

CHARACTERIZATION OF SEED DORMANCY  
OF *NOLANA* (SOLANACEAE) IN THE COASTAL  
ATACAMA DESERT OF CHILE

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## **Chapter I. General introduction**

The environmental conditions that seeds require to germinate in diverse plant populations determine the main limits for their establishment at different times and habitats (Bansal and Sen, 1981). The existence of a variety of complex mechanisms that regulate the germination of seeds proves that they are ecophysiological adaptations, that is, differential physiological responses of organisms according to the environment in which they live and the prevailing environmental factors. These adaptations increase the survival potential of the species and have been shaped in the normal pattern of evolution (Bansal and Sen, 1981).

Deserts are found on planet Earth north and south of the Equator, forming two belts of arid ecosystems with species specifically adapted to those areas. Despite being regions of very rigorous climates and often unfavorable conditions for life, together they occupy a quarter of the surface of the Earth and provide important services to support life on the planet and resources for human populations (UNEP, 2006).

The objective of this work is to understand the germination strategies and dormancy mechanisms presented by plants in desert areas, in particular species of the genus *Nolana* (Solanaceae), which would allow them to ensure their continuity in extreme and unpredictable environments, especially the Atacama Desert in northern Chile.

### **1.1 Definition of desert**

According to Shmida (1985), land deserts can be separated into three categories in terms of the amount of rainfall they receive:

- Semi-deserts: rainfall between 150 and 300 or 400 mm per year.
- True deserts: less than 120 mm of annual rainfall.
- Extreme deserts: less than 70 mm of annual rainfall.

Arid ecosystems usually combine true and extreme deserts, while a semi-arid ecosystem corresponds to a semi-desert (Shmida, 1985). The limits set between one category and another are not arbitrary; it is generally known that these tree formations are not found in regions where the amount of annual rainfall is less than 400 mm. The 120 mm mark the difference between steppe (semi-desert) and desert vegetation, which differ significantly in coverage density; in steppes, coverage is around 20 to 50%, while in deserts, it is less than 10%. As for the 70 mm of rainfall, they would establish the difference between “diffuse” vegetation and “contracted”, or restricted to only certain places (Shmida, 1985).

The arid and semi-arid zones, apart from being distinguished by the low amount of annual rainfall, are also differentiated by the season or seasons in which rainfall occurs (Griffiths, 1972, cited in Guterman, 1994). The more extreme the desert, the more unpredictable are the low amounts and the distribution of rainfall, as well as the beginning and length of the rainy season or seasons (Guterman, 1994).

It is interesting to note that, although a desert is considered “extreme” if it presents an average of less than 70 mm of annual rainfall, there are deserts where rainfall is much lower. In the coastal Atacama Desert, the annual average rainfall is between 0.2 and 2 mm; in the last century, not a drop of water fell in 60% of the years (Cereceda, 2012). The Atacama Desert is considered the most arid in the world; this means that the hydrological balance (balance between the contributions of water to the system by rainfall, and evaporation / evapotranspiration outflows) is the most negative when compared to that of other deserts (Weischet, 1975).

### 1.1.1 The coastal Atacama Desert in Chile

The Atacama and Peruvian Desert forms a nearly continuous, hyper-arid belt for around 3,500 km along the south-western coast of South America (7°-29°S; Dillon *et al.*, 2009). In Chile, the Atacama Desert occupies about 180,000 km<sup>2</sup> of the national territory; here, it extends from the Arica and Parinacota region (18°34'S, 69°27'W) to the north of the Atacama region (26°10'S, 70°00'W), but sometimes the semi-desert area is also considered, which includes the south of the Atacama region and the Coquimbo and Valparaíso regions (Cereceda, 2012). According to Rundel *et al.* (1991), and from a floristic point of view, the southern limit of the Atacama Desert should correspond to La Serena (29° 55'S). Inland, there is an area that corresponds to the absolute desert, which almost completely lacks plant life, except in some sectors with presence of groundwater (Luebert and Pliscoff, 2006).

The climate in the coastal desert of Chile is moderate in terms of temperature, which in general is quite stable along the latitudinal range along the coast (Rundel *et al.*, 1997). The atmospheric humidity is high (annual average of 65-80%), and annual rainfall ranges from less than 1 mm in the northernmost weather stations to around 80 mm in La Serena (Fig. 1, Table 1). Rains generally occur during the southern winter (from May to September), but some sectors also receive summer rains (Schultz *et al.*, 2011).

