

# Sinfonevada: Dataset of Floristic diversity in Sierra Nevada forests (SE Spain)

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## Resource Citation

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## Abstract

The Sinfonevada database is a forest inventory that contains information on the forest ecosystem in the Sierra Nevada mountains (SE Spain). The Sinfonevada dataset contains more than 7,500 occurrence records belonging to 270 taxa (24 of these threatened) from floristic inventories of the Sinfonevada Forest inventory. Expert field workers collected the information. The whole dataset underwent a quality control by botanists with broad expertise in Sierra Nevada flora. This floristic inventory was created to gather useful information for the proper management of *Pinus* plantations in Sierra Nevada. This is the only dataset that shows a comprehensive view of the forest flora in Sierra Nevada. This is the reason why it is being used to assess the biodiversity

in the very dense pine plantations on this massif. With this dataset, managers have improved their ability to decide where to apply forest treatments in order to avoid biodiversity loss. The dataset forms part of the Sierra Nevada Global Change Observatory (OBSNEV), a long-term research project designed to compile socio-ecological information on the major ecosystem types in order to identify the impacts of global change in this area.

### **Keywords**

Sierra Nevada, Spain, floristic inventories, vascular plant, Liliopsida, Magnoliopsida, global-change monitoring, occurrence, observation

## **Project details**

### **Project title**

Sierra Nevada Global Change Observatory (OBSNEV)

### **Personnel**

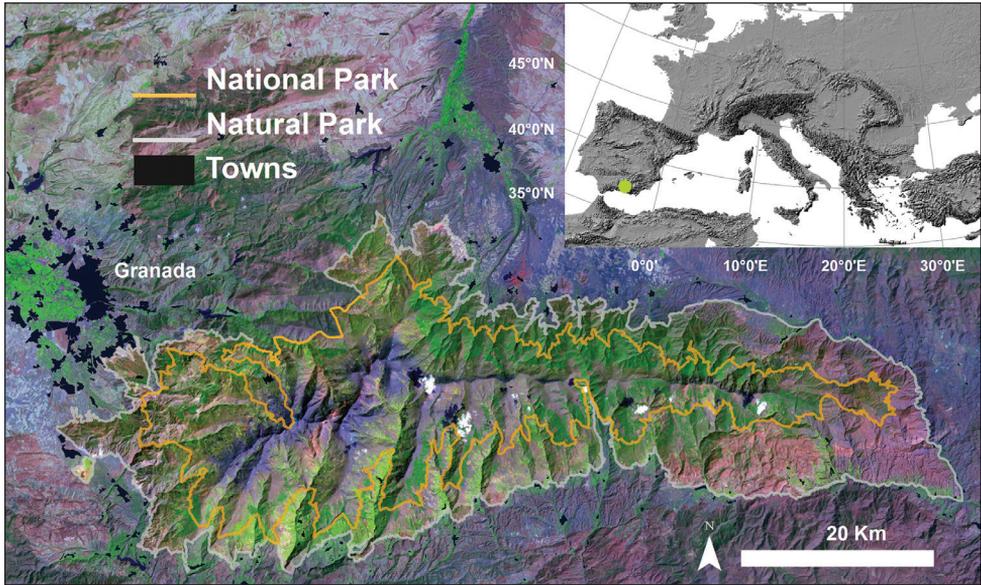
Regino Jesús Zamora Rodríguez (Principal Investigator)

### **Funding**

All the information contained in Sinfonevada was gathered by TRAGSA (Transformación Agraria S.A.), a public company funded by the Spanish Ministry of the Environment. The Sierra Nevada Global Change Observatory is funded by the Andalusian Regional Government (via Environmental Protection Agency) and by the Spanish Government (via “Fundación Biodiversidad”, which is a Public Foundation).

### **Study area descriptions/descriptor**

Sierra Nevada (Andalusia, SE Spain), is a mountainous region with an altitudinal range between 860 m and 3482 m a.s.l. covering more than 2000 km<sup>2</sup> (Figure 1). The climate is Mediterranean, characterized by cold winters and hot summers, with pronounced summer drought (July-August). The annual average temperature decreases in altitude from 12–16°C below 1500 m to 0°C above 3000 m a.s.l., and the annual average precipitation is about 600 mm. Additionally, the complex orography of the mountains causes strong climatic contrasts between the sunny, dry south-facing slopes and the shaded, wetter north-facing slopes. Annual precipitation ranges from less than 250 mm in the lowest parts of the mountain range to more than 700 mm in the summit areas. Winter precipitation is mainly in the form of snow above 2000 m of altitude. The Sierra Nevada mountain range hosts a high number of endemic plant species



**Figure 1.** Location of Sierra Nevada mountain (southern Spain). The boundaries of the National and Natural Parks are shown. We used a Landsat 5 image (2001) as background.

(c. 80; Lorite et al. 2007) for a total of 2,100 species of vascular plants (25% and 20% of Spanish and European flora, respectively), being considered one of the most important biodiversity hotspots in the Mediterranean region (Blanca et al. 1998).

This mountain range has several legal protections: Biosphere Reserve MAB Committee UNESCO; Special Protection Area and Site of Community Importance (Natura 2000 network); and National Park. The area includes 61 municipalities with more than 90,000 inhabitants. The main economic activities are agriculture, tourism, cattle raising, beekeeping, mining, and skiing (Bonet et al. 2010).

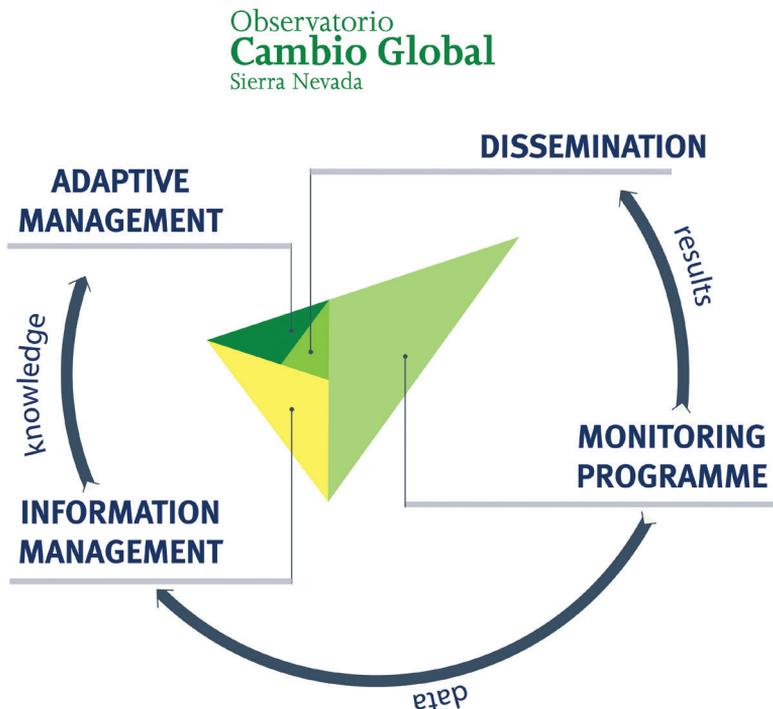
## Design description

Sierra Nevada Global Change Observatory (OBSNEV) (Bonet et al. 2011) is a long-term research project which is being undertaken at Sierra Nevada Biosphere Reserve (SE Spain). It is intended to compile the information necessary for identifying as early as possible the impacts of global change, in order to design management mechanisms to minimize these impacts and adapt the system to new scenarios (Aspizua et al. 2010, Bonet et al. 2010). The general objectives are to:

- Evaluate the functioning of ecosystems in the Sierra Nevada Nature Reserve, their natural processes and dynamics over a medium-term timescale.
- Identify population dynamics, phenological changes, and conservation issues regarding key species that could be considered indicators of ecological processes.

- Identify the impact of global change on monitored species, ecosystems, and natural resources, providing an overview of trends of change that could help foster ecosystem resilience.
- Design mechanisms to assess the effectiveness and efficiency of management activities performed in the Sierra Nevada in order to implement an adaptive management framework.
- Help to disseminate information of general interest concerning the values and importance of Sierra Nevada.

The Sierra Nevada Global Change Observatory has four cornerstones (Figure 2): 1) a monitoring program with 40 methodologies that collect information on ecosystem functioning; 2) an information system to store and manage all the information gathered; 3) a plan to promote adaptive management of natural resources using the knowledge amassed through the monitoring programme; and 4) an outreach program to disseminate all the available information to potential users.



**Figure 2.** Structure of the Sierra Nevada Global Change Observatory. The four cornerstones of the research project are shown: monitoring program, adaptive management, information systems and dissemination. A monitoring program is needed to identify the impacts of global change over Sierra Nevada. The information compiled needs to be transformed into useful knowledge for the managers to carry out an active and adaptive management of natural resources. To achieve this, it is essential that all data be integrated and analysed in an information system. Finally, the general public should be informed of both the results obtained and methodologies used, through effective outreach activities.

The Sierra Nevada Global Change Observatory is linked to other national (Zamora and Bonet 2011) and international monitoring networks: GLOCHAM-ORE (Global Change in Mountain Regions) (Bjørnsen 2005), GLOCHAMOST (Global Change in Mountain Sites) (Schaaf 2009), LTER-Spain (Long-Term Ecological Research).

Sierra Nevada Global Change Observatory is collecting socio-ecological information on the major ecosystem types found in Sierra Nevada. This information is being integrated in an Information System (<http://obsnev.es/linaria.html> - Pérez-Pérez et al. 2012. (Free access upon registration). The dataset described here is a good example of this idea. We have created a relational database to store the floristic inventories prepared in 2004–2005. Thanks to this work, all this valuable and unique information will be available to scientists and environmental managers worldwide.

## Data published through

GBIF: <http://www.gbif.es:8080/ipt/resource.do?r=sinfonevada>

## Taxonomic coverage

### General taxonomic coverage description

Most of the species recorded in the inventories belong to class Magnoliopsida (6,042 records; 76.28 %) and Liliopsida (1,171 records; 14.78 %). The top 10 of the orders (Figure 3) include Poales (1153 records; 14.56 %) for the class Liliopsida, Lamiales (1062 records; 13.41 %) for Magnoliopsida and Pinales (569 records; 7.18 %). In these collection, 57 families are represented, with Poaceae, Fabaceae, and Lamiaceae being the families with major number of records (Table 1) (Figure 3). The collection includes 270 taxa belonging to 159 genera, *Pinus* and *Thymus* being the most represented ones in the database. There are 24 threatened taxa (Table 2).

## Taxonomic ranks

**Kingdom:** Plantae

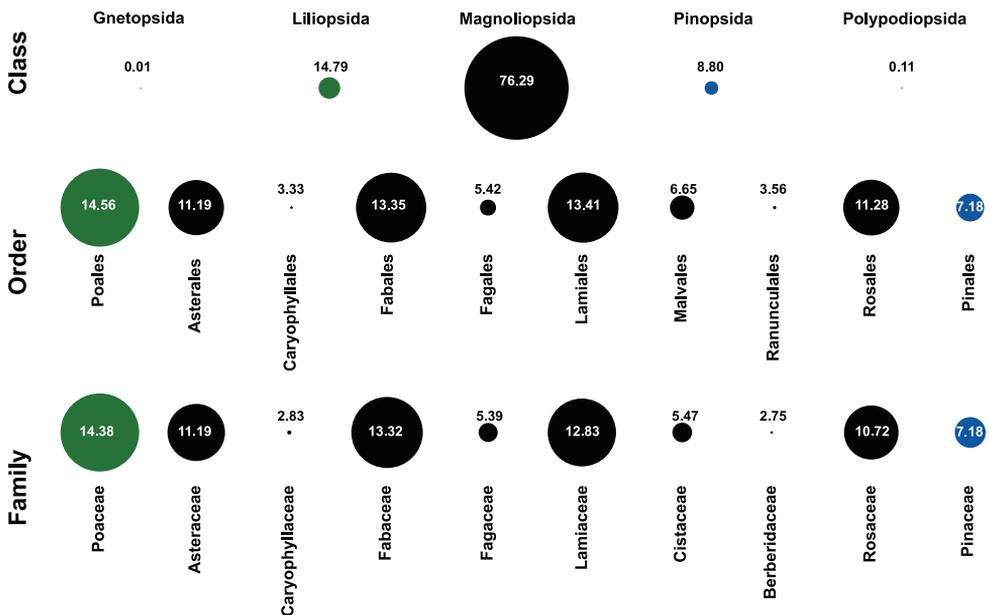
**Phylum:** Pteridophyta, Spermatophyta

**Class:** Gnetopsida, Liliopsida (Monocotyledones), Magnoliopsida (Dicotyledones), Pinopsida, Polypodiopsida

**Order:** Apiales, Asparagales, Asterales, Brassicales, Caryophyllales, Cucurbitales, Cupressales, Dipsacales, Ephedrales, Ericales, Fabales, Fagales, Gentianales, Geraniales, Lamiales, Liliales, Malpighiales, Malvales, Pinales, Poales, Polypodiales, Ranunculales, Rosales, Santalales, Sapindales, Saxifragales, Solanales, Umbellales

**Table 1.** The top 20 of the families represented in the collection.

Family	records	%
Poaceae	1139	14.38
Fabaceae	1055	13.32
Lamiaceae	1016	12.83
Asteraceae	886	11.19
Rosaceae	849	10.72
Pinaceae	569	7.18
Cistaceae	433	5.47
Fagaceae	427	5.39
Caryophyllaceae	224	2.83
Berberidaceae	218	2.75
Apiaceae	160	2.02
Cupressaceae	128	1.62
Thymelaeaceae	94	1.19
Rubiaceae	89	1.12
Brassicaceae	87	1.10
Crassulaceae	78	0.98
Euphorbiaceae	65	0.82
Ranunculaceae	64	0.81
Scrophulariaceae	38	0.48
Rhamnaceae	34	0.43

**Figure 3.** Taxonomic coverage. The figure shows the taxonomic coverage for class, order and family. The circles size are proportional to the number of records in the Sinfonevada database. Numbers indicates the percentage of records. All taxonomic classes included in the database are shown. For order and family rank, only the top 10 are shown. Colour indicates the taxonomic class: green (Liliopsida); black (Magnoliopsida) and blue (Pinopsida).

**Table 2.** Threatened species included in SINFONEVADA dataset.

ScientificName	Bern <sup>a</sup>	CITES <sup>b</sup>	Habitat Directive <sup>c</sup>	Spanish Red List <sup>d</sup>	Andalusian Red List <sup>e</sup>	IUCN <sup>f</sup>
<i>Acer monspessulanum</i> L.					NT	VU
<i>Acer opalus</i> subsp. <i>granatense</i> (Boiss.) Font Quer & Rothm.					NT	VU
<i>Amelanchier ovalis</i> Medik.					NT	LR-lc
<i>Armeria filicaulis</i> Boiss. subsp. <i>nevadensis</i> Nieto Fel., Rosselló & Fuertes				VU	VU	VU
<i>Celtis australis</i> A.Rich.					NT	LR-lc
<i>Centaurea bombycina</i> Boiss. subsp. <i>bombycina</i>				VU	VU	VU
<i>Centaurea monticola</i> Boiss. ex DC.				VU	VU	VU
<i>Centaurea pulvinata</i> (Blanca) Blanca	Appendix I		Annex II	VU	VU	VU
<i>Cephalanthera longifolia</i> (L.) Fritsch		Annex B				
<i>Cotoneaster granatensis</i> Boiss.					NT	VU
<i>Cytisus galianoi</i> Talavera & P.E.Gibbs					NT	
<i>Erica terminalis</i> Klotzsch					NT	VU
<i>Euphorbia nevadensis</i> Boiss. & Reut.			Annex IV		NT	LR-nt
<i>Pinus sylvestris</i> L. var. <i>nevadensis</i> Christ				EN	EN	VU
<i>Potentilla reuteri</i> Boiss.				NT	NT	VU
<i>Prunus avium</i> (L.) L.						VU
<i>Prunus mahaleb</i> L.						VU
<i>Prunus ramburii</i> Boiss.				VU	VU	
<i>Quercus pyrenaica</i> Willd.					NT	LR-cd
<i>Reseda complicata</i> Bory				VU	VU	
<i>Salix caprea</i> L.					EN	EN
<i>Salix eleagnos</i> subsp. <i>angustifolia</i> (Cariot) Rech. f.						LR-cd
<i>Santolina elegans</i> Boiss. ex DC.			Annex IV	VU	VU	VU
<i>Sorbus aria</i> Wimm. ex Nyman					NT	VU

<sup>a</sup> Bern: Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention).

<sup>b</sup> CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora. Species included in its appendices.

<sup>c</sup> Species included in the Habitat Directive Annex (EC 1992)

<sup>d</sup> 2010 Red List of Spanish vascular flora (Moreno 2010)

<sup>e</sup> 2005 Red List of vascular flora of Andalusia (Cabezudo et al. 2005)

<sup>f</sup> IUCN category in Sierra Nevada (IUCN 2001, Blanca et al. 1998, Blanca et al. 2001, Lorite et al. 2007)

EN: Endangered; VU: Vulnerable; NT: Near threatened; LR-nt: Lower Risk-Near Threatened; LR-cd: Lower Risk-Conservation Dependent; LR-lc: Lower Risk-Least Concern

**Family:** Amaryllidaceae, Anacardiaceae, Apiaceae, Apocynaceae, Araliaceae, Asparagaceae, Asteraceae, Berberidaceae, Brassicaceae, Capparaceae, Caprifoliaceae, Caryophyllaceae, Cistaceae, Clusiaceae, Colchicaceae, Convolvulaceae, Coriariaceae, Crassulaceae, Cupressaceae, Cyperaceae, Dennstaedtiaceae, Dipsacaceae, Ephedraceae, Ericaceae, Eu-

phorbiaceae, Fabaceae, Fagaceae, Geraniaceae, Iridaceae, Juglandaceae, Juncaceae, Lamiaceae, Leguminosae, Oleaceae, Orchidaceae, Paeoniaceae, Pinaceae, Plantaginaceae, Plumbaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Resedaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae, Santalaceae, Sapindaceae, Scrophulariaceae, Smilacaceae, Thymelaeaceae, Ulmaceae, Umbelliferae, Urticaceae, Violaceae

## Spatial coverage

### General spatial coverage

The SINFONEVADA forest inventory was conducted in the main forests of Sierra Nevada mountainous region (Figure 1) (for a description of Sierra Nevada see study area of the Project section). The main forest units of Sierra Nevada (Figure 5) are pine plantations (*Pinus halepensis* Mill., *Pinus pinaster* Ait., *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco, and *Pinus sylvestris* L.), evergreen holm oak *Quercus ilex* subsp. *ballota* (Desf.) Samp forests, deciduous broadleaf forests (*Quercus pyrenaica* Willd, *Acer opalus* subsp. *granatense* (Boiss.) Font Quer & Rothm., *Sorbus aria* (L.) Crantz), and autochthonous pine *Pinus sylvestris* L. var. *nevadensis* Christ forests.

