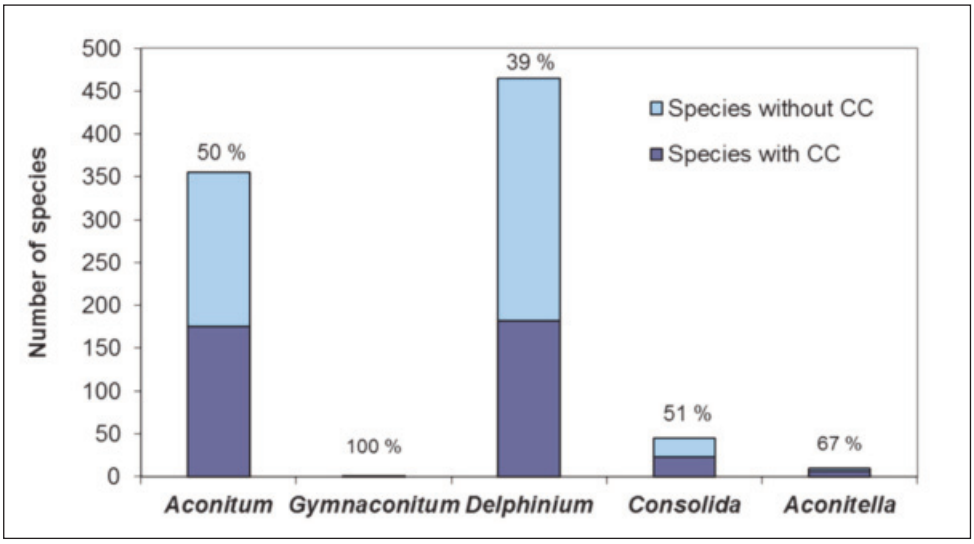

Fig. 1. Number and percentage of species counted per genus in the tribe *Delphinieae* (CC: chromosome count).

The contributions of these precursors were reviewed by Tjebbes (1927) who found the reports of $n = 12$ for *D. ajacis* as a wrong interpretation of V-shaped migrating chromosomes. He demonstrated $n = 8$ in his plates from pollen meiosis, helping to establish since then $x = 8$ as the main basic number, and a characteristic typical karyotype with a first long, submetacentric chromosome pair, for tribe *Delphinieae.*

Figure 2 depicts the evolution of the number of counts reported by year periods and gives us a good picture of the progression of chromosome number research in tribe *Delphinieae*, since the first historical report of Guignard more than 125 years ago. After a dramatic increase by the 1920–1940 authors, and some plain years around WWII, the line follows constantly upwards and has not reached the asymptotic profile yet, thus suggesting still active research in our days.

This increase is due both to chromosome research progress (analysed as counts/year) and an improved information capture system (including checking of populations location through Cyrillic alphabet, and Japanese and Chinese writing systems). Additionally, recent taxonomic advances, synonimization and new phylogenetic criteria have also been taken into account.

If we represent the number of yearly published articles along the same 1889–2016 period (Fig. 3), several major peaks can be identified, corresponding to the most productive authors and papers. Such most numerically significant contributions are those by Propach (1940, 61 counts), Lewis & al. (1951, 299 counts), Kadota (1987, 124 counts), Warnock (1995, 84 counts), Yang (2001, 32 counts), Yuan & Yang (2008, 93 counts), and the relevant contribution of Hong & al. (2016, 60 counts).