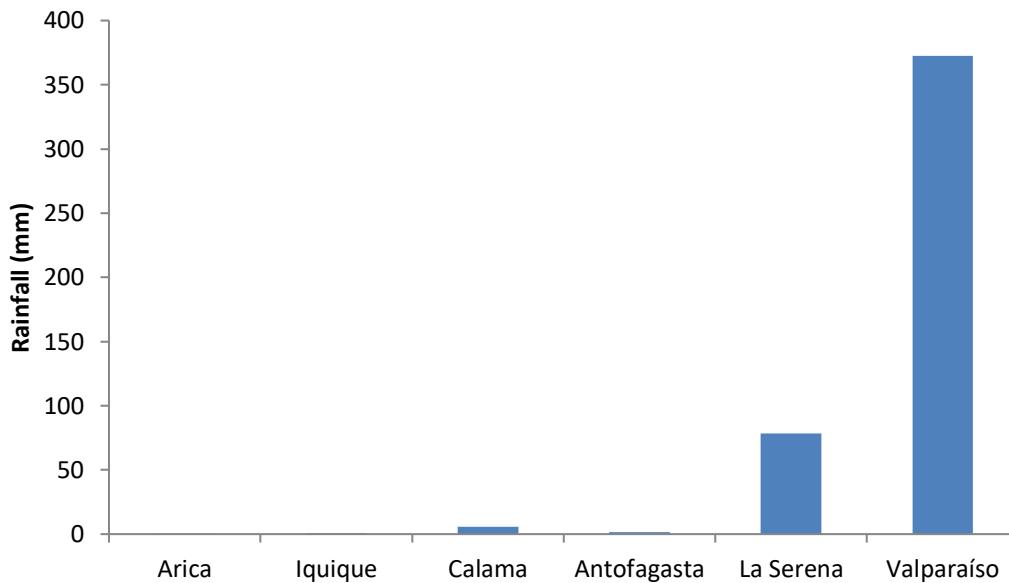


Fig. 1. Amounts of annual rainfall (mm) in a normal year (average of 30 years between 1981 and 2010) in different cities of Chile. Source: Own elaboration based on data from the Chilean Meteorological Office.

Table 1. Normal annual amounts in different coastal cities in northern and central Chile, some of which are included in the Atacama Desert. Average temperature calculated based on data from 1971–2000. Source: Chilean Meteorological Office, Integrated Territorial Information System (ITIS) and Schultz *et al.*, 2011.

City	Annual rainfall (mm)	Mean temperature (°C)
Arica	0,5	18,9
Iquique	0,6	18,4
Antofagasta	1,7	16,7
Copiapó	18,5	15,2
La Serena	78,5	13,7
Valparaíso	372,5	14,0

In the northernmost area (north of 21°30'S) of the coastal desert, vegetation is concentrated in small patches in isolated sites that receive the influence of fog, usually on slopes with south or southwest exposure (Schultz *et al.*, 2011). These small patches are commonly known as “fog oases” (Ellenberg, 1959, cited in Rundel *et al.*, 1991) or “lomas vegetation” (Rundel *et al.*, 1997). Continuing south, up to around 26°S, vegetation increases but remains dispersed. From there towards 30°S, the fog oases extend in a relatively closed formation along the windward slope of the coastal mountain range (Rundel *et al.*, 2007, cited in Schultz *et al.*, 2011).

The high frequency and intensity of the fog is a decisive ecological factor, to which the existence and maintenance of fog oases is attributed. It acts in different ways: by protecting

plants from direct solar radiation and reducing evapotranspiration rates, maintaining low temperatures and high atmospheric humidity, and also by providing water to certain taxa adapted to the collection of fog water (Schultz *et al.*, 2011).

The regeneration and replacement of the annual and perennial seed bank, however, is the consequence of sporadic rains (Gutiérrez *et al.*, 2000; Dillon and Rundel, 1990, cited in Schultz *et al.*, 2011). In years of heavy rains and high temperatures, associated to ENSO (El Niño Southern Oscillation) years, propagule density in the soil seed bank increases 5 to 10 times, compared to normal years (Gutiérrez *et al.*, 2000). This phenomenon occurs approximately every 3.5 to 7 years (Dillon, 2005).