### Coordinates

36°52'12"N and 37°15'36"N Latitude; 3°41'24"W and 2°33'36"W Longitude

### Temporal coverage

2004–2005

## Natural collections description

**Parent collection identifier:** NA

**Collection name:** Sinfonevada: Dataset of floristic diversity in Sierra Nevada forest (SE Spain)

### Collection identifier

<http://www.gbif.es:8080/ipt/manage/metadata-collections.do?r=sinfonevada>

## Methods

### Method step description

This inventory was undertaken in 2004 and the database generated contains information relative to forest attributes and occurrence data (see below). This information, originally stored in a Microsoft Access database, has been integrated into the project's information system.

**Study extent description:** The floristic inventories were conducted at the main forest units of the Sierra Nevada (Andalusia, SE Spain). Forest cover in Sierra Nevada is dominated by pine plantations (*Pinus halepensis* Mill., *Pinus pinaster* Ait., *Pinus nigra* Arnold subsp. *salzmannii* (Dunal) Franco, and *Pinus sylvestris* L.) covering approximately 40,000 ha. Most of them were planted in the period 1960–1980. The main native forests of Sierra Nevada are dominated by the evergreen holm oak *Quercus ilex* subsp. *ballota* (Desf.) Samp. occupying low and medium mountain areas (8,800 ha.) and Pyrenean oak *Quercus pyrenaica* Willd ranging from 1,100 to 2,000 m a.s.l., covering about 2,000 ha. Autochthonous pine *P. sylvestris* var. *nevadensis* forests can also be found in small patches at high altitudes with a characteristically low tree cover.

**Sampling description:** SINFONEVADA Forest Inventory was established over an extensive network of 600 long-term permanent plots distributed within the main forest units of the Sierra Nevada: pine plantations, evergreen *Q. ilex* forests, and deciduous broadleaf forests. The network of plots is a random sample stratified by land cover and altitude, covering a gradient of 974–2439 m a.s.l. (Figure 5).

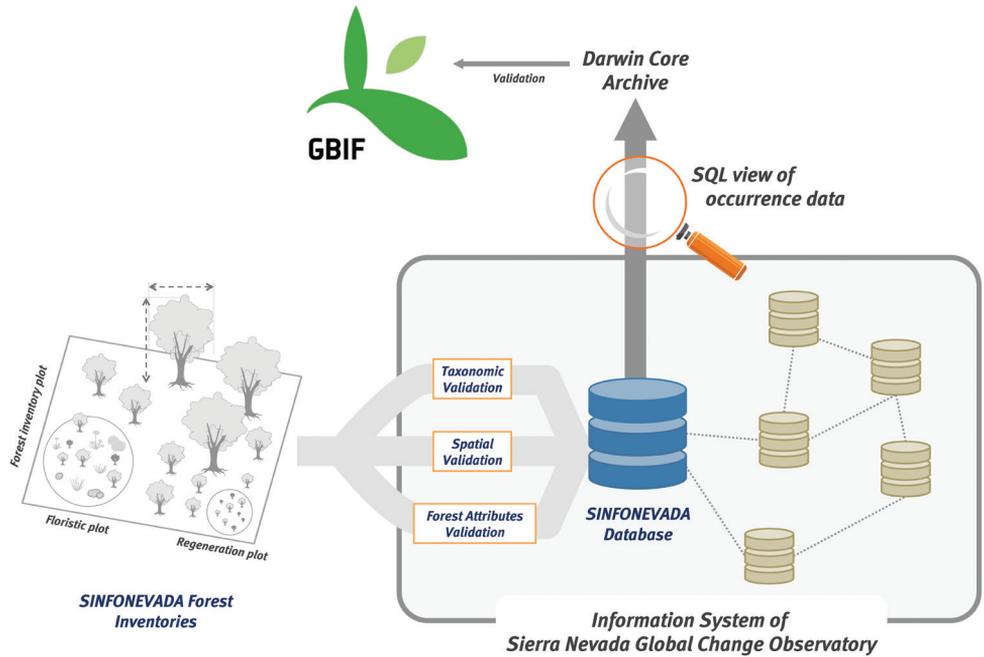
Each inventory plot has three sampling units: i) a forest inventory plot (20 × 20 m); ii) a 5-m radius subplot for the estimation of the regeneration; iii) and a 10-m radius subplot for species composition and abundance.

Each live tree with a diameter at breast height (dbh) > 7.5 cm was tallied by species and dbh in the forest inventory plot. This information was used to calculate forest attributes (tree basal area, tree volume, canopy cover). The regeneration was measured in the 5-m radius subplot (78.5 m<sup>2</sup> in area) as seedling abundance of the main tree species.

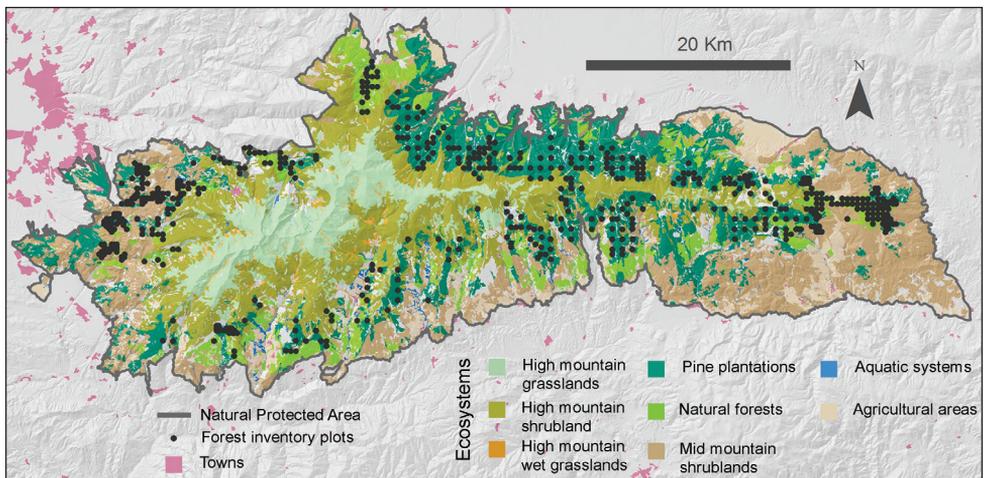
The species composition and diversity was recorded within a 10-m radius subplot (314 m<sup>2</sup> in area) using the Braun-Blanquet cover-abundance scale (Braun-Blanquet 1964).

### Quality control description

Prior to the storing of this information in the database, all the data were assessed by a quality-control process. Each sampling plot was checked to ensure that the geographical coordinates were correct. We used the databases of International Plant Names Index (IPNI 2013) and Catalogue of Life/Species 2000 (Roskov et al. 2013) to verify the taxonomical classification. The specimens were taxonomically identified using *Flora Iberica* (Castroviejo et al. 1986–2005) for the published families while the rest of taxa were identified according to Valdés et al. (1987) and Tutin et al. (1964–1980).



**Figure 4.** Diagram of integration of SINFONEVADA within Information System of Sierra Nevada Global Change Observatory. The original database of SINFONEVADA contains two types of information: forest attributes and related information and occurrence data. This information was integrated into the Information System of Sierra Nevada Global Change Observatory. After a validation process (see Quality Control section) the occurrence data were accommodated to Darwin Core Archive to integrate in GBIF.



**Figure 5.** Location of the forest-inventory plots. This map shows the location of the forest-inventory plots and the distribution of the ecosystem types present in Sierra Nevada. The vegetation is predominantly high-mountain shrublands and pine plantations, with some natural forests (oaks, Pyrenean oaks, maples, etc.).

## Datasets

The original database of SINFONEVADA contains two types of information: forest attributes (and related information), and occurrence data. There are several national forest inventories (Spanish National Forest Inventory, Alberdi et al. 2010) that have partially monitored some forests of Sierra Nevada. However, due to grain size, none have been as exhaustive as the SINFONEVADA inventory.

The original SINFONEVADA database was incorporated into the Information System of Sierra Nevada Global Change Observatory (Figure 4). Taxonomic and spatial validations were made on this database. Also, we carried out quality control procedures for forest attributes (detection of atypical values). A custom-made SQL view of the original SINFONEVADA was performed to gather occurrence data. The view shows occurrence data collected in the floristic inventories associated with the forest inventory. We included only records that had been accepted for publication. The occurrence data were accommodated to the Darwin Core Archive to integrate in GBIF. We used Darwin Core Archive Validator tool (<http://tools.gbif.org/dwca-validator/>) to check whether the dataset meets Darwin Core specifications. The Integrated Publishing Toolkit (IPT v2.0.5) of the Spanish node of the Global Biodiversity Information Facility (GBIF) (<http://www.gbif.es:8080/ipt>) was used both to upload the Darwin Core Archive and to fill out the metadata. Information about forest attributes included in the original SINFONEVADA database is available upon request.

The fields provided by the SINFONEVADA dataset are:

occurrenceId, modified, basisOfRecord, institutionCode, collectionCode, catalogNumber, occurrenceRemarks, scientificName, kingdom, phylum, class, order, family, genus, specificEpithet, infraspecificEpithet, scientificNameAuthorship, continent, country, stateProvince, county, locality, minimumElevationInMeters, maximumElevationInMeters, recordedBy, identifiedBy, dateIdentified, decimalLongitude, decimalLatitude, coordinateUncertaintyInMeters.

The SINFONEVADA dataset represents an exhaustive floristic inventory of diversity of Sierra Nevada forest. It includes occurrences of 270 taxa, of which 24 are considered threatened and 9 endemic (Table 2). Information of SINFONEVADA has been used for the Natural Resources Ordinance Plan of the Sierra Nevada Natural Area (CMA 2011). Also its information provides valuable support to natural-resource managers in their decision making. It is being considered for management actions within strategies of diversification and naturalization of forests in the Sierra Nevada natural area.

## Dataset description

**Object name:** Darwin Core Archive Sinfonevada: Dataset of floristic diversity in Sierra Nevada forest (SE Spain)

**Character encoding:** UTF-8.

**Format name:** Darwin Core Archive format.

**Format version:** 1.0

**Distribution:** <http://www.gbif.es:8080/ipt/archive.do?r=sinfonevada>

**Publication date of data:** 2013-09-24

**Language:** English.

**Licenses of use:** The “Sinfonevada: Dataset of floristic diversity in Sierra Nevada forest (SE Spain)” dataset is made available under the Open Data Commons Attribution License: <http://www.opendatacommons.org/licenses/by/1.0/>.

**Metadata language:** English.

**Date of metadata creation:** 2013-06-18

**Hierarchy level:** Dataset.

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# ***Piptochaetium fuscum* (Nees ex Steud.) Barkworth, Ciald., & Gandhi, a new combination replacing *Piptochaetium setosum* (Trin.) Arechav.**

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## **Abstract**

A new name, *Piptochaetium fuscum*, is provided for a taxon hitherto known as *Piptochaetium setosum* (Trin.) Arechav. Morphological, anatomical, and molecular studies that argue against including *Piptochaetium* in *Stipa*, and hence use of *S. purpurata* (Phil.) Columbus & J.P. Sm., are cited.

## **Keywords**

*Stipeae*, *Stipa*, *Piptochaetium*, nomenclature

## **Introduction**

In February 2010, Gandhi, in responding to an inquiry from Dr. Travis Columbus, noticed that the name *Piptochaetium setosum* (Trin.) Arechav. was superfluous and illegitimate at the time of publication because its basionym, *Urachne setosa* Trin., was itself superfluous and illegitimate at the time of publication, Trinius having included in it two older and validly published names, *Stipa panicoides* Lam. and *Oryzopsis setacea* Rich. *Stipa panicoides* is the basionym of *P. panicoides* (Lam.) Desv., a taxon that is now con-

sidered distinct from *Piptochaetium setosum* (Parodi 1944; Cialdella and Arriaga 1998; Peña et al. 2008). In addition, Parodi, who examined the types of all the names involved, stated that the type of *Oryzopsis setacea* was evidently based on the same material as that of *Stipa panicoides* (Parodi 1944, p. 299). Thus neither of the names Trinius treated as synonyms of *Urachne setosa* can be used as the basionym for *P. setosum* when this taxon is considered to be distinct from *P. panicoides*.

Columbus and Smith have published a new name for the taxon, but they placed it in *Stipa* L. as *Stipa purpurata* (Phil.) Columbus & J.P. Sm. (Columbus and Smith 2010). We strongly disagree with their generic interpretation. *Piptochaetium* J. Presl, as interpreted by Parodi (1944; Parodi and Freier 1945; Thomasson 1978, 1979; Cialdella and Arriaga 1998; Cialdella and Guissani 2002; Cialdella et al. 2007; Jacobs et al. 2007; Barber et al. 2009) has been shown to be morphologically, anatomically, and molecularly distinct from other genera of the Stipeae as well as monophyletic. The purpose of this paper is to provide a valid combination in *Piptochaetium* for the taxon hitherto known as *P. setosum* in *Piptochaetium*.

Parodi (1944), in his revision of *Piptochaetium*, listed four synonyms for *Piptochaetium setosum*: *Urachne fusca* Steud. (Steudel 1854) (the basionym of *P. fuscum*), *Piptochaetium purpuratum* Phil. (Philippi 1857), *Piptochaetium pallidum* Phil. ex Griseb. (Grisebach 1879), and *P. macrocarpum* Phil. (Philippi 1896). In describing *Urachne fusca*, Steudel cited a specimen collected by Cuming near Valparaiso, Chile. Parodi stated that he had examined a specimen in B that Nees had annotated as *Piptatherum fuscum*, Valparaiso, Cuming, Herb. Lindley. He also examined two other specimens, one from K and one from CGE, that were labeled Cuming 453. He stated that all three specimens were identical to each other and to the type material of *Urachne setosa* Trin.

## Results and discussion

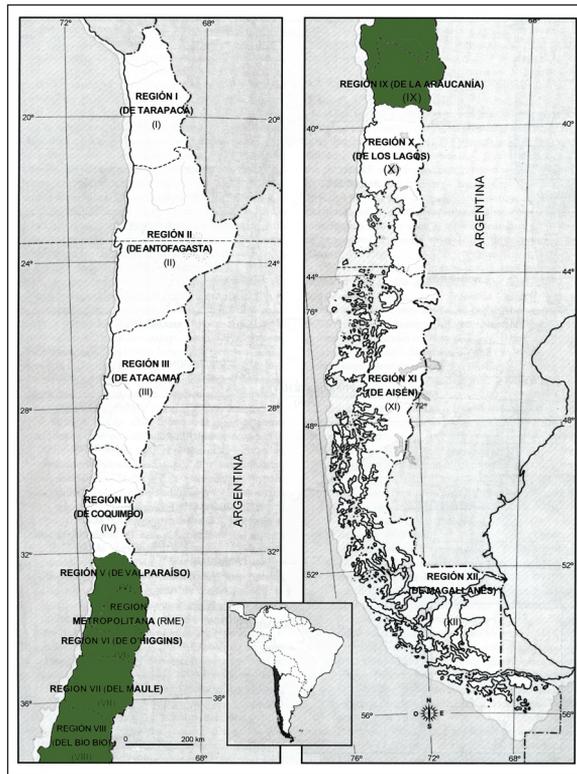
We have examined images from each of the CGE and K specimens cited above. We agree with Parodi that they belong to *Piptochaetium setosum* as recognized by Arechavaleta (1896) and Parodi (1944) and, on that basis, present the following new combination:

***Piptochaetium fuscum* (Nees ex Steud.) Barkworth, Ciald., & Gandhi, comb. nov.**  
urn:lsid:ipni.org:names:77136235-1  
[http://species-id.net/wiki/Piptochaetium\\_fuscum](http://species-id.net/wiki/Piptochaetium_fuscum)

**Basionym.** *Urachne fusca* Nees ex Steud., Syn. Pl. Glumac. 1(2): 123. 1854 [1855 publ. 2–3 Mar 1854]. Lectotypus: K000433539, Herbarium Hookerianum 1867 (<http://specimens.kew.org/herbarium/K000433539>), imaginem videmus; Isolectotypi K000433540, Herbarium Benthianum 1854 (<http://specimens.kew.org/herbarium/K000433540>), imaginem videmus; CGE, Herb. J. Lindley, purchased 1866 (figs 1, 2, 3); “prope Valparaiso, Chili; H. Cuming 453, 1831; Imagines videmus.



**Figure 1.** Isotype of *Piptochaetium fuscum* deposited at CGE, the Cambridge University Herbarium, England. Image used with permission.



**Figure 2.** Native distribution of *Piptochaetium fuscum*. Information and base map from Zuloaga et al. (2008), used with permission of Missouri Botanical Garden Press.

The three specimens are from the same gathering and conform to the protologue. We chose K000433539 as the lectotype because it has more material, both reproductive and vegetative, than the other specimens. Columbus and Smith (2010) were forced to base their name on *P. purpuratum* Phil. because the name *Stipa fusca* had already been used for an Australian taxon by Hubbard (1925).

Parodi (1944) stated that *Piptochaetium fuscum* grew in central Chile, extending from Valparaiso and Santiago to Valdivia. This statement was confirmed by Zuloaga et al. (2008) who added that it grew at 0–800 m. There are only three South American records with latitude and longitude in the Global Biodiversity Information Facility. They were collected at 37.41S, 72.01W [SI 268952]; 36.48S, 72.71W [BAA 416344], and 36.56S, 72.49W [BAA 416345]. Zuloaga et al. (2008) provide information in terms of Chile's regions (Fig. 2). The species is also known from one locality in Marin County, California, where it was first collected in 1978 (Consortium of California Herbaria 2014). The origin of the population is unknown. It does not appear to have spread since its introduction.

To determine the conservation status of *Piptochaetium fuscum*, a search should be made for specimens in Chilean herbaria and field work conducted to locate natural populations. Such activities were beyond the scope of our study.

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We thank Christine Bartram and Maria Vorontsova, at CGE and K, respectively, for their rapid response to our request for images of the Cuming 453 specimens at their institutions and Victoria Hollowell of Missouri Botanical Garden Press for giving permission to use the map on p. xix of Zuloaga et al. (2008) to illustrate the native distribution of the species. This research was supported by the Utah Agricultural Experiment Station, Utah State University, and approved as journal paper number 8622.

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# *Tryonia*, a new taenitidoid fern genus segregated from *Jamesonia* and *Eriosorus* (Pteridaceae)

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## Abstract

The Neotropical fern genera *Eriosorus* and *Jamesonia* have long been thought of as close relatives. Molecular phylogenetic studies have confirmed this notion but have also revealed that neither genus is monophyletic with respect to the other. As a result, all known species of *Eriosorus* were recently subsumed under the older generic name *Jamesonia*. Here, through an analysis of a four-gene plastid dataset, we show that several species traditionally treated in *Eriosorus* are in fact more closely related to other taenitidoid fern genera (namely *Austrogramme*, *Pterozonium*, *Syngamma*, and *Taenitis*) than they are to the large *Jamesonia sensu lato* clade. *Tryonia* Schuettp., J.Prado & A.T.Cochran **gen. nov.** is described to accommodate these species and four new combinations are provided. *Tryonia* is confined to southeastern Brazil and adjacent Uruguay; it is distinct (from most species of *Jamesonia*) in having stramineous rachises.