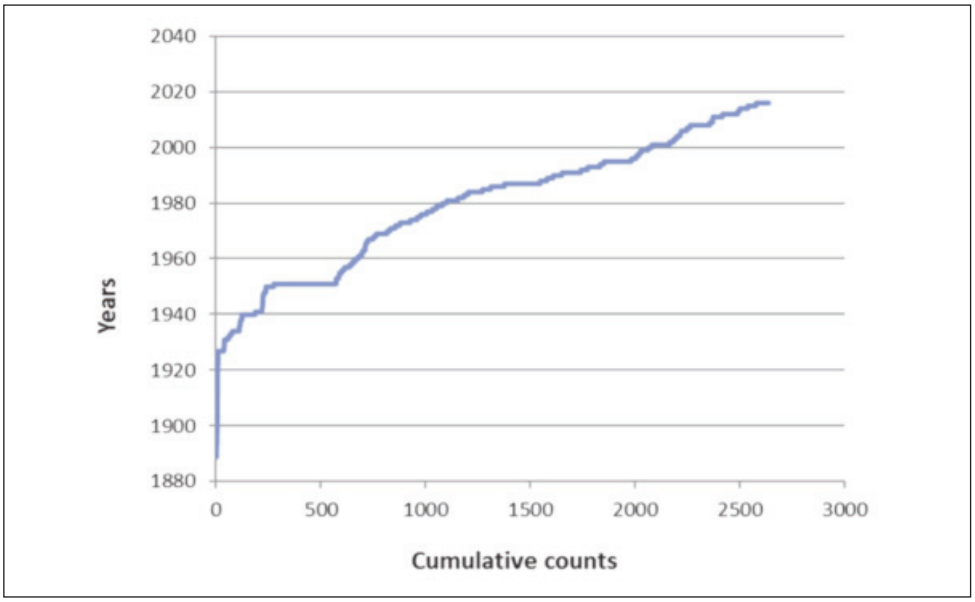

Fig. 2. Historical evolution of cumulative number of chromosome counts published on tribe *Delphinieae* (1889–2016) from data stored in DCDB.

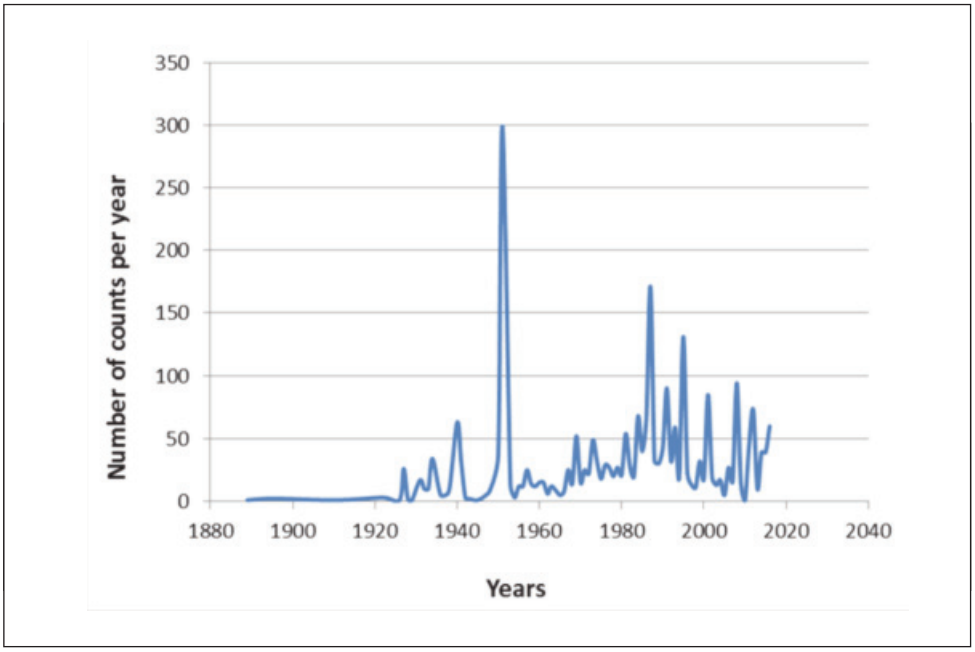

Fig. 3. Number of published papers containing chromosome counts per year in tribe *Delphinieae* (1889-2016) from data stored in DCDB. Peaks denote significant contributions (see text).

Geographic origin of chromosome counts – The geographic location of chromosome counts stored (Fig. 4) fits with the diversity of *Delphinieae* species in a given area together with the situation of the main research teams devoted to this study area: USA (18.8%), China (16%), Russia (13.9%), Japan (8.1%), and the Mediterranean Basin (12.6% including data from Catalan Countries, Spain, Morocco, Turkey, France, Greece, Italy, etc.) are the most represented regions in the DCDB records. Six percent of reports give no clear indication of origin—many of them on material from botanic gardens—and 1% needs to be confirmed.

Chromosome numbers, polyploidy and dysploidy – The number of chromosome counts reported for each listed species varies from one to more than 100, including both haploid and diploid counts. The most studied taxa are *Aconitum septentrionale* Koelle (117 counts), *Delphinium elatum* L. (67 counts), *Delphinium hansenii* (Greene) Greene (66 counts), and *Consolida ajacis* (L.) Schur (49 counts). The most frequent counts found in *Delphinieae* are $2n = 16$ and 32, but reports of $2n = 12$, 14, 17, 18, 20, 24, 26, 28, 30, 34, 40, 46, 48, 52, and 64 have also been recorded. In 20 species more than one different chromosome number have been reported; the species with more different chromosome numbers is *Aconitum palmatum* D. Don ($2n = 30$, 32, 46, 48, and 52).
The main basic number $x = 8$ is found at $2x$, $3x$, $4x$, $5x$, $6x$, and $8x$ ploidy levels, whereas $x = 6$, 7, 9, 10, and 13 are much rarer and reported for a reduced number of species (Table 1 and Figs. 5 and 6).