An increasing number of evidences indicate a decline in the oasis vegetation, especially in the northernmost part of the coastal desert, which manifests in the loss of vitality of the plants, withdrawal of individual populations, and probably also loss of species (Rundel *et al.* 1997; Muñoz-Schick *et al.* 2001; Pinto *et al.* 2001; Egaña *et al.*, 2004; Pinto and Luebert, 2009; Schultz *et al.*, 2011). Schultz *et al.* (2011), in their study of fog oases in Chile and Peru between 2005 and 2009, conclude that this decline would be the product of recent changes in the climate: less abundant and less frequent rainfall, and greater sunstroke caused by less cloudiness in the area. They also mention local pollution as an additional factor in vegetation degradation.

Since projections indicate that rainfall will continue to decline in the arid and semi-arid zone of northern Chile towards the end of the 21st century (while temperatures may increase on average) (Santibañez *et al.*, 2017), the opportunity for an improvement in the conditions of development of these plant formations is scarce, and on the contrary, they are expected to continue receding (Schultz *et al.*, 2011). Therefore, it is necessary to find ways to preserve these ecosystems and species; an alternative is *ex situ* cultivation in Botanic Gardens, and storage in seed banks (León-Lobos *et al.*, 2012). Therefore, it is necessary to understand the dormancy mechanisms and germination requirements presented by the stored seed collections and their cultivation.

## 1.2 Germination in desert environments

Plants that grow in desert environments have a set of strategies that allow them to continue existing in these complex habitats (Gutterman, 1994). The more extreme the desert, and the more unpredictable and scarce the annual rainfall, the more important the complementary adaptations and survival strategies during the different stages of the life cycle of a plant, particularly for annual species (Gutterman, 2002).

Obviously, the availability of water, through rainfall, is the main limiting factor for the germination, growth and productivity of plants in arid ecosystems (Noy-Meir, 1973; Baskin and Baskin, 1998). There is a high variability in terms of quantity and temporality of rainfall in desert ecosystems, which means that when a habitat is favourable for the germination of seeds, and in particular for the completion of the life cycle of annual species, is unpredictable (Baskin *et al.*, 1993).

Guterman (1994, 2000, 2002) has described two main types of dispersal and germination strategies for annual species, based on observations in the Negev and Judea desert: the "escape" strategy for seed dispersal and "opportunism" in germination; and "cautious" dispersion and germination. Several species show combinations of these two extreme strategies (Guterman, 1994), demonstrating the high variability that exists in the effort to secure the future of new generations of annual plants. For example, in a two-year study by Loria and Noy-Meir (1980) on the demography of six species (*Schismus arabicus*, *Spergularia diandra*, *Erodium bryoniifolium*, *Gymnarrhena micrantha*, *Carrichtera annua*, *Filago desertorum*) in the Negev desert, he found that there were no two species identical or even similar in terms of population distribution and behaviour: they all showed different patterns of germination, dispersion, survival and seed production (size and shape), in time and space (Loria and Noy-Meir, 1980, cited in Guterman, 1994). Schwienbacher *et al.* (2011) also studied the dormancy types present in 28 species of alpine plants (Austria), and concluded that species in this alpine zone had different germination requirements, that is, there was no "common" strategy or mechanism to all of them.

### **1.3 Dormancy mechanisms and their ecological importance**

Seeds, the plant structures with the greatest capacity to withstand extreme environmental conditions, eventually give way to seedlings, which are the most sensitive. For this reason, it is very relevant for the survival of the species that fractions of the total seed population germinate at the right time and place, especially for those that inhabit more extreme deserts (Guterman, 1994).

Dormancy can be defined as "an innate property of the seed that defines the environmental conditions that must be met before the seed can germinate" (Finch-Savage and Leubner-Metzger, 2006). It is a quantitative genetic character that involves several genes and exhibits continuous phenotypic variation (Baskin and Baskin, 2004). The induction of dormancy is controlled both genetically and by the environment. Genetic control can be associated with both the genotype of the embryo and of the mother plant (Contreras and Rojas, 2010); the dormancy state is influenced by the environment in which the seed develops and matures (maternal environment), but also by the external environment when dispersed, so it can change over time (Finch-Savage and Leubner-Metzger, 2006). To break dormancy, and depending on its type and level, a seed must undergo certain environmental factors for minimum periods of time, which induce metabolic and structural changes within the seed and eventually lead to germination (Bewley *et al.*, 2013).