## Keywords

Brazil, phylogeny, pteridophytes, Taenitidoideae, taxonomy

## Introduction

The Neotropical genus *Jamesonia* Hook. & Grev. *sensu stricto* is among the most distinctive of all fern genera. It has linear, indeterminate leaves bearing highly reduced, coriaceous pinnae covered with dense pubescence (Tryon 1962; Fig. 1). These morphological characteristics are generally considered to be an adaptation to the high-



**Figure 1.** *Jamesonia pulchra* Hook. & Grev., the type species of *Jamesonia*. Ewan 16100 (US), inset detail of (castaneous) rachis magnified 4x.

elevation Andean páramo habitats where most *Jamesonia* species reside (Tryon et al. 1990). Based on reproductive and other cryptic morphological characteristics, *Jamesonia* has long been thought to be closely related to the genus *Eriosorus* Fée (Tryon 1962, 1970, Tryon and Tryon 1982). *Eriosorus* mostly occupies middle-elevation habitats in the Andes and its leaves are much more typical of ferns, usually being very dissected and rather delicate in texture (Tryon 1970; Figs 2, 3). Recent analyses have demonstrated that *Jamesonia* is both nested within *Eriosorus* and polyphyletic (Prado et al. 2007, Sánchez-Baracaldo 2004a, 2004b, Schneider et al. 2013, Schuettpelz et al. 2007), supporting the hypothesis of Tryon (1962, 1970) that the unique morphology of *Jamesonia* evolved independently multiple times. This finding prompted the recent recombination of all known species of *Eriosorus* into *Jamesonia* (*sensu lato*, Christenhusz et al. 2011).

Although it is clear that species of *Jamesonia sensu stricto* are intermixed with those previously assigned to *Eriosorus*, relationships remain rather poorly supported and additional studies are needed to better resolve the evolutionary history of this group. With that said, the isolated phylogenetic position revealed for one Brazilian species requires special attention. In the most comprehensive study of *Jamesonia sensu lato* to date (Sánchez-Baracaldo 2004b), two accessions of *E. myriophyllus* (Sw.) Copel. (Fig. 4) were resolved together and well supported as sister to the remainder of *Jamesonia sensu lato*. However, it is clear from the phylogram included in the Sánchez-Baracaldo (2004b) study that these accessions are genetically more similar to the outgroup used than they are to the remainder of the ingroup, suggesting that the phylogenetic position of *E. myriophyllus* may be an artifact of including a single outgroup genus (*Pterozonium* Fée). Subsequent analyses with a broader phylogenetic context but including fewer exemplars from within *Jamesonia sensu lato*, actually found *E. myriophyllus* to be most closely related to the genus *Taenitis* Willd. ex Schkuhr (Prado et al. 2007, Schneider et al. 2013).

Here, through analyses of a four-gene (*atpA*, *chlL*, *rbcL*, and *rps4*) plastid dataset that incorporates many *Eriosorus* and *Jamesonia sensu stricto* species, as well as a broad sampling of related genera, we aim to better resolve the phylogenetic position of *E. myriophyllus* and allied species. Based on our results, we describe a new genus, *Tryonia* Schuettp., J.Prado & A.T.Cochran, to accommodate this species and its closest allies.

## Methods

### Sampling

A total of thirty-eight collections were sampled for the phylogenetic analysis, including four individuals of *Eriosorus myriophyllus*, nine other species of *Eriosorus*, eight *Jamesonia sensu stricto* species, and seventeen additional species representing other genera in the taenitidoid clade (Prado et al. 2007, Sánchez-Baracaldo 2004a, Schuettpelz et al. 2007, Table 1).



**Figure 2.** *Jamesonia aureonitens* (Hook.) Christenh., the type species of *Eriosorus*. Hutchison 5504 (US), inset detail of (castaneous) rachis magnified 4x.



**Figure 3.** *Jamesonia congesta* (Christ) Christenh., a species with generalized morphology (Tryon 1970) previously classified in *Eriosorus*. Lellinger 1711 (US), inset detail of (castaneous) rachis magnified 4x.



**Figure 4.** *Tryonia myriophylla* (Sw.) Schuettp., J.Prado & A.T.Cochran, the type species of *Tryonia*. Smith 1795 (US), inset detail of (stramineous) rachis magnified 4x.

**Table 1.** Collections included in our phylogenetic analyses supporting the recognition of *Tryonia*, with voucher information and corresponding GenBank accession numbers.

Species	Voucher	<i>atpA</i>	<i>chlL</i>	<i>rbcL</i>	<i>rps4</i>	FLDB <sup>1</sup>
<i>Actiniopteris dimorpha</i> Pic.Serm.	Schneider s.n. (GOET)	EF452066	KJ416295	EF452130	KJ416352	3515
<i>Actiniopteris semiflabellata</i> Pic.Serm.	Smith s.n. (UC)	KJ416270	KJ416296	KJ416326	KJ416353	3742
<i>Anogramma leptophylla</i> (L.) Link	Schuettpelz 1079 (DUKE)	KJ416271	KJ416297	KJ416327	KJ416354	4822
<i>Austrogramme decipiens</i> (Mett.) HENNIPMAN	van der Werff 16114 (UC)	NA	NA	NA	AF321702	NA
<i>Austrogramme marginata</i> (Mett.) E.FOURN.	Hodel 1454 (UC)	NA	NA	NA	AY357704	NA
<i>Cosentinia vellea</i> (Aiton) Tod.	Larsson 55 (UPS)	KJ416272	KJ416298	KJ416328	KJ416355	8670
<i>Jamesonia alstonii</i> A.F.TRYON	Moran 8248 (DUKE)	KJ416273	KJ416299	KJ416329	KJ416356	5587
<i>Jamesonia blepharum</i> A.F.TRYON	Schuettpelz 269 (DUKE)	KJ416274	KJ416300	EF452154	KJ416357	2437
<i>Jamesonia brasiliensis</i> Christ	Schuettpelz 1444 (SP)	KJ416275	KJ416301	KJ416330	KJ416358	8379
<i>Jamesonia cheilanthoides</i> (Sw.) Christenh.	Rothfels 3964 (DUKE)	KJ416276	KJ416302	KJ416331	KJ416359	7694
<i>Jamesonia congesta</i> (Christ) Christenh.	Grusz 08-036 (DUKE)	KJ416277	KJ416303	KJ416332	KJ416360	5272
<i>Jamesonia elongata</i> (Grev. & Hook.) J.Sm.	Rothfels 3602 (DUKE)	KJ416278	KJ416304	KJ416333	KJ416361	7362
<i>Jamesonia flexuosa</i> (Kunth) Christenh.	Rothfels 08-042 (DUKE)	KJ416279	KJ416305	KJ416334	KJ416362	5273
<i>Jamesonia goudotii</i> (Hieron.) C.Chr.	Rothfels 3694 (DUKE)	KJ416280	KJ416306	KJ416335	KJ416363	7414
<i>Jamesonia hirta</i> (Kunth) Christenh.	Rothfels 3669 (DUKE)	KJ416281	KJ416307	KJ416336	KJ416364	7397
<i>Jamesonia insignis</i> (Kuhn) Christenh.	Salino 3010 (UC)	NA	NA	NA	AF321708	NA
<i>Jamesonia pulchra</i> Hook. & Grev.	Sánchez-Baracaldo 306 (UC)	NA	NA	NA	AF321746	NA
<i>Jamesonia rotundifolia</i> Fée	Sundue 1357 (DUKE)	KJ416282	KJ416308	KJ416337	KJ416365	6049
<i>Jamesonia scammaniae</i> A.F.TRYON	Rothfels 2631 (DUKE)	KJ416283	KJ416309	KJ416338	KJ416366	5588
<i>Jamesonia verticalis</i> Kunze	Rothfels 3638 (DUKE)	KJ416284	KJ416310	KJ416339	KJ416367	7386
<i>Jamesonia warszewiczii</i> (Mett.) Christenh.	Grusz 08-039 (DUKE)	KJ416285	KJ416311	KJ416340	KJ416368	5275
<i>Onychium japonicum</i> (Thunb.) Kunze	Schneider s.n. (GOET)	EF452107	KJ416312	KJ416341	NA	3463
<i>Onychium lucidum</i> (D.Don) Spreng.	Schuettpelz 1161 (DUKE)	KJ416286	KJ416313	KJ416342	NA	4904
<i>Pityrogramma austroamericana</i> Domin	Schuettpelz 301 (DUKE)	EF452112	KJ416314	EF452166	KJ416369	2561
<i>Pityrogramma chaerophylla</i> (Desv.) Domin	Prado 2178 (SP)	KJ416287	KJ416315	KJ416343	KJ416370	8755
<i>Pityrogramma jamesonii</i> (Baker) Domin	Moran 7592 (NY)	EF463857	KJ416316	EF452167	KJ416371	3769
<i>Pterozonium brevifrons</i> (A.C.Sm.) LELLINGER	Schuettpelz 285 (DUKE)	EF452124	KJ416317	EF452175	KJ416372	2453
<i>Pterozonium cyclosorum</i> A.C.Sm.	Brewer 1006 (UC)	NA	NA	NA	AF321703	NA
<i>Pterozonium reniforme</i> (Mart.) Fée	Brewer 1005 (UC)	NA	NA	NA	AF321704	NA
<i>Syngamma quinata</i> (Hook.) Carr.	Kessler 2273 (L)	NA	NA	NA	AF321701	NA
<i>Taenitis blechnoides</i> (Willd.) Sw.	Schuettpelz 689 (DUKE)	KJ416288	KJ416318	KJ416344	KJ416373	4102
<i>Taenitis interrupta</i> Hook. & Grev.	Schuettpelz 851 (DUKE)	KJ416289	KJ416319	KJ416345	KJ416374	4270

<i>Tryonia areniticola</i> (Schwartzsb. & Labiak) Schuettp., J.Prado & A.T.Cochran	Prado 2169 (SP)	NA	KJ416320	KJ416346	KJ416375	8433
<i>Tryonia myriophylla</i> (Sw.) Schuettp., J.Prado & A.T.Cochran	Schuettpelz 1411 (SP)	KJ416290	KJ416321	KJ416347	KJ416376	8345
<i>Tryonia myriophylla</i> (Sw.) Schuettp., J.Prado & A.T.Cochran	Schuettpelz 1449 (SP)	KJ416291	KJ416322	KJ416348	KJ416377	8384
<i>Tryonia myriophylla</i> (Sw.) Schuettp., J.Prado & A.T.Cochran	Schuettpelz 1461 (SP)	KJ416292	KJ416323	KJ416349	KJ416378	8396
<i>Tryonia myriophylla</i> (Sw.) Schuettp., J.Prado & A.T.Cochran	Prado 2186 (SP)	KJ416293	KJ416324	KJ416350	NA	8753
<i>Tryonia schwackeana</i> (Christ) Schuettp., J.Prado & A.T.Cochran	Schuettpelz 1433 (SP)	KJ416294	KJ416325	KJ416351	KJ416379	8367

<sup>†</sup>Fern Lab Database voucher number (see <http://fernlab.biology.duke.edu> for additional information concerning these collections)

### DNA extraction, amplification, and sequencing

Genomic DNA was typically extracted using a modified CTAB protocol (Doyle and Doyle 1987), as described in detail in Beck et al. (2011). Four plastid gene regions (*atpA*, *chlL*, *rbcL*, and *rps4*) were amplified using the polymerase chain reaction (PCR). Each reaction incorporated 13.6 µl ultrapure water, 2 µl buffer (10×), 2 µl dNTPs (2 mM each), 0.2 µl Choice-Taq DNA Polymerase (5 units/µl, Denville Scientific), 0.2 µl BSA (10 mg/ml), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), and 1 µl template DNA (primer details are provided for each gene in Table 2). All thermal cycling protocols employed an initial denaturation step (95 °C for 2 min), 35 amplification cycles, and a final elongation step (71 °C for 5 min). Each amplification cycle involved a denaturation step (95 °C for 0.5 min), an annealing step (50 °C for 0.5 min for *atpA*, *chlL*, and *rps4*; 45 °C for 0.5 min for *rbcL*), and an elongation step (71 °C for 1 min for *atpA* and *chlL*; 71 °C for 1.5 min for *rps4* and *rbcL*).

Amplifications were visualized using standard gel electrophoresis and imaging approaches. Unincorporated nucleotides and primers were removed from successful reactions by adding 1.0 µl Shrimp Alkaline Phosphatase (1 unit/µl) and 0.5 µl Exonuclease I (10 units/µl) to each reaction and incubating at 37 °C for 15 min. Reactions were then heated to 80 °C for 15 min to inactivate the enzymes.

Sequencing reactions were carried out, in both directions, with the amplification primers, following a standard protocol (Schuettpelz and Pryer 2007). For *rbcL*, two additional (internal) sequencing primers were utilized (Table 2). Sequencing reactions were cleaned using the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research), according to the manufacturer's protocol. Sealed plates were submitted to Operon (Huntsville, Alabama) for sequencing.

Sequencing reads were independently (for each PCR product) assembled and edited using Sequencher (Gene Codes Corporation). The 110 new consensus sequences were added to the Fern Lab Database (<http://fernlab.biology.duke.edu>) and deposited into GenBank (Table 1). For four (of thirty-eight) collections, we could only obtain three of the four gene regions targeted (Table 1). For six collections, an *atpA* and/or

**Table 2.** Primers utilized in this study supporting the recognition of *Tryonia*.

Region	Name	Type	Sequence	Reference
<i>atpA</i>	atpA-F1	Forward	GAATCTGATAATGTTGGGGCTG	This study
<i>atpA</i>	atpA-R1	Reverse	AAACATCTCCNGGATAYGCTTC	This study
<i>chlL</i>	chlL-F1	Forward	GRATTGGMAARTCAACAACCTAGCTG	This study
<i>chlL</i>	chlL-R1	Reverse	CBAGTACRGGCATGGGRCAAGCTTC	This study
<i>rbcL</i>	ES-rbcL-1F	Forward	ATGTCACCACAAACGGAGACTAAAGC	Schuettpelz and Pryer 2007
<i>rbcL</i>	ES-rbcL-1361R	Reverse	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz and Pryer 2007
<i>rbcL</i>	ES-rbcL-628F	Forward <sup>†</sup>	CCATTYATGCGTTGGAGAGATCG	Schuettpelz and Pryer 2007
<i>rbcL</i>	ES-rbcL-654R	Reverse <sup>†</sup>	GAARCGATCTCTCCAACGCAT	Schuettpelz and Pryer 2007
<i>rps4</i>	rps5	Forward	ATGTCCCCTTATCGAGGACCT	Souza-Chies et al. 1997
<i>rps4</i>	trnS	Reverse	TACCGAGGGTTCGAATC	Souza-Chies et al. 1997

<sup>†</sup>Primer used only for sequencing.

*rbcL* sequence had already been published; these existing sequences (from Schuettpelz and Pryer 2007 and Schuettpelz et al. 2007) were obtained directly from GenBank, as were seven *rps4* sequences (from Sánchez-Baracaldo 2004a, 2004b) corresponding to species not otherwise available to us (Table 1). All new and existing sequences were aligned, by gene region, using Mesquite (Maddison and Maddison 2011). The final *atpA*, *chlL*, *rbcL*, and *rps4* datasets included 30, 31, 31, and 35 taxa, respectively (see Table 3 for additional details concerning our alignments).

**Table 3.** Details for the alignments analyzed in this study supporting the recognition of *Tryonia*.

Dataset	Taxa	Characters			Data	Bipartitions
		Total	Included	Variable	Missing <sup>†</sup>	Supported <sup>‡</sup>
<i>atpA</i>	30	1506	629	113	1.04%	11
<i>chlL</i>	31	523	523	120	0.92%	15
<i>rbcL</i>	31	1309	1309	250	0.39%	15
<i>rps4</i>	35	1176	560	177	1.77%	17
Combined	38	4514	3021	660	17.76%	25

<sup>†</sup>Calculation based on included characters

<sup>‡</sup>Bayesian posterior probability  $\geq 0.95$

## Phylogenetic analyses

Bayesian phylogenetic analyses were conducted independently for each of the four single-gene datasets using MRBAYES version 3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). These Bayesian analyses utilized the GTR+ $\Gamma$ +I model of sequence evolution (the most complex model available) and consisted of four independent runs per dataset, each utilizing four chains and proceeding for five million generations, with trees sampled every 4000 generations. After completion of each analysis, we examined the standard deviation of split frequencies among the runs, plot-

ted the output parameter estimates using Tracer 1.5 (Rambaut and Drummond 2009), and very conservatively excluded the first 250 trees (one million generations) from each run. A majority-rule consensus phylogeny with clade posterior probabilities was then calculated from the remaining 4000 trees, for each gene. Based on earlier studies with broader sampling (Prado et al. 2007, Sánchez-Baracaldo 2004a), we rooted our resulting gene trees with *Actiniopteris* and *Onychium*.

We compared the results of our single-gene analyses, looking for conflicts that were supported by a Bayesian posterior probability  $\geq 0.95$ . Finding none, we concatenated the four datasets. The resulting 38-taxon combined dataset was analyzed as above, but with model parameters estimated and optimized separately for each gene and each run proceeding for 20 million generations. We sampled trees every 16,000 generations and excluded the first four million generations from each run prior to calculating a majority-rule consensus phylogeny with clade posterior probabilities.

## Results

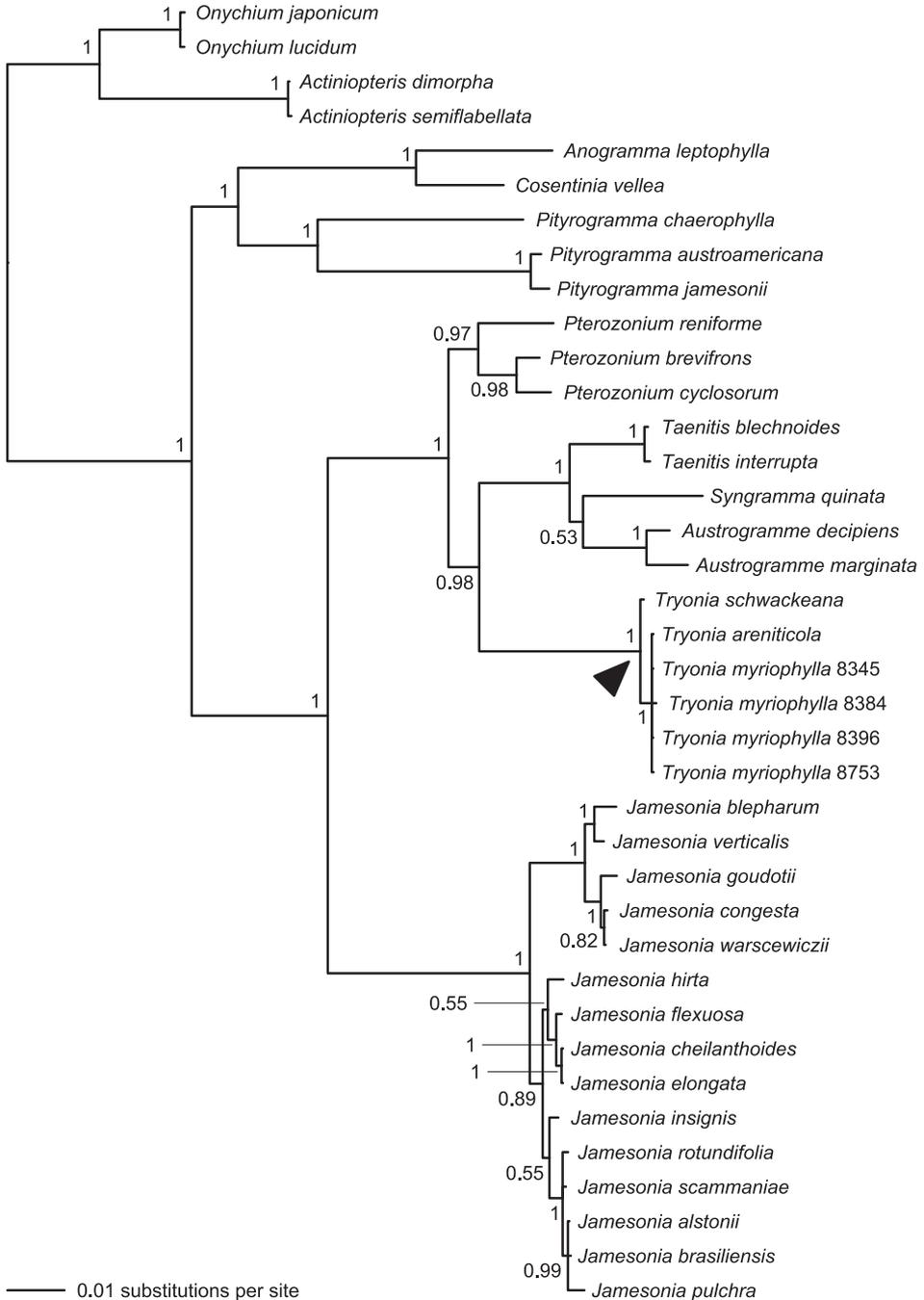
The four single-gene (*atpA*, *chlL*, *rbcL*, and *rps4*) datasets contained varying amounts of phylogenetic signal, providing significant support (Bayesian posterior probability, BPP  $\geq 0.95$ ) for as few as 11 and as many as 17 bipartitions (Table 3). The single-gene trees were largely consistent in their resolved relationships (trees not shown) and there were no well-supported (BPP  $\geq 0.95$ ) conflicts among them.