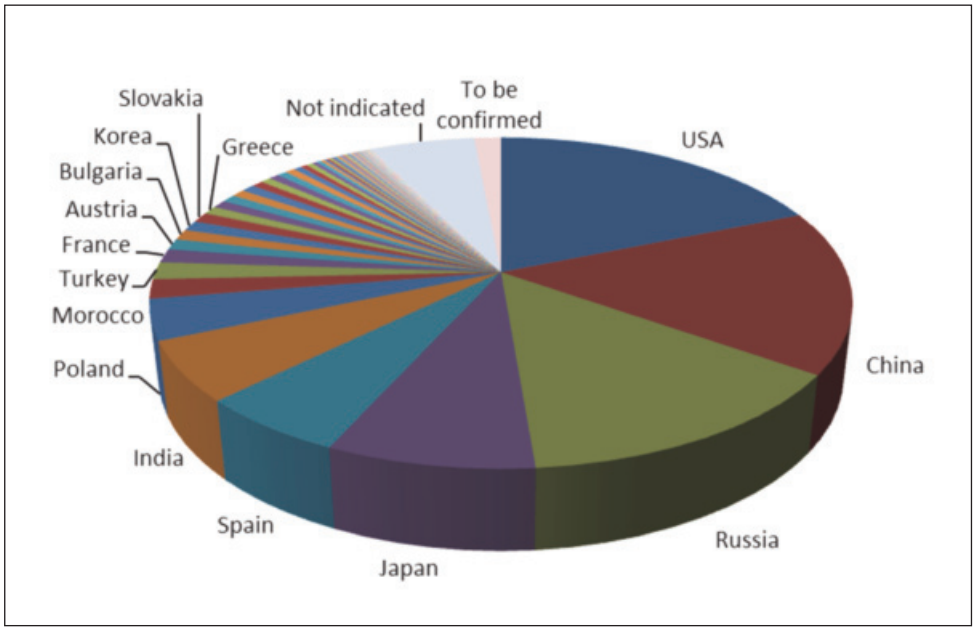

Fig. 4. Top-15 countries with more chromosome number reports of tribe *Delphinieae* from data stored in DCDB.

Table 1. Basic numbers and chromosome numbers in the tribe *Delphinieae* from data stored in DCDB.

GENUS	BASIC NUMBER (*x*)	CHROMOSOME NUMBER (2*n*)
Aconitum	6*, 8 , 9*, 10*, 13*	12, 16, 17, 18, 20, 24, 26, 28, 30, 32, 34, 40, 46, 48, 52, 64
Gymnaconitum	8	16
Delphinium	8, 9*, 10*	16, 18, 20, 24, 32, 48
Consolida	7*, 8, 9*, 10*	14, 16, 18, 20, 24
Aconitella	6*, 8, 9*	12, 16, 18

* uncommon

Polyploidy is more frequent in perennial taxa (*Aconitum* and *Delphinium* s. str.) but dysploidy (both increasing and decreasing) takes more importance (Figs. 5 and 6) in annuals and should be considered as a source of new evolutionary opportunities. Up to 81 species showed different ploidy levels.
B-chromosomes have been only found in *Aconitum* (in 17 species). Some rare numbers, especially in perennials, are coming from anomalous, experimental or ornamental plants and some other deviating counts are doubtful or coming from very old literature.

Concluding remarks – This compilation provides scientists interested in tribe *Delphinieae* convenient access to the chromosome numbers of species published to date, and contains updated information allowing direct consultation through the digital repository of the