The dormancy of seeds in environments with high temporal variability, such as deserts, is often described as a bet-hedging strategy, or bet diversification (Eberhart and Tielbörger, 2012). Not all mature seeds produced by a plant germinate immediately after dispersion, or in the following season; in many cases, as a survival strategy for the species, a significant amount of seeds remains dormant in the soil, for a period that can last for years or even decades, forming what is known as a seed bank (Fenner, 1985, cited in Aguado *et al.*, 2012).

There are essentially two types of soil seed banks: transitory, and persistent or permanent. The first are those in which all seeds germinate or lose viability during the same year of

production; in the latter, a variable fraction of seeds germinates during the first year and the rest remains viable for several more years (Aguado *et al.*, 2012). Persistent banks are considered one of the main survival strategies for desert plants, particularly for annual or ephemeral species (Baskin and Baskin, 1998; Figueroa *et al.*, 2004; Facelli *et al.*, 2005).

According to Baskin and Baskin (1998, 2004), seed dormancy can be classified into: a) physiological dormancy, which in turn has three levels of depth (non-deep, intermediate and deep); b) morphological dormancy; c) morphophysiological dormancy (with several levels); d) physical dormancy; and e) combined dormancy (physical and physiological). Table 3 summarizes the main characteristics of each type of dormancy.

Baskin and Baskin (1998), in a general review on the scientific knowledge about dormancy, found that the percentage of species with seeds that have some type of dormancy, increased from 40% in tropical rain forests to 84% in hot deserts (Gutterman, 2002). The most common type of dormancy would be physiological, then physical.

Table 3. Types of dormancy, description and treatments to break dormancy, according to Baskin and Baskin (1998, 2004).

Type of dormancy	Description
Non-dormant (ND)	A non-dormant seed is able to germinate at a specific time if the environmental conditions are favourable; otherwise, it is said to be dormant.
Physiological dormancy (PD)	It is an inhibitory physiological mechanism from the embryo that prevents the emergence of the radicle. The structures surrounding the embryo (endosperm, testa, fruit walls) may also have a role in preventing germination. There are three levels of physiological dormancy depth: non deep, intermediate and deep; the first is the most common form of dormancy, and it occurs both in gymnosperms and angiosperms. Non deep PD is broken with relatively short periods of cold stratification (days to weeks); intermediate PD needs longer periods of cold stratification (months), and deep PD requires cold stratification for even longer periods. The exogenous application of gibberellins could replace stratification in the case of several species; in the case of deep PD, embryo removal would be necessary for this application to take effect.
Morphological dormancy (MD)	Embryos are differentiated (it is possible to distinguish radicle and cotyledons), but not fully developed. For germination to take place, the embryo needs time to complete its development. Hence, it is the morphological characteristics of the embryo that prevent germination.

Morphophysiological (MPD)	It is a combination of morphological and physiological dormancy, that is, underdeveloped embryos that also have physiological dormancy. Two things must happen so that a seed with MPD can germinate: (1) the embryo must grow to a critical and specific size (depending on the species); and (2) physiological dormancy must be overcome. The key is to determine what environmental condition each event can promote; for some species, the same condition promotes embryo growth and dormancy rupture; in others, specific conditions are required.
Physical (PY)	The impermeability of the testa (or walls of the fruit) to water is the main reason why germination does not occur. All areas where water could enter (micropyle, hilum, chalazal area) become impermeable. To break this type of dormancy, the following methods are cited: cold stratification, mechanical scarification, acid treatments, hot water (Ren and Tao, 2004) and temperature treatments (Baskin and Baskin, 1998).
Combinada (CD)	An embryo with physiological dormancy of the non-deep type is added to an impermeable testa. Germination does not occur until both types of dormancy have been overcome.

There are several studies in which dormancy patterns have been investigated for different populations of related species. Meyer and Monsen (1991) studied the mature seeds of 15 populations of mountain sagebrush (*Artemisia tridentata* spp. *Vaseyana*), which were located in an altitudinal gradient. They were interested in finding out if it was possible to predict germination responses in seeds from populations of different environments. They found that in general, the seeds of more risky environments germinated more incompletely and more slowly than those of less risky environments, that is, they were more dormant. This variation in the germination response of collections from different environments could be representing “germination ecotypes”, although a genetic study was not done; their results suggest that, for this species, the variation increased the probability of seedling survival in each habitat within a wide range (Meyer and Monsen, 1991).