Our combined four-gene dataset comprised a total of 4514 characters, of which 660 were variable (Table 3). Analysis of this dataset resulted in a phylogeny with considerably improved support relative to the single-gene phylogenies; 25 bipartitions had a BPP  $\geq 0.95$  (Fig. 5). The separation of *Actiniopteris* and *Onychium* from the remaining taenitoid genera was well supported (BPP = 1.00). *Anogramma*, *Cosentinia*, and *Pityrogramma* formed a well-supported clade that was, in turn, well-supported as sister to a robust clade including *Austrogramme*, *Pterozonium*, *Syngramma*, *Taenitis*, and all sampled species previously assigned to either *Jamesonia* or *Eriosorus* (Fig. 5).

The vast majority of our *Jamesonia sensu lato* collections come together in a clade on a rather long branch; within this clade branches are short and support is frequently lacking. Six samples previously included within *Jamesonia sensu lato* are not allied to that larger clade, but rather are embedded within a well-supported clade that also contains *Austrogramme*, *Pterozonium*, *Syngramma*, and *Taenitis* (Fig. 5).

## Discussion

Most species previously assigned to *Eriosorus* and *Jamesonia sensu stricto* have been consistently resolved together in a well-supported clade (Prado et al. 2007, Sánchez-Baracaldo 2004a, 2004b, Schneider et al. 2013, Schuettpelz et al. 2007). And, although support for relationships within this large clade has been generally lacking, the



**Figure 5.** Phylogeny resulting from Bayesian analysis of our combined four-gene (*atpA*, *chlL*, *rbcL*, and *rps4*) plastid dataset. Posterior probabilities ( $\geq 0.50$ ) are provided at the nodes. Note that species now treated in *Tryonia* (black arrowhead) are distinct from *Jamesonia*, the genus in which these species were most recently placed. Numbers provided for *Tryonia myriophylla* samples are Fern Lab Database voucher numbers (Table 1).

hypothesis that *Jamesonia sensu stricto* was derived from within *Eriosorus* (Tryon 1962, 1970) has received considerable backing. In our combined analysis, we too find strong support for a clade containing most sampled *Eriosorus* and *Jamesonia sensu stricto* species (Fig. 5). Additionally, we find strong support for some of its constituent internal nodes, which indicate that neither *Eriosorus* nor *Jamesonia sensu stricto* is monophyletic. Phylogenetic analyses incorporating a more comprehensive sample of taxa and a greater number of markers will ultimately be necessary to fully understand evolutionary relationships within this clade. However, based solely on the evidence to date, it is abundantly clear that *Jamesonia* and *Eriosorus* (as typically circumscribed) cannot both be recognized, assuming monophyly as a criterion for generic delimitation. With *Jamesonia* being the older name (published in 1830, versus 1852 for *Eriosorus*), the recombination of all known species of *Eriosorus* into *Jamesonia* in Christenhusz et al. (2011) was mostly warranted.

*Eriosorus myriophyllus* was shown by Prado et al. (2007), Sánchez-Baracaldo (2004b), and Schneider et al. (2013) to be isolated relative to most other species previously assigned to *Eriosorus* or *Jamesonia sensu stricto*. Here, we find *E. myriophyllus* and two previously unsampled species of *Eriosorus* to be more closely related to *Austrogramme*, *Pterozonium*, *Syngamma*, and *Taenitis* than to *Jamesonia* (as newly circumscribed herein, Fig. 5). Support for this relationship is strong (BPP = 1.00) and the implications are significant if monophyly is used as a criterion for generic delimitation. Because the type of *Jamesonia* (*Jamesonia pulchra* Hook. & Grev.) is resolved well within the large *Jamesonia* clade and the type of *Eriosorus* (*E. aureonitens* (Hook.) Copel.) shows clear morphological and geographical affinities to this clade, and because there are no other generic names available for the *E. myriophyllus* group, we here describe a new genus—*Tryonia* (see below)—to accommodate the isolated species.

In her monograph of *Eriosorus*, Tryon (1970) identified several small groups of closely allied species. Among these was the species pair of *E. myriophyllus* and *E. sellowianus* (with *E. schwackeanus* considered by her to be a synonym of *E. sellowianus*). This group corresponds perfectly to our proposed circumscription of *Tryonia*. We find *E. myriophyllus*, *E. schwackeanus* (which we consider to be distinct from *E. sellowianus*), and the recently described *E. arenitcola* (Schwartzburd and Labiak 2008) to form a genetically isolated clade of closely related species (Fig. 5). New combinations for these species, along with the unsampled *E. sellowianus*, are provided below.

Based on our current dataset, we do not consider the precise phylogenetic position of *Tryonia* (within the *Austrogramme*, *Pterozonium*, *Syngamma*, *Taenitis*, and *Tryonia* clade) to be fully resolved. Although our combined analysis clearly places *Tryonia* sister to *Austrogramme*, *Syngamma*, and *Taenitis* (collectively), this relationship is not well supported in any single-gene analysis. The *atpA* and *rbcL* datasets do place *Tryonia* sister to *Taenitis* (*atpA* and *rbcL* sequences were not available for *Austrogramme* and *Syngamma*), but support is lacking (BPP = 0.61 and 0.83, respectively). Likewise, the *rps4* dataset resolves *Tryonia* as sister to *Austrogramme*, *Syngamma*, and *Taenitis* without significant support (BPP = 0.88). Strong single-gene support for the precise

position of *Tryonia* only comes from the *chlL* dataset, where *Tryonia* is most closely related to *Pterozonium* (BPP = 1.00).

Two of the species of *Tryonia* included in our phylogenetic analysis (*T. areniticola* and *T. schwackeana*) are endemic to Brazil; the third sampled species (*T. myriophylla*) also occurs in Uruguay, near its border with the Brazilian state of Rio Grande do Sul. Although the Andes are the center of diversity for *Jamesonia* (as newly circumscribed herein), this genus is not entirely geographically distinct from *Tryonia*. In the recently published *Catálogo de Plantas e Fungos do Brasil*, a total of nine species are ascribed to *Eriosorus* or *Jamesonia* (Prado 2010). Only three of these species noted for Brazil (*E. areniticola*, *E. myriophyllus*, and *E. schwackeanus*) are resolved as sister to *Austrogramme*, *Syngramma*, and *Taenitis*. We found *Eriosorus cheilanthoides*, *E. insignis*, and *J. brasiliensis* to be embedded within the *Jamesonia* clade (Fig. 5) and *E. rufescens* was resolved within *Jamesonia* in an earlier study (Sánchez-Baracaldo 2004b). As for the remaining Brazilian species that have yet to be included in a phylogenetic study, one (*E. sellowianus*) shows clear morphological affinities to, and is here considered to be a member of, *Tryonia*; the other (*E. biardii*) appears, based on morphology, to be best accommodated in *Jamesonia*. Regardless of the ultimate phylogenetic placement of these two unsampled species, the genus *Tryonia* can be described as wholly endemic to Brazil and Uruguay.

## Taxonomy

### *Tryonia* Schuettp., J.Prado & A.T.Cochran, gen. nov.

urn:lsid:ipni.org:names:77136217-1

<http://species-id.net/wiki/Tryonia>

Figs 4, 6–9

*Similar to some species of Jamesonia, but with stramineous rather than castaneous rachises.*

**Type.** *Tryonia myriophylla* (Sw.) Schuettp., J.Prado & A.T.Cochran, comb. nov., *Gymnogramma myriophylla* Sw., Kongl. Vetensk. Acad. Handl. 1817(1): 58. 1817.

**Description.** Plants terrestrial, rupicolous, or saxicolous. Rhizomes creeping to erect at apex, compact, with appressed hairs or crispate bristles, sometimes rigid, ruddy brown, darker at the base. Fronds erect, 6–100 cm long; petioles terete or sulcate adaxially, brown at base and stramineous distally, from 1/8 as long to equal the length of the lamina, densely to sparsely pubescent, the hairs short and erect or long and crispate, hyaline or reddish brown at the cell junctions, glandular or non-glandular; laminae linear to elongate-triangular, 1 or 2-pinnate-pinnatisect to 1–3-pinnate-pinnatifid, 4.0–48 cm long, 1.0–14 cm wide, determinate; rachises straight, sometimes slightly flexuous, terete or sulcate adaxially, stramineous, pubescent, the hairs like those of the petioles; pinnae ascending to patent to the rachis, oblong to deltate, 0.5–10 cm long, 0.5–5 cm wide, membranaceous to herbaceous, densely to sparsely pubescent on both surfaces, the hairs glandular, hyaline or with the terminal cell light to dark red-



**Figure 6.** *Tryonia arenitcola* (Schwartzsb. & Labiak) Schuettp., J.Prado & A.T.Cochran. Schwartzburd 487 (SP), inset detail of (stramineous) rachis magnified 4x.

dish brown, 2–5-celled, or hairs non-glandular, hyaline or reddish brown at the cell junctions, 2–5(–7)-celled; ultimate segments entire and round or emarginate; veins free. Sporangia borne along the veins, short-stalked, stalks 1–2-celled, stomia with 2–4 indurated cells; spores trilete, tetrahedral-globose, with an equatorial flange, distal face coarsely tuberculate, proximal face with prominent ridges, brown, 40–60 µm (Fig. 9).

**Etymology.** The generic name honors Dr. Alice Faber Tryon, who made extraordinary contributions to fern systematics and published taxonomic revisions of both *Jamesonia sensu stricto* and *Eriosorus* (from which *Tryonia* is segregated herein).

**Distribution.** *Tryonia* occurs primarily in southeastern Brazil. However, one species (*T. myriophylla*) can also be found in Uruguay (Cerro Largo: Sierra Souza), near the Brazilian border. The genus is mostly restricted to the Atlantic Forest, along shaded streams, on damp shaded sandstone, or in more open places (but here shaded by shrubs); 600–2300 m.

**Discussion.** *Tryonia* can be distinguished most readily from *Jamesonia* by its stramineous rachises, but its gross morphology is also reasonably distinct. Tryon (1970) referred to the leaves of *T. myriophylla* as “generalized” (i.e., elongate-triangular and well developed). She drew a distinction between them and the “specialized” (i.e., either complex and scandent or compact and linear) leaves of *Jamesonia sensu stricto* and many other species at the time placed in *Eriosorus*, as well as between them and the “intermediate” (i.e., falling between the two extremes) leaves of other species she treated in *Eriosorus*. Although the Andean *Jamesonia congesta* also has “generalized” leaves, it is readily distinguished from *Tryonia* by its rachis color. The only species of *Jamesonia* with occasionally stramineous rachises (*J. flexuosa*) has “specialized” (complex and scandent) leaves. Spores of *Tryonia* (Fig. 9) and *Jamesonia* are basically indistinguishable.

*Tryonia* comprises the following species.

***Tryonia areniticola* (Schwartzb. & Labiak) Schuettp., J.Prado & A.T.Cochran, comb. nov.**

urn:lsid:ipni.org:names:77136218-1

[http://species-id.net/wiki/Tryonia\\_areniticola](http://species-id.net/wiki/Tryonia_areniticola)

Figs 6, 9

Synonym: *Jamesonia areniticola* (Schwartzb. & Labiak) Christenh. (Phytotaxa 19: 20. 2011).

**Basionym.** *Eriosorus areniticola* Schwartzb. & Labiak (Amer. Fern J. 98: 160. 2008).

**Type. Brazil:** Paraná: Jaguariaíva: Parque Estadual do Cerrado, 12 April 1994, P.H. Labiak 182 (holotype: UPCB; isotypes: SP!, UC).

**Distribution.** Brazil: Paraná, Rio Grande do Sul, Santa Catarina (probably), and São Paulo.

**Discussion.** Based on the gene regions included in our analysis, we found *Tryonia areniticola* to be genetically indistinguishable from *T. myriophylla*, despite the presence of several morphological differences (Schwartzburd and Labiak 2008). Further studies that include nuclear markers will be necessary.



**Figure 7.** *Tryonia schwackeana* (Christ) Schuettp., J.Prado & A.T.Cochran. Schuettelpelz 1433 (MO), inset detail of (stramineous) rachis magnified 4×. Image modified from <http://www.tropicos.org/Image/100140486>.



**Figure 8.** *Tryonia sellowiana* (Kuhn) Schuettp., J.Prado & A.T.Cochran. Mulford 710 (US), inset detail of (stramineous) rachis magnified 4x.

***Tryonia myriophylla* (Sw.) Schuettp., J.Prado & A.T.Cochran, comb. nov.**

urn:lsid:ipni.org:names:77136219-1

[http://species-id.net/wiki/Tryonia\\_myriophylla](http://species-id.net/wiki/Tryonia_myriophylla)

Figs 4, 9

Synonyms: *Psilogramme myriophylla* (Sw.) Kuhn (Festschr. 50 Jähr. Jub. Königstädt. Realschule Berlin 339. 1882); *Eriosorus myriophyllus* (Sw.) Copel. (Gen. Fil. 58. 1947); *Jamesonia myriophylla* (Sw.) Christenh. (Phytotaxa 19: 21. 2011).

**Basionym.** *Gymnogramma myriophylla* Sw. (Kongl. Vetensk. Acad. Handl. 1817(1): 58. 1817).

**Type. Brazil:** [Minas Gerais]: Villa Rica [now Ouro Preto], Aug 1815, *G. W. Frey-riss s.n.* (lectotype [designated by Tryon, 1970]: S-R-2467, image!; isolectotypes: BM 000936677, image!, S-R-2469, image!).

**Distribution.** Brazil: Bahia, Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina, São Paulo, and Rio Grande do Sul. Uruguay: Cerro Largo.

***Tryonia schwackeana* (Christ) Schuettp., J.Prado & A.T.Cochran, comb. nov.**

urn:lsid:ipni.org:names:77136220-1

[http://species-id.net/wiki/Tryonia\\_schwackeana](http://species-id.net/wiki/Tryonia_schwackeana)

Fig. 7

Synonym: *Eriosorus schwackeanus* (Christ) Copel. (Gen. Fil. 59. 1947).

**Basionym.** *Gymnogramma schwackeana* Christ in Schwacke (Pl. Nov. Mineiras 2.18. 1900).

**Type. Brazil:** [Minas Gerais]: Ouro Preto, *C.A.W. Schwacke 9389* (lectotype [designated by Tryon, 1970]: P 00603566, image!; isolectotype: GH 00021287, image!).

**Distribution.** Brazil: Bahia and Minas Gerais.

***Tryonia sellowiana* (Kuhn) Schuettp., J.Prado & A.T.Cochran, comb. nov.**

urn:lsid:ipni.org:names:77136221-1

[http://species-id.net/wiki/Tryonia\\_sellowiana](http://species-id.net/wiki/Tryonia_sellowiana)

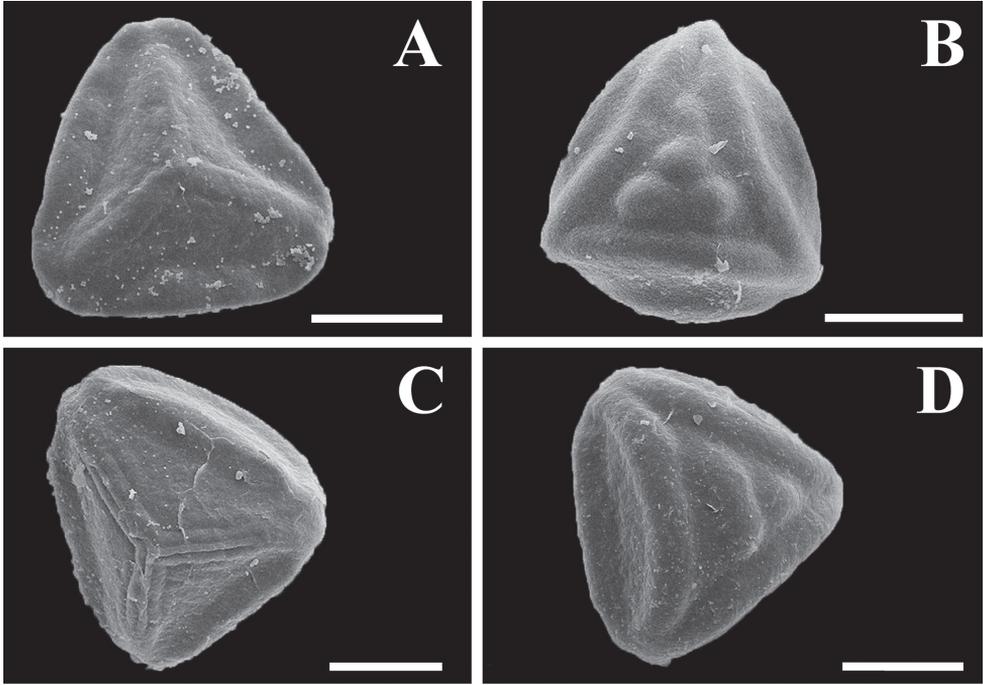
Fig. 8

Synonyms: *Psilogramme sellowiana* (Mett. ex Kuhn) Kuhn (Festschr. 50 Jähr. Jub. Königstädt. Realschule Berlin 339. 1882); *Eriosorus sellowianus* (Mett. ex Kuhn) Copel. (Gen. Fil. 59. 1947); *Jamesonia sellowiana* (Mett. ex Kuhn) Christenh. (Phytotaxa 19: 21. 2011).