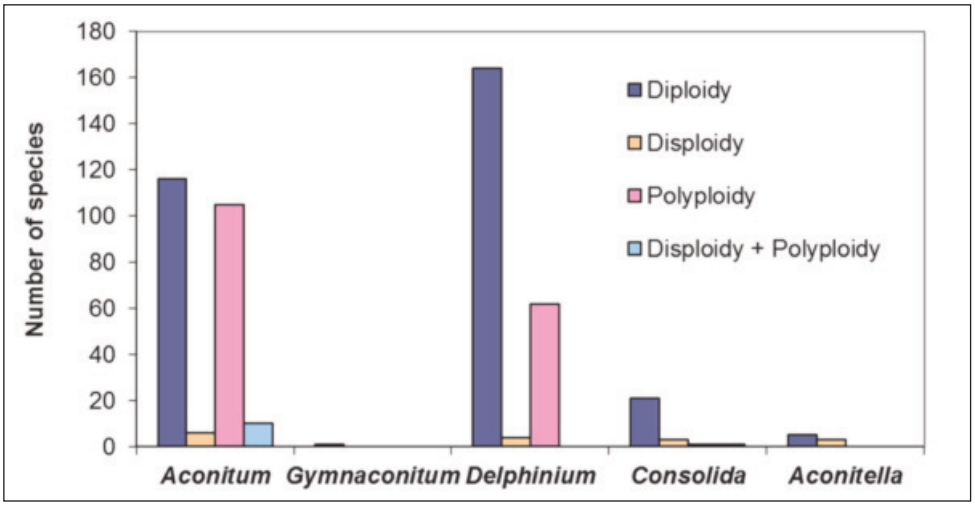

Fig. 5. Number of species with diploidy, dysploidy, polyploidy, or dysploidy + polyploidy per genus in the tribe *Delphinieae*.

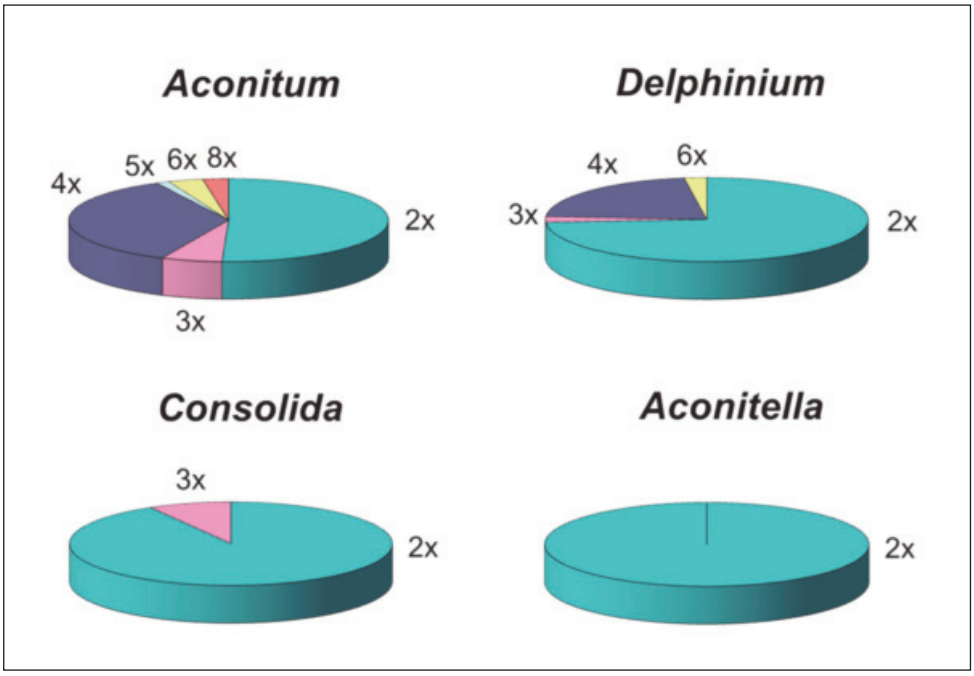

Fig. 6. Polyploidy levels per genus in the tribe *Delphinieae* (*Gymnaconitum* is not represented because it contains only one diploid species).

University of Barcelona. It places the tribe *Delphinieae*, with 2589 counts, within the taxonomic under-family level plant groups with available online chromosome database as the genus *Cardamine* (*Brassicaceae*, 2966 counts, Kučera & al. 2005), the genus *Hieracium* (*Compositae*, 356 records, Schuhwerk 1996) or the tribe *Alysseae* (*Brassicaceae*, 780 records, Spaniel & al. 2015).

The current state of knowledge is presented and the gaps that need further research are made evident. Although counts have been reported for about 44% of *Delphinieae* species, some of the single counts need confirmation, specially dysploid and rare numbers. The remaining 56% of species needs to be investigated. We hope this report will stimulate an interest in additional cytological and taxonomical studies that will contribute to elucidation of nature of the species in this large tribe. The online version of the DCDB data base containing the Chromosome counts in the tribe *Delphinieae* reported in the literature (1889–2016) with references is freely available under a CC BY-NC-ND 3.0 ES license at Dipòsit Digital de la Universitat de Barcelona [URI: http://hdl.handle.net/2445/98702]

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