Seed germination patterns of 135 populations (28 species and 13 sections) of the genus *Penstemon* (Scrophulariaceae) were examined in the laboratory by Meyer *et al.* (1995). The environments in which these seeds were found ranged from warm desert to alpine tundra. Most species had dormant seeds at the time of dispersal and required a wet stratification period to germinate. The response to stratification was related to the probable duration of cold at the collection site; thus, populations of environments with severe winters produced seeds with prolonged stratification requirements, while populations of environments with mild winters produced seeds with short stratification requirements. The seeds that came from intermediate altitude habitats, presented intermediate cold requirements, but there was a considerable fraction that did not respond to any stratification period. Species with a wide distribution in terms of habitats showed contrasting germination patterns among populations, with a wide range of habitat-specific mechanisms. In fact, habitat-related patterns were observed (similar mechanisms for the same habitat in seeds of different sections). The common garden experiments showed a strong genetic basis for differences in germination, among populations and also among individuals in a population. The *Penstemon* genus thus

shows that the multiple lineages within the genus have adapted and evolved in different habitats with different dormancy mechanisms, specific to those environments (Meyer *et al.*, 1995).

Baskin and Baskin (2014) compile other studies that also investigate species with variation in level/depth of dormancy in terms of their populations. For example, Japanese populations of *Cardamine hirsuta* (Brassicaceae) had stronger dormancy (less germination) than those from Europe (Kudoh *et al.*, 2007, cited in Baskin and Baskin, 2014). Depth of dormancy in 16 ecotypes of *Hordeum spontaneum* (Poaceae) from Israel ranged from low in mesic sites to high in dry sites (Yan *et al.*, 2008, cited in Baskin and Baskin, 2014). In the case of *Biscutella didyma* (Brassicaceae), dormancy increased with an increase in aridity and decreased with an increase in soil moisture (Lampei and Tielborger, 2010, cited in Baskin and Baskin, 2014).

There is little information on germination requirements or dormancy mechanisms in Chilean desert species. Figueroa *et al.* (2004) mention that physical dormancy would be important in species of the genus *Prosopis* (Fabaceae) and other legumes such as *Caesalpinia*, *Balsamocarpum*, *Adesmia* and *Cassia*, and eventually also in *Nolana* (Solanaceae). Physiological dormancy has been reported in *Atriplex* (Chenopodiaceae), *Solanum* (Solanaceae) and *Rhodophiala* (*Myostemma*; Amaryllidaceae). For their part, Jara *et al.* (2006) studied the behavior of six endemic and native herbaceous species of northern Chile (*Cistanthe salsoloides* (Montiaceae), *Leucocoryne purpurea* (Amaryllidaceae), *Pasithea coerulea* (Asphodelaceae), *Placea amoena* (Amaryllidaceae), *Schizanthus litoralis* (Solanaceae) and *Trichopetalum plumosum* (Asparagaceae). The trials determined that for all the species studied, the defined factors (hydration time, lighting, temperature and scarification) and their interactions were highly significant, and that in fact the seeds of the species had similar germination thresholds (Jara *et al.*, 2006). The most important conclusion of this study is that the combinations of factors with which the highest percentage of seed germination was achieved, coincide with the environmental conditions of the sites from where the species are native (Jara *et al.*, 2006).

Given the scenario of decline of vegetation in the Chilean coastal desert, it is relevant to increase the knowledge regarding germination requirements and dormancy mechanisms that species present, and to study in particular whether there are patterns that would allow prediction of their levels or depths of dormancy and therefore, their responses to germination experiments.

### 1.3 The genus *Nolana*

*Nolana* is the fifth genus with more species in the Solanaceae family, after *Solanum*, *Lycianthes*, *Cestrum* and *Nicotiana* (D'Arcy, 1991, cited in Dillon, 2005). It is composed of 89 species, of which 49 are distributed in Chile and 43 in Peru; four species have been registered in both countries and one in the Galapagos Islands (Dillon *et al.*, 2007a). Around 70 are considered endemic to oasis formations in Chile and Peru; 13 other species occur in higher altitude habitats (> 1,000 m) and one of them, *N. paradoxa*, has an extensive

distribution from the center to the south of coastal Chile ( $33^{\circ}$  S -  $42^{\circ} 30'$ S) (Dillon, 2005). According to Dillon *et al.* (2009), the genus *Nolana* is the only one found throughout the entire range of hill formations.