**Basionym.** *Gymnogramma sellowiana* Mett. ex Kuhn (Linnaea 36:69. 1869).

**Type.** Brazil, *Sello 1365* (lectotype [designated by Tryon, 1970]: B-Herb. Mett., image!; isolectotype: B, image!)

**Distribution.** Brazil: Minas Gerais.



**Figure 9.** Spores of *Tryonia*. **A** *Tryonia myriophylla* proximal view, Wacket s.n. (US) **B** *Tryonia myriophylla* distal view, Wacket s.n. (US) **C** *Tryonia areniticola* proximal view, Kummrow 2773 (US) **D** *Tryonia areniticola* distal view, Kummrow 2773 (US). All scale bars are 20  $\mu$ m.

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# Two new desert *Eschscholzia* (Papaveraceae) from southwestern North America

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## Abstract

Two new species of *Eschscholzia* are described. Both are found in the deserts of California and one extends outside the state boundary into Arizona. *Eschscholzia androuxii* Still, **sp. nov.** is found mainly in and around Joshua Tree National Park in Riverside and San Bernardino counties. *Eschscholzia papastillii* Still, **sp. nov.** is found from the northern Mojave south through Joshua Tree National Park to central Imperial County. Both are annuals found in coarse, sandy soil and have yellow flowers typical of desert *Eschscholzia*. *Eschscholzia papastillii* has an expanded receptacular rim similar to that of *E. californica*. *Eschscholzia androuxii* has anthocyanin bands around the stamen filaments.

## Keywords

Arizona, California, *Eschscholzia*, Papaveraceae, new species, *Eschscholzia androuxii*, Joshua Tree poppy, *Eschscholzia papastillii*, cryptic desert poppy, Mojave Desert, Colorado Desert, Sonoran Desert

## Introduction

*Eschscholzia* Cham. (1820) is a genus in the Papaveraceae tribe Eschscholtzieae along with the genera *Hunnemannia* Sweet and *Dendromecon* Benth. The type genus is native to the mainland and islands of western North America in both the United States and Mexico, but the type species, *Eschscholzia californica* Cham. has invaded Mediterranean regions around the world. The taxa are native to mesic and xeric landscapes. Recent treatments for *Eschscholzia* (Clark 1997, Roberts 1989) recognize 12 species

and several subspecies for a total of 16 taxa. Recent phylogenetic work (Still and Potter 2013) indicates 18 distinct taxa. While the genus is fairly small, there are nearly 198 taxon names and 168 type specimens, with the majority described by EL Greene (1905). The majority of described taxa are synonymous with *E. californica*.

All taxa are herbaceous annuals or perennials with taproots and basal rosettes. The leaves are ternately-dissected 2–many times and range from bright green, dark green to glaucous grey-green. Flowers are bisexual, have two sepals fused into a single cap structure, four petals and many stamens. The sepals fall from the flower upon opening. The flowers are yellow, orange or can be yellow with an orange basipetal spot on each petal. The desert taxa of the genus can be difficult to identify (personal experience) and this resulted in further morphological and molecular examination of *Eschscholzia* (Still 2011, Still and Potter 2013). In the course of study, two new taxa were discovered among *Eschscholzia* native to desert regions (Still 2011). These two taxa exhibited both morphological traits and mutations in nucleotide sequences from nuclear and plastid regions that distinguish them from one another and from all previously described species of *Eschscholzia*. The two new taxa are here described as new species.

## Taxonomy

### *Eschscholzia androuxii* Still, sp. nov.

urn:lsid:ipni.org:names:77136479-1

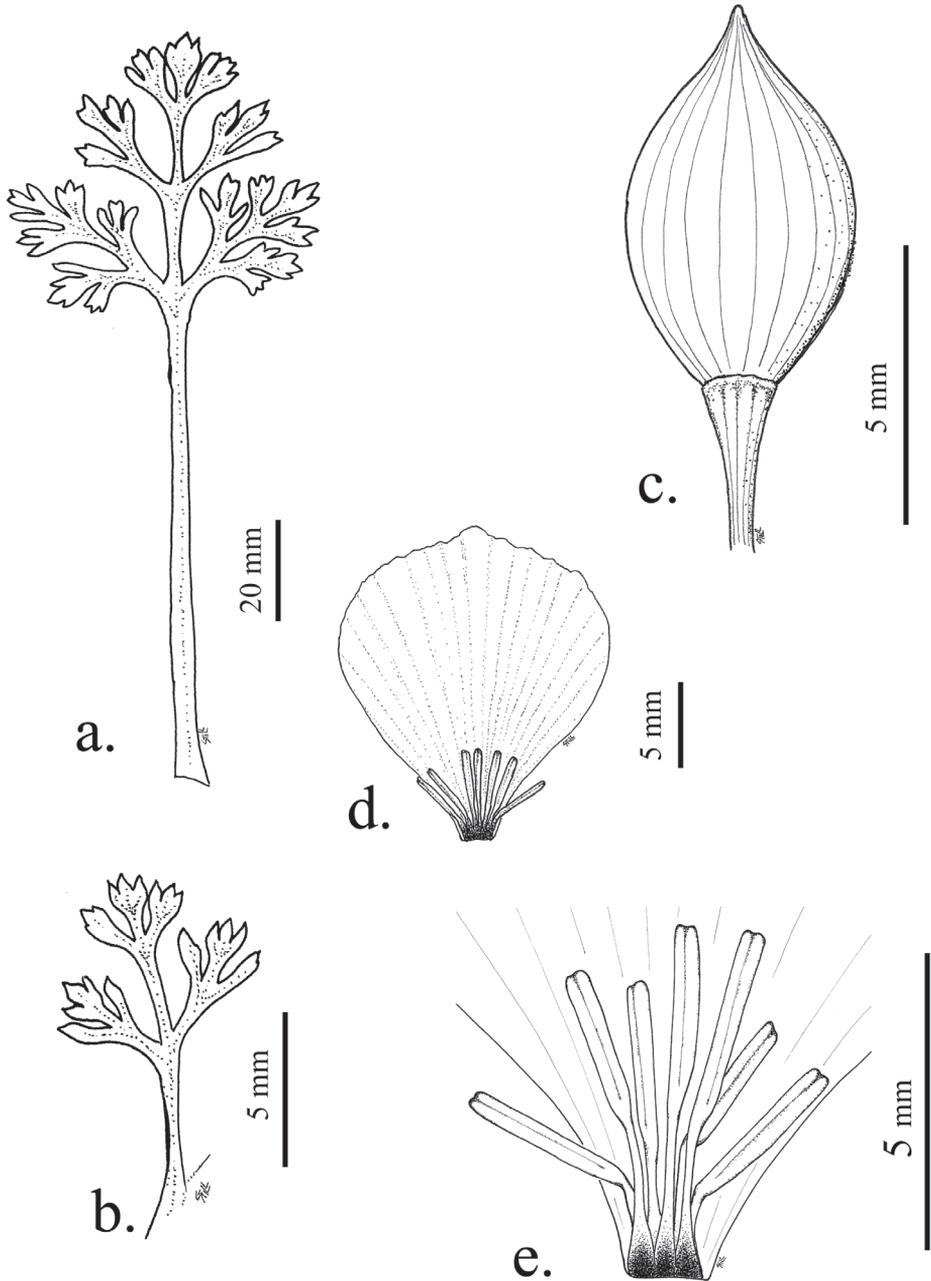
[http://species-id.net/wiki/Eschscholzia\\_androuxii](http://species-id.net/wiki/Eschscholzia_androuxii)

Figs 1–3

**Type.** UNITED STATES, California: Riverside County, just south of entrance sign to Joshua Tree National Park heading north on Cottonwood Springs Road from US Interstate-10, [33°41.188'N, 115°48.103'W], 610 m alt., 14 Feb 2008, *Shannon M. Still 258A with Jennifer Still and Charles Still* (holotype: DAV!).

**Diagnosis.** *Eschscholzia androuxii* is similar to *E. minutiflora* subsp. *twisselmannii* C. Clark & M. Faull but with ultimate lobes of the dissected leaves more numerous and narrower than *E. minutiflora* subsp. *twisselmannii*. *Eschscholzia androuxii* is similar to *E. minutiflora* subsp. *minutiflora* S. Watson and *E. minutiflora* subsp. *covillei* (E. Greene) C. Clark but with larger flowers and consistently appearing, pronounced black-blue or darkened anthocyanin area or spot basipetally located on the fused filament bases of the stamens. *Eschscholzia androuxii* differs from *E. papastillii* and *E. parishii* with the aforementioned stamen spot and basal foliage that appears more compact in habit.

**Description.** Annual herb, erect or spreading with a basal rosette of leaves from a taproot. *Leaves* highly ternately-dissected into a great number of ultimate lobes, which may number to 100 on larger specimens. Leaves glaucous-green with ultimate lobes more rounded than pointed. Basal leaves are 3–11 cm long and 0.8–3.2 cm wide and held on a petiole comprising 2/3 the entire leaf length. Younger plants will have fewer ultimate lobes and shorter, narrower leaves. *Inflorescence* with few flowers held above



**Figure 1.** Illustrations of leaves, buds and flowers of *Eschscholzia androuxii*. **A** Basal leaf **B** Cauline leaf **C** Bud **D** Petal **E** Stamens showing diagnostic anthocyanin spot at base of the fused filaments.

the foliage and to 4 dm above the ground. Leaves on the inflorescence are 2–20 mm long and are divided into 2–23 ultimate lobes. *Buds* nodding and 4.5–11.5 mm long with an apiculate bud tip less than 25% of the total bud length. Less mature buds may

be shorter than average with a longer bud tip by percentage. *Flowers* held upright and are yellow with four petals 10.5–23 mm long. Each flower has 20–36 stamens fused at the base. There is a darkened area or patch, often black-blue, located at the fused filament bases of the stamens. Receptacles obconic and 2.5–5.5 mm long and 1.1–3 mm wide and often have a scarious inner hyaline rim. *Fruit* 3.5–6.5 cm long with 10–12 nerves, dehiscing at maturity. Seeds with reticulate ridges.

**Distribution** (Fig. 3). Found in and around Joshua Tree National Park in both Riverside and San Bernardino counties of California.

**Habitat and ecology.** Desert washes, flats, and slopes in coarse, sandy soil.

**Phenology.** *Eschscholzia androuxii* typically flowers between late-February and early-May but may flower earlier in the season, including in the fall, during years with a summer rain and cool fall temperatures.

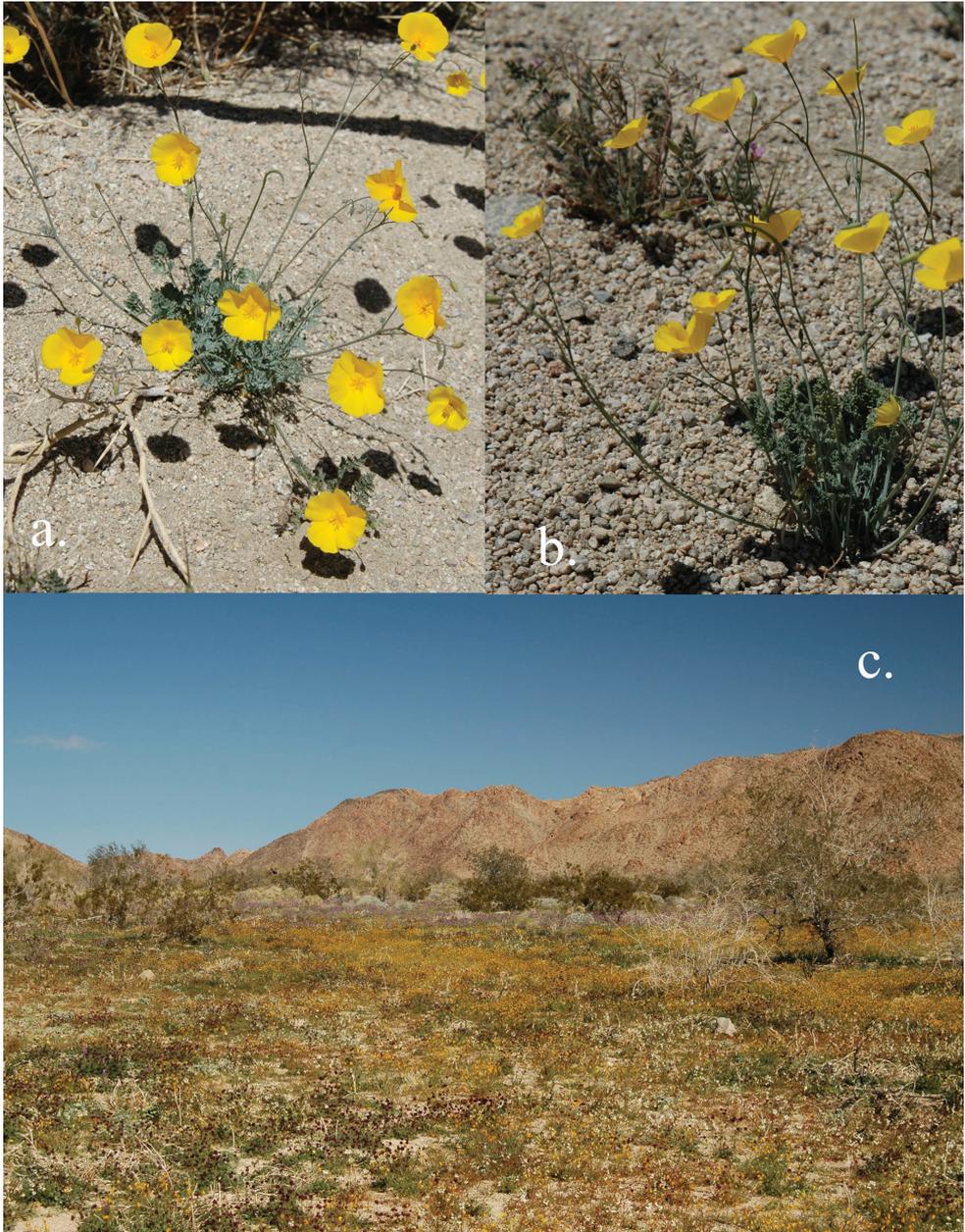
**Etymology.** The species is named for James André and Tasha La Doux, two desert botanists and friends that helped point to the problems with desert *Eschscholzia* identification.

**Suggested common name.** Joshua Tree poppy.

**Conservation status.** As this is a new taxon it has yet to be considered for conservation status. Due to the limited range and low number of occurrences, the author suggests the California Native Plant Society consider this taxon for listing as a rare plant.

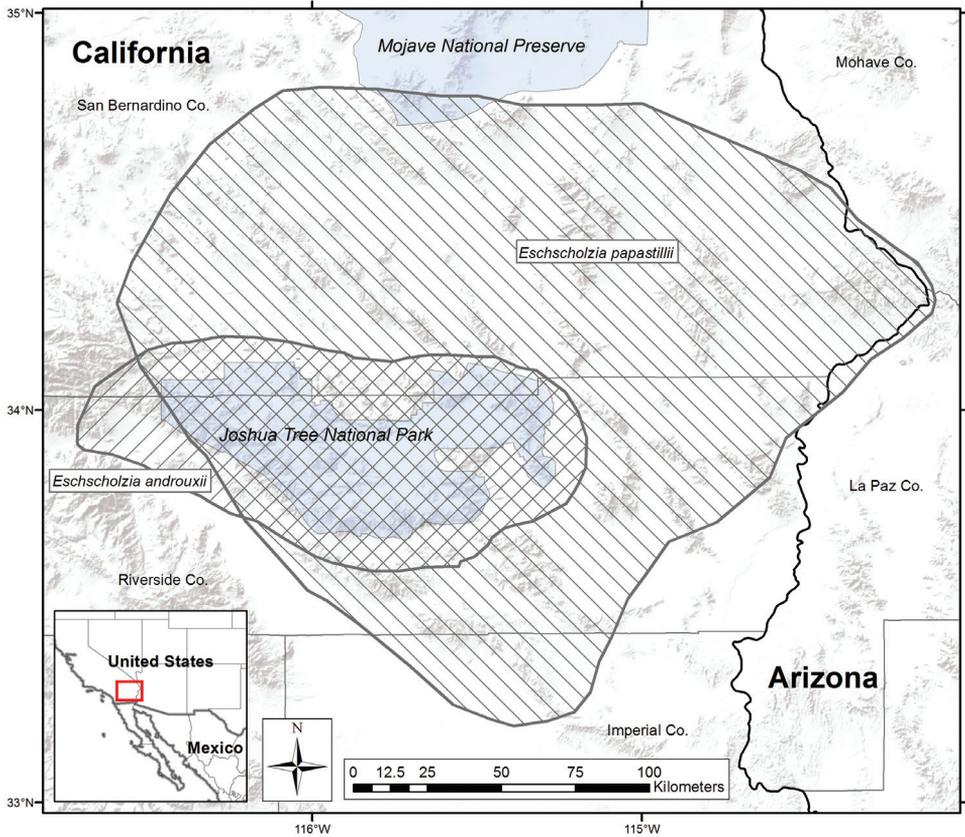
**Specimens examined. U.S.A. California:** Riverside Co.: White Water, Apr 1907, *S.B. Parish 6103* (DS!); slope of hill at west side of mouth of Whitewater Canyon, 18 Mar 1962, *D.W. Kybos 62-43* (DS!); Cottonwood Pass, Joshua Tree National Monument, 19 Mar 1949, *C. Francis Shutts 58* (ASU!, DES!); Coachella Valley, Desert Hot Springs, N of intersection of Pierson Blvd. and Atlantic, north of flood control ditch, 6 Apr 2001, *A.C. Sanders, Mitch Provance & T.B. Salvato 23939* (DES!); Joshua Tree National Park, [33°41.18333'N, 115°48.1'W], 15 Feb 2008, *Shannon M. Still, Jennifer R. Still, & Charles M. Still 258B* (DAV!); id., Cottonwood Wash on west side of road, [33°41.21299'N, 115°48.15100'W], 22 Feb 2009, *Shannon M. Still 444* (DAV!); id., [33°41.833'N, 115°48.17598'W], 3 Mar 2009, *Shannon M. Still & Robert Lee 457* (DAV!); id., [33°50.22799'N, 115°45.174'W], 28 Mar 2009, *Shannon M. Still, Steven M. Still & Carolyn M. Still 512* (DAV!); San Bernardino Co.: 1.4 mi N of Yucca Valley on road to Lucerne Valley. About 19 mi west of town of Twentynine Palms, 6 Apr 1957, *John H. Thomas 6627* (DS!).

**Discussion.** This new taxon has a darkened area basipetally located on the stamen filaments (Fig. 1e), which are fused at the base. *Eschscholzia minutiflora* subsp. *twisselmannii* also has regularly occurring stamen spots, but only on approximately 70% of specimens examined. No other closely related taxa have these stamen spots. The flower size for this new species is similar to that of the diploid *E. minutiflora* subsp. *twisselmannii* but larger than both the hexaploid *E. minutiflora* subsp. *minutiflora* and the tetraploid *E. minutiflora* subsp. *covillei*. The petal size for *E. minutiflora* subsp. *covillei*, described in Flora of North America as 6–18 mm long, does overlap with the petal size of *E. androuxii*, with petals 10–23 mm long. But more recent morphological study of the genus (Still 2011, Still in preparation) indicates that the petals in *E. minutiflora*



**Figure 2.** Photographs of *Eschscholzia androuxii*. **A** Species profile shot **B** Species profile shot **C** *Eschscholzia androuxii* in the type area in a heavy-flowering year.

subsp. *covillei* range from 4.5–12.5 mm. The reason for this discrepancy may be that some of the larger-flowered *E. minutiflora* subsp. *covillei* specimens are actually the new taxon, *E. androuxii*. The Joshua Tree poppy has an overlapping range with several species but is found only in Riverside Co. and the southern part of San Bernardino Co.



**Figure 3.** Distribution map showing the range for the two new *Eschscholzia*. *Eschscholzia androuxii* is represented by right-slanting cross-hatching. *Eschscholzia papastillii* is represented by left-slanting cross-hatching.

and not much further north or south of Joshua Tree National Park. The range does not overlap, and there are more basal leaf ultimate lobes, than with *E. minutiflora* subsp. *twisselmannii*. The tips of the basal leaf ultimate lobes are more rounded (Fig. 1a) than what is found in either *E. parishii* or *E. papastillii* (Fig. 4a), and *E. androuxii* has three times the number of cauline leaf ultimate lobes (Fig. 1b) as these two taxa.

***Eschscholzia papastillii* Still, sp. nov.**

urn:lsid:ipni.org:names:77136480-1

[http://species-id.net/wiki/Eschscholzia\\_papastillii](http://species-id.net/wiki/Eschscholzia_papastillii)

Figs 3–5

**Type. UNITED STATES, California:** Riverside County, Joshua Tree National Park next to stone outcropping off Old Dale Road. [33°50.232'N, 115°45.12'W], 724 m alt., 19 Apr 2009, *Shannon M. Still 546A* (holotype: DAV!).

**Diagnosis.** *Eschscholzia papastillii* is similar to *E. parishii* Greene but with more basal leaf ultimate lobes and more broadly spreading leaves. *Eschscholzia papastillii* has an enlarged receptacle (Fig. 4d) that is widely-obconic or bell-shaped, and wider at the midpoint of the receptacle than *E. parishii*, *E. androuxii* or any of the subspecies of *E. minutiflora*, which are usually more obconic or funnel-shaped. The expanded receptacular rim of *E. papastillii* is similar, but typically smaller, than the expanded receptacular rim of *E. californica*. *Eschscholzia papastillii* differs from *E. androuxii* and *E. minutiflora* with basal foliage that appears less compact in habit.

**Description.** Annual herb, erect or spreading with a basal rosette of leaves from a taproot. *Leaves* highly ternately-dissected into 17–70 ultimate lobes with the higher number on larger specimens. Leaves glaucous-green to green with ultimate lobes more pointed than rounded. Basal leaves are 2.7–16 cm long and 0.9–7 cm wide and held on a petiole comprising 2/3 the entire leaf length. Younger plants have few basal leaf ultimate lobes and shorter, narrower leaves. *Inflorescence* with few flowers held above the foliage and to 5 dm above the ground. Leaves on the inflorescence are 3–50 mm long and are divided into 1–13 ultimate lobes. *Buds* nodding to erect and 2.5–16 mm long with an apiculate bud tip greater than 30% of the total bud length. Less mature buds may be shorter than average with a longer bud tip by percentage. *Flowers* held upright and are yellow with four petals 5–24 mm long. Each flower has 12–32 stamens fused at the base. Receptacles widely-obconic or funnel-shaped to nearly bell-shaped, 3–9 mm long and 1.5–4.7 mm wide. Receptacular rim typically noticeable and often thick but can be scarious, expanded laterally up to 1.2 mm from the top of the receptacle. The receptacle often has a scarious inner hyaline rim in addition to the outer rim diagnostic of the species. *Fruit* 4–8 cm long with 10–12 nerves, dehiscing at maturity. Seeds with reticulate ridges.

**Distribution** (Fig. 3). Found north to the northern Mojave Desert; south into northern Colorado Desert of San Diego Co., and possibly south along the east side of the Sea of Cortez in Mexico; east to the California-Arizona border (Whipple Mountains); west to the western end of Joshua Tree National Park.

**Habitat and ecology.** Desert washes, flats, and gentle slopes in coarse, sandy soil.

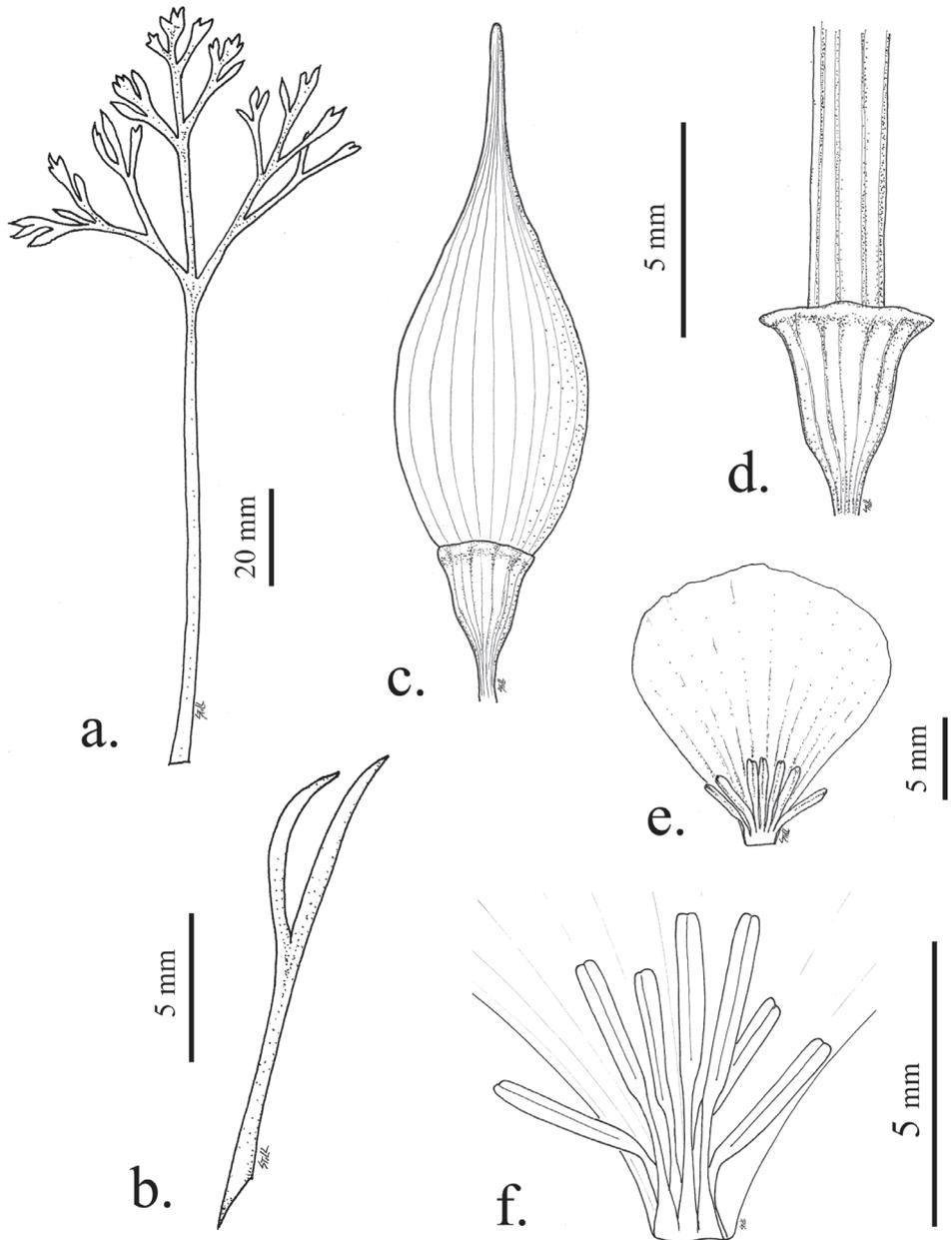
**Phenology.** *Eschscholzia papastillii* typically flowers between late-February and early-May but may flower earlier in the season, and in the fall, during years with a summer rain and cool fall temperatures.

**Etymology.** The species is named in honor of Dr. Steven Still, my father and mentor and the reason for which I study plants.

**Suggested common name.** Cryptic desert poppy.

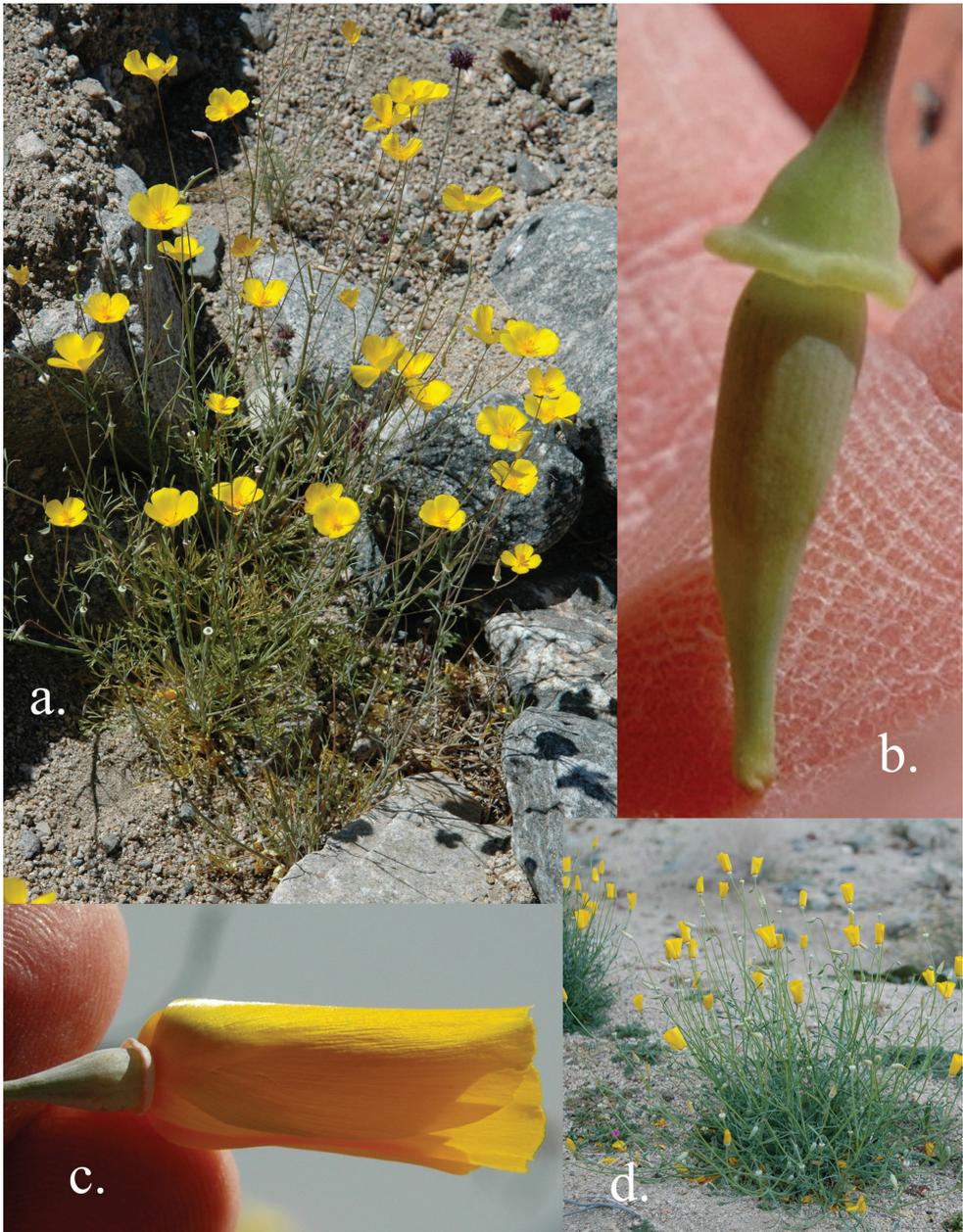
**Conservation status.** As this is a new taxon it has yet to be considered for conservation status. Due to the range and number of occurrences the author does not suggest this taxon be considered for conservation status.

**Specimens examined. U.S.A. California:** Kern Co.: Hidden Springs Rd., 6 May 1930, *Lester Rowntree s.n.* (CAS!); Riverside Co.: Painted Canyon, 4 Mar 1922, *Edmund C. Jaeger s.n.* (DS!); Painted Canyon, 12 Apr 1927, *Frank W. Peirson 7167* (CAS!); near Shavers Well, 6 Apr 1930, *R.A. Piebles and H.F. Loomis 188* (DS!);



**Figure 4.** Illustrations of leaves, buds and flowers of *Eschscholzia papastillii*. **A** Basal leaf **B** Cauline leaf **C** Bud showing widened receptacle **D** Enlarged receptacle with expanded receptacular rim common in the species **E** Petal **F** Stamens lacking the anthocyanin spot at base of the fused filaments common to *E. androuxii*.

Box Canyon, Coachella Valley, 21 Mar 1937, *Ynez Whilton Winblad s.n.* (CAS!); Coachella Valley, 21 Mar 1937, *Ynez Whilton Winblad s.n.* (CAS!); east slope of Chocolate Mnts, 22 Mar 1937, *Ynez Whilton Winblad s.n.* (CAS!); above Cotton-



**Figure 5.** Photographs of *Eschscholzia papastillii*. **A** Species profile shot **B** Buds with enlarged receptacle common in the species **C** Flower with enlarged receptacle common in the species **D** Species profile shot.

wood Springs, west end of Eagle Mnts, 13 Apr 1949, *Philip A. Munz 13056* (CAS!); Road to Morongo Valley, 7.8 mi from junction with Highway 99/60/70, 14 Apr 1952, *Richard Snow* (DS!); Box Canyon, 5 Apr 1953, *Richard Snow 51a* (DS!); 0.8

mi east of Cactus City, 13 mi east of Coachella, near U.S. Highways 60 and 70. Colorado Desert, 30 Mar 1957, *John H. Thomas 6523A* (DS!); U.S. Highway 60/70, Indio to Blythe, 1 mi. W of Cactus City, 29 Apr 1958, *P.C. Everett and E.K. Balls 23013* (CAS!, DAV!); East of Indio on Highway 60/70, about 6 mi west of Cactus City, 15 Mar 1960, *W.R. Ernst 720* (CAS!); Mecca-Joshua Tree Road, 7 miles southwest of junction with Interstate Highway 10, 13 Apr 1976, *Curtis Clark 527* (DAV!); 2 mi S.E. of Desert Center, 1 Jul 1981, *J.C. Roos s.n.* (ASU!, CAS!); wash along Eagle Mountain Rd., north along I-10, 3 Mar 1995, *John Wear s.n.* (DAV!); Joshua Tree National Park, [33°43.68999'N, 115°49.317'W], 14 Feb 2008, *Shannon M. Still 253 with Jennifer R. Still, Charles M. Still* (DAV!); id., [33°55.012'N, 115°52.60305'W], 3 Mar 2009, *Shannon M. Still 452 with Robert Lee* (DAV!); id., [33°50.22799'N, 115°45.174'W], 28 Mar 2009, *Shannon M. Still 513 with Steven M. Still, Carolyn M. Still* (DAV!); San Bernardino Co.: Sheephole Mnts., 8 Apr 1935, *P.A. Munz 13823* (DS!); Cave Spring, Lower Sonoran zone, 16 Apr 1940, *C.L. Hitchcock 6073* (DS!); Bristol Lake Basin 8.8 mi N. of summit of Sheephole Pass on Amboy Rd., 16 Mar 2001, *A.C. Sanders, Mitch Provance & Petra Wester 23753* (CAS!); Sheephole Pass just to south of the top of the pass, [34°13.711'N, 115°43.19599'W], 25 Nov 2007, *Shannon M. Still 222A* (DAV!); Base of Old Dad Mountains in wash, [34°44.512'N, 115°45.081'W], 26 Nov 2007, *Shannon M. Still, Jim André & Tasha La Doux 248* (DAV!); Base of Old Dad Mountains in wash, [34°44.512'N, 115°45.082'W], 6 Apr 2008, *Shannon M. Still & Steven M. Still 377A* (DAV!); Clipper Mountains, just off the pipeline road, [34°40.573'N, 115°22.73502'W], 18 Apr 2009, *Shannon M. Still, Jim André, Jeff Galvin & Amy Toulson 536* (DAV!).

**Discussion.** While the buds of all desert *Eschscholzia* appear similar, those of *E. papastillii* most resemble *E. parishii*, as the bud tip is typically more than 25% of the total bud length. The receptacular rim is prominent in this species and *E. californica* is the only other species that has a pronounced receptacular rim. The range of *E. papastillii* extends from San Bernardino County south to northern Imperial County. Most collections of *E. parishii* collected north of San Diego and Imperial Counties are likely the new *E. papastillii*. *Eschscholzia papastillii* extends at least into easternmost San Bernardino County that contains the Whipple Mountains, and likely well into Arizona.

#### Key to the desert *Eschscholzia* species

- 1 Basal leaf ultimate lobes long-linear; leaves ternately-dissected 2–3×; flower scapes typically without cauline leaves; seed coats pitted without reticulations ..... *E. glyptosperma*
- 1' Basal leaf ultimate lobes not long-linear; leaves ternately-dissected 3–7×; flowers typically borne on few-flowered racemes with a cauline leaf at each flower axil; seeds coats reticulate
- 2 Receptacular rim prominent when in fruit, 0.25–5 mm

- 3 Basal leaf ultimate lobes with length < 3× width, with acute or rounded tips; leaf blades often deep green with a glaucous patch at the crotch of the leaf dissections; cauline leaf ultimate lobes many (range 5–30) with rounded to acute tips; Petals yellow, often with a basipetal orange spot, or petals orange
- 4 Petals yellow, often with a basipetal orange spot, or petals orange, or rarely white (Arizona mountains); cotyledons entire; annual; limited to eastern Mojave Desert in California and through Arizona ..... *E. californica* subsp. *mexicana*
- 4' Petals yellow, often with a basipetal orange spot, or petals orange; cotyledons bifid (2-lobed); annual or perennial; widespread but mostly along highways, railways, and planted areas ..... *E. californica* subsp. *californica*
- 3' Basal leaf ultimate lobes with length 3.5 (2–8)× width, with acute tips; leaf blades bright-green to yellow-green; cauline leaf ultimate lobes 3 (rarely 5–13) with acute tips; petals yellow without basipetal orange spot ..... *E. papastillii*
- 2' Receptacular rim not prominent in fruit, < 0.25 mm
- 4 Petal < 1 cm long
- 5 Buds with tip < 25% total bud length; cauline leaves generally with > 5 (rarely < 6) ultimate lobes, ± rounded to acute; 2n=24 or 36
- 6 Basal leaf ultimate lobes ± narrow, length ca. 4.5× the width; petals generally less than 5.5 (rarely 2–9) mm long, stamens 6–18, typ. 12; 2n=36 .....  
..... *E. minutiflora* subsp. *minutiflora*
- 6' Basal leaf ultimate lobes widened, length ca. 2.5× the width; petals generally greater than (5–) 9 (–12) mm long; stamens 6–18, typ. 14–16; 2n=24.....  
..... *E. minutiflora* subsp. *covillei*
- 5' Buds with tip > 25% total bud length; cauline leaves generally with ≤ 3 (rarely to 8) ultimate lobes, ± acute to acuminate; 2n=12 ..... *E. parishii*
- 4' Petals > 1 cm long
- 8 Bud tip generally > 30% length of bud; leaves bright-green to yellow-green, ultimate lobes ± acute to acuminate; cauline leaf reduced to one-few ultimate lobes
- 9 Receptacle 1–2 mm wide, obconic to funnel-shaped..... *E. parishii*
- 9' Receptacle 1.5–5 mm wide, widely-obconic to bell-shaped, often flaring at the end of the receptacle ..... *E. papastillii*
- 8' Bud tip generally < 20% length of bud; leaves more glaucous to grey-green, ultimate lobes ± round to acute; terminal cauline leaf typically with 5+ ultimate lobes
- 10 Basal leaves generally with 35–40 (rarely 26–60) ultimate lobes, and ultimate lobes ± widened appearance, length of ultimate lobes less than 2× width, cuneiform; (12–) 18–20 (–28) stamens often with anthocyanin spot at basipetal end of filaments fused at the base; plants of El Paso and Rand Mountains in Kern Co., California..... *E. minutiflora* subsp. *twisselmannii*
- 10' Basal leaves generally with 45–70 (rarely 26–55) ultimate lobes, length of ultimate lobes more than 2× width; stamens (16–) 22–24 (–32), with anthocyanin spot at basipetal end of filaments fused at the base; plants of Riverside and San Bernardino Counties in and around Joshua Tree National Park... *E. androuxii*

## Acknowledgements

I would like to thank James André, Tasha La Doux, Dan Potter, Steven Still, and the curatorial staff at the herbaria DAV, RSA, ND-G, SD, DES, ASU and UC for their inspiration and help with my research. I would also like to thank a few anonymous reviewers who helped edit the manuscript, species descriptions, and taxonomic key.

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# URJC GB dataset: Community-based seed bank of Mediterranean high-mountain and semi-arid plant species at Universidad Rey Juan Carlos (Spain)

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## Abstract

The Germplasm Bank of Universidad Rey Juan Carlos was created in 2008 and currently holds 235 accessions and 96 species. This bank focuses on the conservation of wild-plant communities and aims to conserve *ex situ* a representative sample of the plant biodiversity present in a habitat, emphasizing priority ecosystems identified by the Habitats Directive. It is also used to store plant material for research and teaching purposes. The collection consists of three subcollections, two representative of typical habitats in the center of the Iberian Peninsula: high-mountain pastures (psicroxerophylous pastures) and semi-arid habitats (gypsophylic steppes), and a third representative of the genus *Lupinus*. The high-mountain subcollection currently holds 153 accessions (63 species), the semi-arid subcollection has 76 accessions (29 species,) and the *Lupinus* subcollection has 6 accessions (4 species). All accessions are stored in a freezer at -18 °C in Kilner jars with silica gel. The Germplasm Bank of Universidad Rey Juan Carlos follows a qual-

ity control protocol which describes the workflow performed with seeds from seed collection to storage. All collectors are members of research groups with great experience in species identification. Herbarium specimens associated with seed accessions are preserved and 63% of the records have been georeferenced with GPS and radio points. The dataset provides unique information concerning the location of populations of plant species that form part of the psicroxerophylous pastures and gypsophylic steppes of Central Spain as well as populations of genus *Lupinus* in the Iberian Peninsula. It also provides relevant information concerning mean seed weight and seed germination values under specific incubation conditions. This dataset has already been used by researchers of the Area of Biodiversity and Conservation of URJC as a source of information for the design and implementation of experimental designs in these plant communities. Since they are all active subcollections in continuous growth, data is updated regularly every six months and the latest version can be accessed through the GBIF data portal at <http://www.gbif.es:8080/ipt/resource.do?r=germoplasma-urjc>. This paper describes the URJC Germplasm Bank and its associated dataset with the aim of disseminating the dataset and explaining how it was derived.

### Keywords

Accessions, germplasm bank, gypsum, high-mountain, Madrid, seeds, seedbank, semi-arid, Spain, Universidad Rey Juan Carlos, wild species

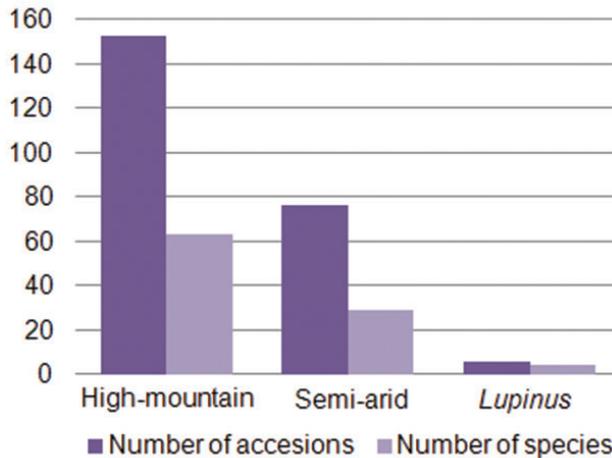
### Data published through GBIF

<http://www.gbif.es:8080/ipt/resource.do?r=germoplasma-urjc>

### Introduction

#### The URJC Germplasm Bank

The URJC Germplasm Bank (URJC GB) was created in 2008 and focuses on the conservation of wild plant communities. Until now, germplasm banks have conserved plant biodiversity and have promoted the use of plant genetic resources at the species, subspecies or variety level, conserving threatened taxa or taxa with some important traits. In these cases, the aim is to conserve the species or variety regardless of its origin or natural habitat. However, as the most appropriate way to conserve a biological entity is within the ecosystem that it naturally forms part of (Gómez-Campo 1985, Prance 1997), the concept of seed banks can be reconsidered, at least for wild species. In this context, the objective of this germplasm bank is to conserve *ex situ* a representative sample of the entire plant biodiversity characteristic of a habitat with special emphasis on habitats defined as priority habitats by the Habitats Directive. The URJC GB is used to conserve seeds for research projects and teaching purposes. It currently holds 235 accessions and 96 species. Figure 1 shows the number of accessions and species in each subcollection. Thus, there are 153 accessions and 63 species in the high-mountain subcollection, 76 accessions and 29 species in the semi-arid subcollection and 6 accessions and 4 species in the *Lupinus* subcollection.

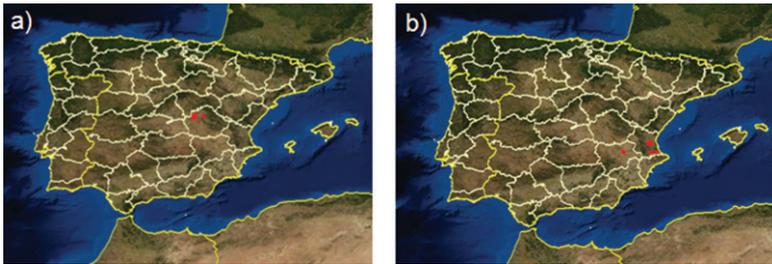


**Figure 1.** Number of accessions and species in the different subcollections of the URJC GB.

### The URJC GB dataset

Creating and managing a germplasm bank not only entails keeping the seed material under proper storage conditions, but also ensuring the correct administration of all the related data. After all, seed accessions with no associated information are virtually useless from a conservation and research perspective. Thus, it is essential for seed banks to obtain reliable data and record it in a database accessible to potential users. In addition to passport data on the location, time and other features of seed collection, additional data need to be gathered throughout the different activities involved in seed preservation, including seed processing, testing and storage. These data must be accurately compiled and efficiently managed to ensure the accuracy, consistency and wider relevance of the dataset. This information is necessary for both the management of the seed bank and for conservation and research studies on the species included in the seed bank.

Most global and national efforts in the dissemination of seed bank datasets are related to plant species of agricultural value. In this sense, it is worthy to mention the Germplasm Resources Information Network (GRIN) of the United States Department of Agriculture (<http://www.ars-grin.gov/>), and the EURISCO catalogue of the European Cooperative Programme for Plant Genetic Resources (ECPGR) (<http://eurisco.ecpgr.org/>), which provides information about *ex situ* plant collections maintained in Europe. Furthermore, many datasets are available at the genebank, national, sub-regional and regional levels (see Bettencourt (2011) for a detailed account). Concerning wild plant species seed banks, dataset availability is scarce. For instance, of the 13 wild plant germplasm banks in Spain, only BG JBB (Germplasm Bank of Botanic Garden of Barcelona) and URJC GB have currently published their dataset through GBIF, although there are ongoing efforts to make all this information available through the Spanish Network of Seed banks (REDBAG, <http://www.redbag.es/>). A good example



**Figure 2.** Distribution map of the two species of URJC GB dataset endemic to Spain: **a** *Helianthemum marifolium* ssp. *conquense* **b** *Lupinus mariae-josephae* (Source: <http://www.anthos.es>).

of a dataset of this type of seed banks is provided by the Data Warehouse of the Millennium Seed bank Partnership coordinated by Royal Botanic Gardens, Kew (<http://herbaria.plants.ox.ac.uk/bol/msbp>).

This dataset provides unique information on the occurrence of plant species in two characteristic habitats of Spain that are currently protected by the Habitats Directive of the European Union (Council Directive 92/43/EEC). Some of the species recorded in the dataset are endemic (i.e., *Helianthemum marifolium* ssp. *conquense*, *Lupinus mariae-josephae*) (Figure 2) or have special threat status. The information contained in this dataset has already been used in several research articles published in scientific journals as a source of information on chorology, seed germination and phenology. Furthermore, the storage of seeds in the seed bank allows experiments to be replicated in these studies if necessary.

## Dataset description

**Object name:** Darwin Core Archive Banco de Germoplasma de la Universidad Rey Juan Carlos

**Character encoding:** UTF-8

**Format name:** Darwin Core Archive format

**Format version:** 1.0

**Distribution:** <http://www.gbif.es:8080/ipt/archive.do?r=germoplasma-urjc>

**Publication date of data:** 2013-12-05

**Language:** English

**Licenses of use:** This database “URJC GB dataset” is made available under the Open Data Commons Attribution License: <http://www.opendatacommons.org/licenses/by/1.0/>.

## Project description

**Project title:** URJC GB dataset: Community-based seed bank of Mediterranean high-mountain and semi-arid plant species at Universidad Rey Juan Carlos.

**Personnel:** José María Iriondo (Principal Investigator) and Patricia Alonso.

**Funding:** This project is financed by LIMITES (CGL2009-07229) and AdAptA (CGL2012-33528) research projects of the Spanish Ministry of Science and Innovation and Remedial-2 project of the Autonomous Community of Madrid.

**Study area description:** The study area includes high-mountain Mediterranean systems and semi-arid Mediterranean ecosystems of Peninsular Spain. It also comprises ruderal habitats linked to the occurrence of *Lupinus* species. The sampled high-mountain Mediterranean systems are psicroxerophyllous pastures in the Sierra de Guadarrama mountain range located between 1550 and 2438 m elevation. The sampled semi-arid Mediterranean systems correspond to plant communities of gypsum steppes in the river Tajo valley located between 489 and 939 m elevation. Finally, the ruderal habitats of *Lupinus* spp. are scattered in Central Spain between 440 and 743 m elevation. Further details concerning the climate, geologic substrate and vegetation of these habitats are provided in the spatial coverage section. The study area is currently represented by the provinces of Ávila, Cuenca, Guadalajara, Madrid, Salamanca, Segovia, Valencia and Zaragoza in Spain.

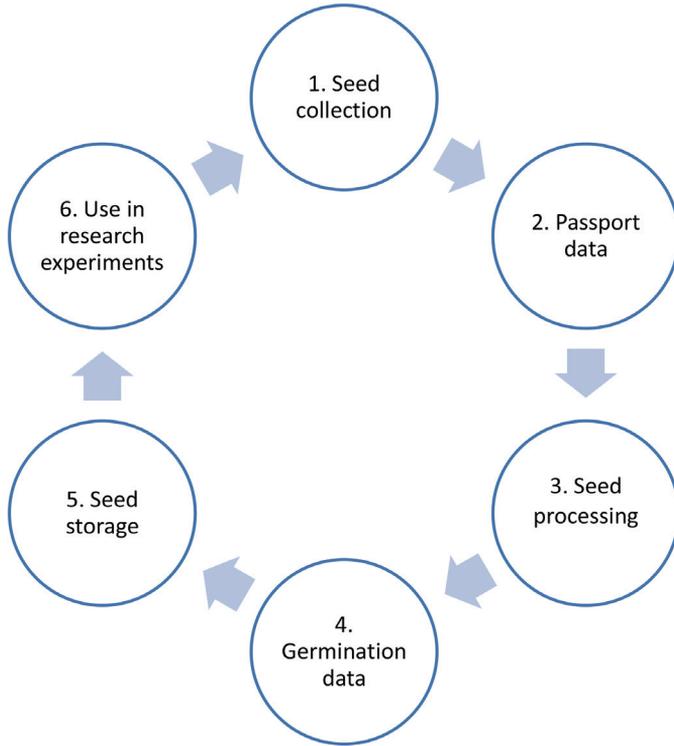
**Design description:** The Germplasm Bank of Universidad Rey Juan Carlos is a long-term research infrastructure project which aims to collect and conserve seeds of plant species that are found in the ecosystems where most of the research of the Biodiversity and Conservation Area of URJC takes place. In addition to conserving seed accessions of the most representative species of these plant communities, this project aims to gather and manage data describing the geographic, physical and biotic characteristics of the associated habitat of each accession and the morphological and physiological traits of the seeds. Collected and preserved seeds can also be used in research experiments focused on comparing the performance of germplasm across a spatial or temporal range. Figure 3 summarizes the basic elements of the project design. Further details on the seed collection and preservation process are given in the Sampling Methods section.

## Taxonomic coverage

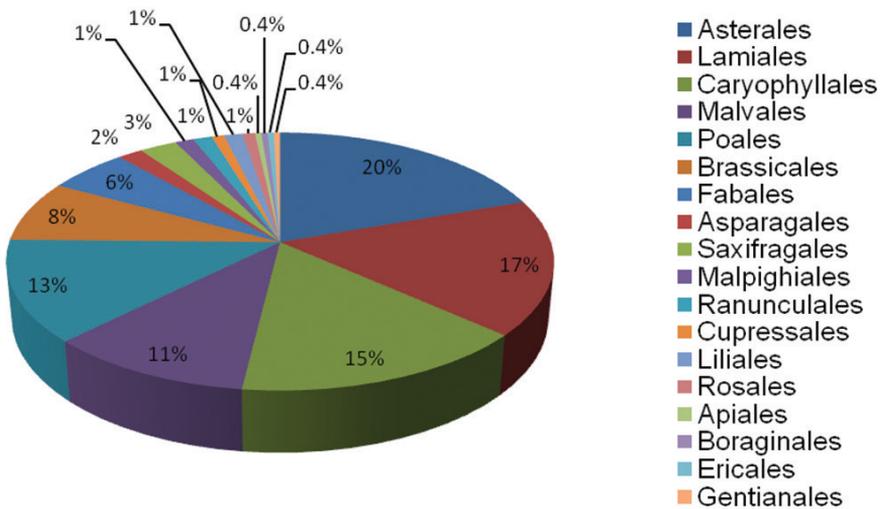
### General taxonomic coverage description

The URJC GB holds the seeds of vascular plants from specific habitats. The high-mountain subcollection is composed of communities of siliceous psicroxerophilous pastures dominated by *Festuca curvifolia* and rich in hemicryptophytes and chamaephytes. The semi-arid subcollection is composed of gypsophytes characteristic of semi-arid environments dominated by *Cistaceae*, *Asteraceae* and *Labiatae*. Finally, the *Lupinus* subcollection is composed of seeds from different species in this genus collected in different regions in Spain.

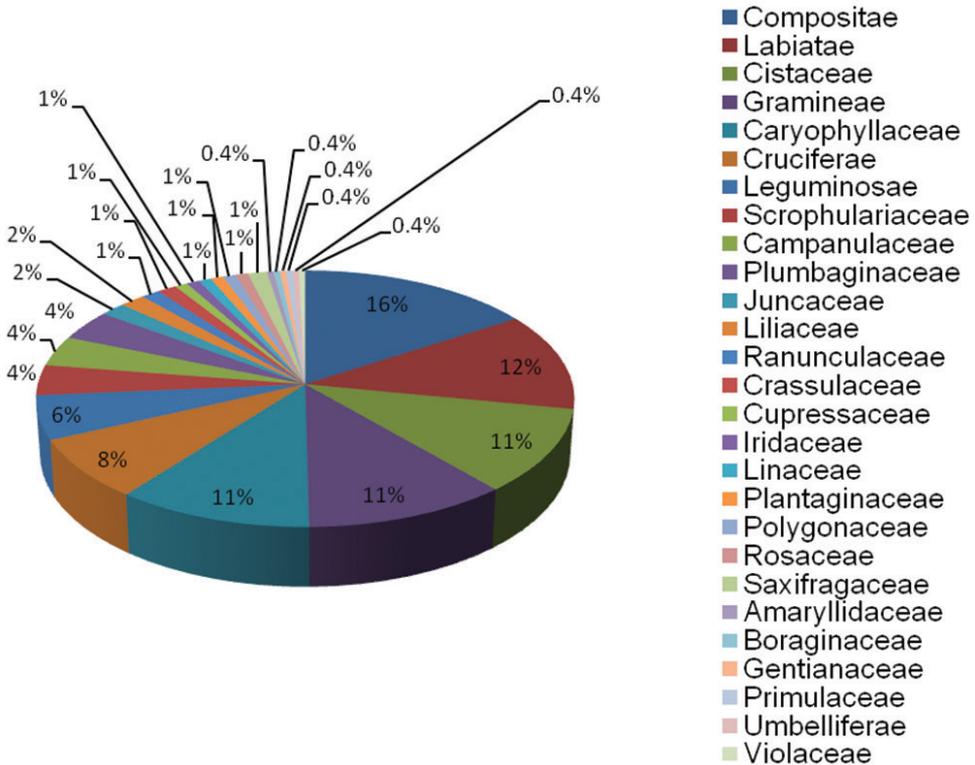
Two phyla are represented in the URJC GB: Magnoliophyta (198; 99%) and Pinophyta (2; 1%). Of the three classes found, the most representative is Magnoliopsida (167; 84%), followed by Liliopsida (31; 15%) and Pinopsida (2; 1%). 18 orders are represented in the Germplasm Bank, and those with the highest number of accessions are Asterales,



**Figure 3.** Basic elements of the project design. **1** Seed collection in the target plant communities **2** Gathering of passport data on the geographical, physical and biotic features of the locality of seed collection **3** Seed processing to prepare seed accessions **4** Initial germination experiments to obtain seed germinability **5** Seed storage in cold-dry conditions **6** Use in research experiments.



**Figure 4.** Taxonomic coverage (percentage per order) of URJC GB.



**Figure 5.** Taxonomic coverage (percentage per family) of URJC GB.

Lamiales, Poales, Malvales, Caryophyllales, Brassicales and Fabales (Figure 4). Of the 27 families in the collection, those with the highest representation are Compositae, Labiatae, Cistaceae, Gramineae, Caryophyllaceae, Cruciferae and Leguminosae (Figure 5).

### Taxonomic ranks

**Kingdom:** Plantae

**Phylum:** Magnoliophyta, Pinophyta

**Class:** Liliopsida, Magnoliposida, Pinopsida

**Order:** Apiales, Asparagales, Asterales, Boraginales, Brassicales, Caryophyllales, Cupressales, Ericales, Fabales, Gentianales, Lamiales, Liliales, Malpighiales, Malvales, Poales, Ranunculales, Rosales, Saxifragales

**Family:** Compositae, Labiatae, Cistaceae, Gramineae, Caryophyllaceae, Cruciferae, Leguminosae, Scrophulariaceae, Campanulaceae, Plumbaginaceae, Juncaceae, Liliaceae, Ranunculaceae, Crassulaceae, Cupressaceae, Iridaceae, Linaceae, Plantaginaceae, Polygonaceae, Rosaceae, Saxifragaceae, Amaryllidaceae, Boraginaceae, Gentianaceae, Primulaceae, Umbelliferae, Violaceae.

## Spatial coverage

### High-mountain subcollection

The seeds in this subcollection are from different areas along the altitudinal gradient of the Sierra de Guadarrama between the provinces of Madrid, Segovia and Ávila in Central Spain (Figure 6). This mountain range follows a southwest-northeast orientation and is approximately 80 Km long. It forms part of the Sistema Central, which delimits the hydrographic basins of the Tajo and Duero Rivers. With regard to its lithologic composition, it is dominated by plutonic and metamorphic siliceous rocks like granite, gneiss, slate and quartzite.

Peñalara Natural Park extends over 768 hectares and is located in the municipality of Rascafría (Madrid). The altitude of the Park ranges from 1640 m to 2428 m (Peñalara Peak), and the highest peaks of the Sierra de Guadarrama are found here. Habitats vary along the altitudinal gradient: from lower to higher elevations, *Pinus sylvestris* woods, shrublands dominated by *Cytisus oromediterraneus* and *Juniperus communis* subsp. *alpina*, and different summit pastures dominated by *Festuca curvifolia*. There are also azonal habitats that are dependent on geomorphology and hydrological and edaphic conditions, such as scree, rocky areas and fens (Vielva et al. 2004).

Climate conditions as well as its relative geographic isolation have made Peñalara Peak a biogeographic island where species characteristic of colder areas and higher latitudes have found refuge, forming the southern limit of their distribution. These species are difficult to find in other parts of Central Spain and include species such as *Senecio boissieri*, *Coincya orophila*, *Erysimum penyalarensense* and *Armeria caespitosa* (Fernández-González 1987).

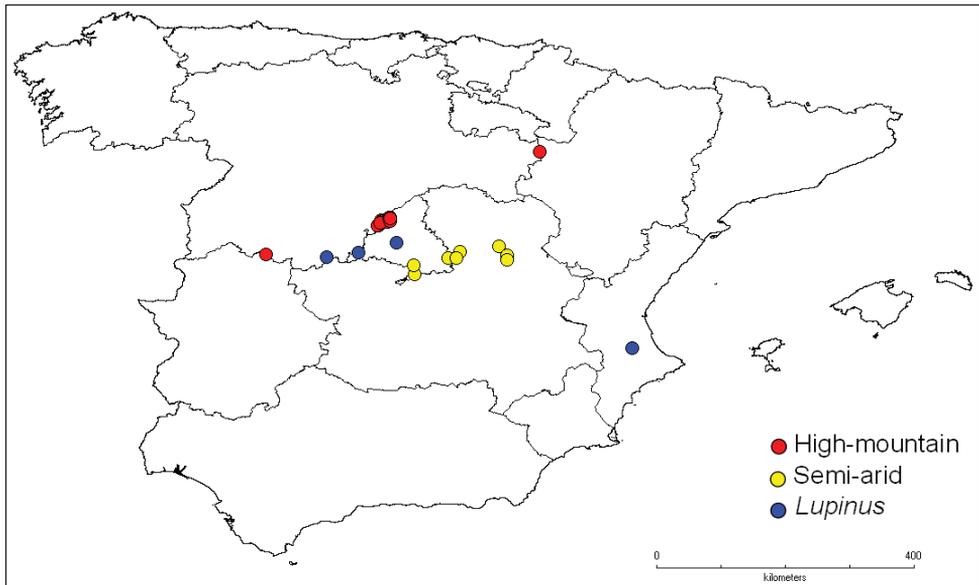
Mean monthly temperatures on the highest peaks range between 0 °C in the winter months and 6 °C in the summer months. Minimum and maximum temperatures range between -18 °C and 26 °C, respectively. Annual precipitation is around 1300 mm, with a large proportion in the form of snow (Peñalara Natural Park, pers. com.).

The altitude of this area confers climatic conditions characteristic of high mountains. This is shown by the high contrast in daily temperatures, strong winds, extreme minimum temperatures, the accumulation of snow during long periods, high insolation in summer accompanied by seasonal drought and high UV radiation (Giménez-Benavides et al. 2007, García-Romero and Muñoz-Jiménez 2010).

This habitat (number 6160) is included in the Habitats Directive of the European Union (Council Directive 92/43/EEC) due to its singularity and reduced area (Rivas Martínez 1963, European Community 1992, Giménez-Benavides 2006).

### Semi-arid subcollection

The seeds in this subcollection are from different areas along the altitudinal gradient in the Tajo valley. These communities occur at 700–900 m elevation in the gypsum steppes in the southeastern area of the Madrid Autonomous Region and in the northeastern area of the Castilla - La Mancha Autonomous Region (Guadalajara and Cuenca) (Figure 6). These gypsophyte communities are characteristic of dry, poorly



**Figure 6.** Geographical distribution of the germplasm subcollections in Peninsular Spain.

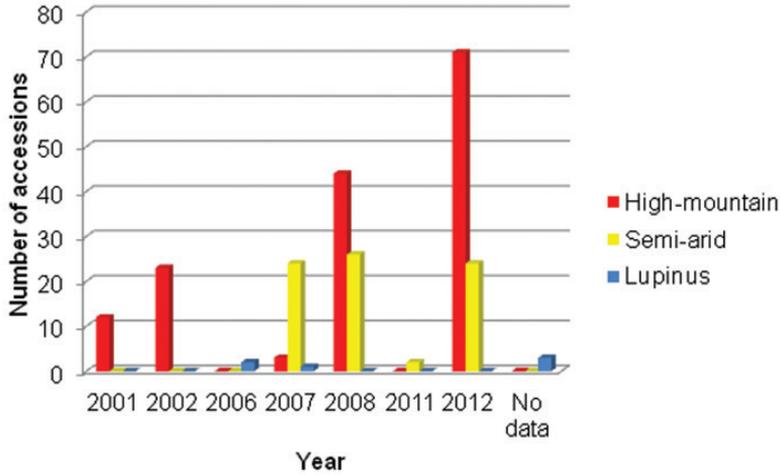
developed gypsum soils, which often have a lichen crust. They are generally open communities dominated by chamaephytes and small bushes, sometimes accompanied by *Stipa tenacissima*. Among the gypsophilous shrubs, gypsophyte communities of annual ephemeral plants can also be found (Martín-Herrero et al. 2003).

These communities generally occur in the mesomediterranean floor in semi-arid or dry ombroclimates. They rarely penetrate the supramediterranean floor or occur in subhumid ombroclimates, where conditions are no longer favourable for their development, and they are substituted by other communities from base-rich soils (Martín-Herrero et al. 2003). Mean annual precipitation ranges between 429 and 596 mm, mean annual temperature between 12 and 13.7 °C and mean minimum temperature in the coldest month between 0.8 and 2.2 °C (Elías, Ruiz 1981, in Ferrandis et al. 2005).

The different texture and composition of gypsum soils influence the floristic composition of this vegetation. These peculiar communities are exclusive to Iberian steppes and have a high number of plants, including several endemics. This, together with their adaptation to a substrate that is very selective for other plant communities, makes them of singular interest. They are considered a “priority habitat” (number 1520) according to Council Directive 92/43/EEC (European Community 1992, Martín-Herrero et al. 2003).

### Lupinus subcollection

The seeds in this subcollection are from different locations in Spain: Madrid, Valencia y Ávila (Figure 6). In this case, they are not from a specific ecosystem, and samples are collected from any region within the territory.



**Figure 7.** Temporal coverage in the different subcollections of the URJC GB.

## Coordinates

38°58'58,8"S and 40°51'3,6"N Latitude; 5°43'4,8"W and 0°31'1,2"E Longitude.

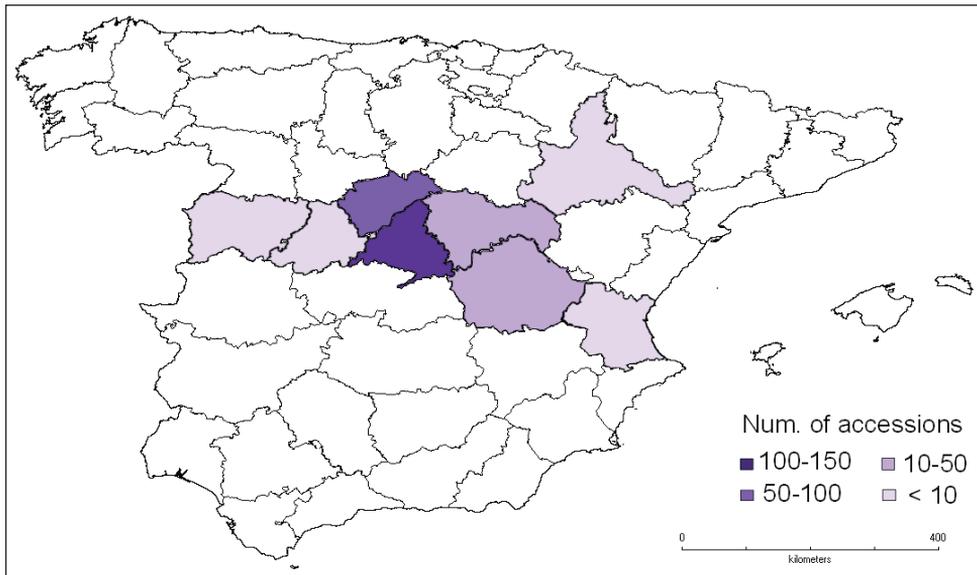
## Temporal coverage

2001 – 2012. The earliest collection event dates back to 2001 and the latest to 2012. The highest number of accessions was collected in 2012, 2008 and 2006 for the High-mountain subcollection, the Semi-arid subcollection and the *Lupinus* subcollection, respectively (Figure 7). Three accessions in the *Lupinus* subcollection lack year data. There are significant variations in the number of seeds collected between years due to the number and type of research projects active at the institution at a given time. Thus, seed collecting expeditions are carried out according to the experimental design of each project involved. It is worth noting that all subcollections are active and in continuous growth. Therefore, temporal coverage will be regularly updated.

## Sampling methods

### Study extent description

The Sierra de Guadarrama and gypsophylic steppes of Central Spain are the most significant areas represented in URJC GB. Seeds have been collected from 8 provinces in Spain: Ávila, Cuenca, Guadalajara, Madrid, Salamanca, Segovia, Valencia and Zaragoza (Figure 8). 84% of the accessions come from Madrid and Segovia.



**Figure 8.** Geographical distribution of accessions in Peninsular Spain.

### Sampling description

In the collection phase, seed samples were collected from several different localities to cover the distribution range of the communities in the study area and thereby obtain genetic material from the different species with the potential to adapt to different local conditions.

To plan seed collection, it was essential to determine the timing of seed dispersal. In this sense, the literature related to the phenology of the collected species was consulted. Other morphological indicators of the timing of natural dispersal were also used. These included tissue hardness, changes in color in seeds and fruits, dryness of pods and capsules, among others.

The physical quality of the seeds was evaluated at the time of collection to avoid collecting specimens that were infected or clearly unviable. We aimed to collect between 3000 and 5000 seeds per accession to guarantee that there would be enough material for germination assays, long-term conservation and, if necessary, propagation (Gold et al. 2004). In order not to compromise the viability of the population *in situ*, no more than 20% of the seeds present at the time of collection were sampled (Gold et al. 2004).

Once samples were collected for each species, they were placed in paper bags and labeled with the name of the species, the site where they were collected, altitude, site coordinates, the date of collection and the name of the collector. This information constituted the passport data for each seed accession.

## Quality control

The URJC GB has a protocol which describes the seed processing methods for collection, processing in the laboratory, germination assays, desiccation, recording accessions, labeling, scanning and freezing. Seed collectors are professors and researchers with great knowledge of the flora characteristic of each ecosystem. They are all members of research groups that have worked in this field for years and, therefore, have great experience in the phenology and identification of the species. Herbarium specimens associated with the seed accessions are preserved at the Department of Biology and Geology of URJC or at the Herbarium of the Royal Botanic Garden of Madrid. 63% of the records are georeferenced with GPS and radio points. When this information is not available, the geographic coordinates of the site and the extension of the municipality are used as a surrogate of this measure. Since the subcollections are active and continuously growing, data are updated regularly every six months.

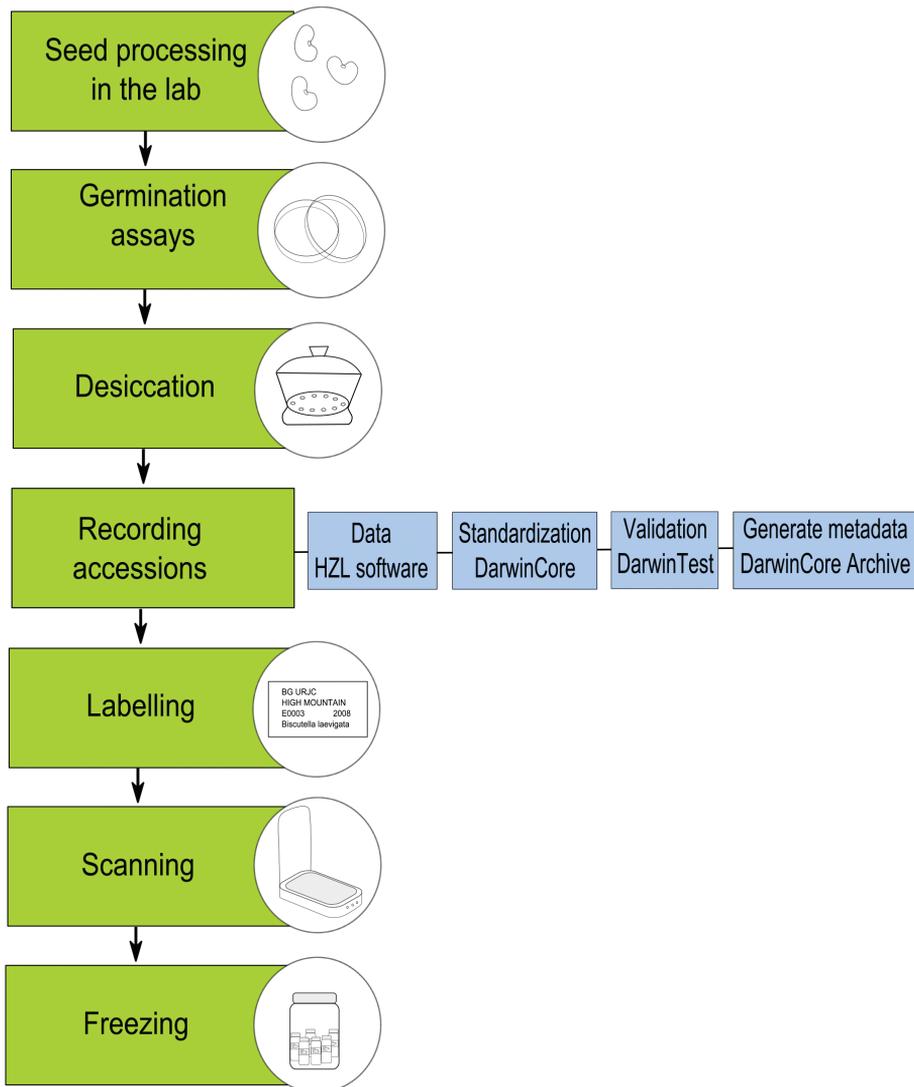
## Description of steps

The different steps of the process are summarized in Figure 9 and are described as follows:

**Seed processing in the laboratory:** The first treatment consisted of cleaning the seeds. Sieves with stainless steel meshes of different sizes (mesh holes between 2 mm–0.5 cm) were used to separate as much undesired material as possible (soil, stones, small leaves, stems, flowers, etc.) from the seed sample. The separated seeds were then introduced in tubes identified with the passport data (species name, date, site, altitude and collector) and stored in a cupboard at room temperature under ambient moisture conditions.

**Germination assays:** Germination assays were carried out with 100 seeds of each accession. Four replicates of 25 seeds were placed in Petri dishes on two pieces of filter paper. Distilled water was added until the surface was wet, and the dishes were placed in a germination chamber (Selecta Hotcold GL, Barcelona, Spain). Accessions from the high-mountain subcollection were incubated at 15 °C with a 16/8 hour light/dark photoperiod (Giménez-Benavides et al. 2005), while accessions from the semi-arid subcollection were incubated at 20 °C with the same photoperiod (Pérez-García and Durán 1989, Escudero et al. 1997, Herranz et al. 2002). Assays were monitored every 2–3 days for a minimum period of one month, and the assay was considered to have finished when no germination was observed for four subsequent censuses (8-day period). Germinated seeds were counted and removed in each census.

**Desiccation:** Silica gel (SiO<sub>2</sub>) was used to desiccate the samples. As desiccation rates increase with the amount of gel used, the base of the desiccator was filled to a 1:1 proportion with the seeds. The seeds, which were separated by accessions in the Petri dishes, were placed uncovered on the metal plate in the desiccator. They were then placed in a cool place to dry for at least two months.



**Figure 9.** URJC Germplasm Bank workflow.

**Recording accessions:** Accessions were added to the database of the URJC GB using Herbar Zoorbar Ligero (HZL) software. Each accession was assigned a reference number allocated consecutively. The information contained in the record of each accession is: dataset, institution, catalogue number, scientific name, family, genus, specific epithet, taxon rank, infraspecific epithet, scientific name authorship, collection code, subcollection, number of mother plants, number of tubes (in which the accession is stored), number of seeds, seed weight, community name, country, country code, state province, municipality, locality, decimal latitude, decimal longitude, geodetic datum,

coordinate uncertainty in meters, elevation, collector, number of jar (in which the accession is stored), number of drawer (in which the accession is stored), collection date, sample acquisition date, % germination and observations. All data are standardized by DarwinCore 1.2 and validated by Darwin Test. We generated metadata with DarwinCore Archive in order to publish data in GBIF IPT.

**Labelling:** After registering the accession in the database, seed accessions were labelled for proper identification.

**Scanning:** Approximately 50 seeds were uniformly distributed avoiding contact between them and scanned. A resolution of 300 ppp was used, except in the case of very small seeds when a 600 ppp resolution was used.

**Freezing:** All of the subcollections in the URJC GB are base collections; i.e. they are stored for long periods of time and are only used in regeneration processes. Storage is carried out under optimal conditions to maximize seed viability. The procedure followed at the URJC GB was to store seeds in glass tubes in Kilner jars with silica gel. These jars were sealed hermetically and placed in a freezer at -18 °C.

## Acknowledgements

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