In addition to contributing to the knowledge of the regeneration dynamics of a group of plants representative of the Atacama Desert, there are several reasons that make the study of the *Nolana* genus particularly interesting: a) its high degree of endemism and presence in habitats with extreme conditions of aridity and salinity, which gives it a high conservation value (Tu *et al.*, 2008; Dillon *et al.*, 2009); b) the abundance of species with high ornamental potential due to their foliage and flowering characteristics, such as *N. acuminata*, *N. aplocaryoides*, *N. baccata*, *N. balsamiflua*, *N. carnosa*, *N. coelestis*, *N. crassulifolia*, *N. elegans*, *N. filifolia*, *N. linearifolia*, *N. parviflora*, *N. pterocarpa*, *N. ramosissima*, *N. reichei*, *N. rostrata*, *N. rupicola*, *N. salsoloides* and *N. sedifolia* (Freyre *et al.*, 2005; Riedemann *et al.*, 2006); c) the presence, in at least one species, of compounds with fungicidal activity in fungi of agricultural importance (*N. sedifolia*; Vio *et al.*, 2012); and d) its relation with species of economic importance, such as potato, tomato, pepper and petunia, which makes it a possible source of genes of interest for development of cultivars better adapted to aridity and/or salinity conditions. Despite these reasons, the genus *Nolana* have generally been poorly studied: there are still many species for which relevant aspects of their reproductive biology and establishment strategies are unknown. This knowledge is essential for their conservation and potential use.

*Nolanas*, or "suspiros," as they are commonly known, are annual, perennial or shrubby herbaceous species (Tu *et al.*, 2008). Leaf succulence is a characteristic feature of these species (Dillon *et al.*, 2007a); their corollas can be regular, irregular or slightly zgomorphic, with wide variations in their shape, size and colour (Dillon *et al.*, 2007a). The most frequent colour is lavender or blue, then white; in addition, in populations of species with bluish flowers, sometimes individuals with white flowers occur (Dillon *et al.*, 2007a). These species may exhibit allogamy (obligatory or not) (Freyre *et al.*, 2005) or autogamy.

Until relatively recently, the genus *Nolana* was considered as belonging to the Nolanaceae family; Johnston, who described several *Nolana* species, noted in 1936 that there was an obvious relationship with the Solanaceae family, but also an important difference in terms of the nature and structure of the fruit. It is currently included in the Solanaceae family, after analyzes based on chloroplastidial DNA, which also suggested that the *Nolana* genus would be deeply inserted in the Solanoideae subfamily (Olmstead and Palmer, 1992; Olmstead *et al.*, 2008), to which tomato and pepper also belong.

*Nolana* is considered a monophyletic genus and is distinguished by the 5-15 carpellar ovary that develops fibrous fruits (Knapp, 2002) that correspond to schizocarps (indehiscent fruit originated by a gynoecium of two or more concrescent carpels; Font Quer, 2000), which split up into mericarps when ripe (Fig. 2). Therefore, in *Nolana*, dispersal units are not seeds but mericarps, which can vary in number between species from 2 to 30 per schizocarp and from laterally united and multi-seeded to completely free and single-seeded (Tago-Nakazawa and Dillon, 1999; Knapp, 2002). The seeds in the mericarp are separated by parenchymal tissue, so that each one remains in an independent "embryonic chamber" (Saunders, 1936). Although variable in size, shape and degree of fusion between species, the mericarp is a

convincing synapomorphy for *Nolana* (Dillon *et al.*, 2007a), an evolutionary novelty that allows the identification of the members of the group.

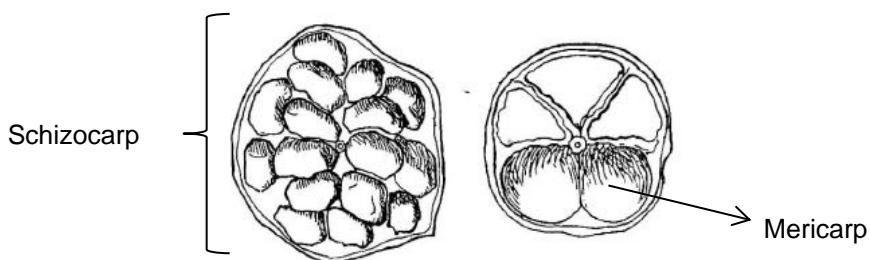


Fig. 2. General scheme of schizocarp and mericarp in *Nolana* (taken from Saunders, 1936).

As for germination experiments, little is known for *Nolana* species. However, Freyre *et al.* (2005) and Douglas and Freyre (2006) mention that germination occurs when the “germination plugs” are opened in the mericarps of eight *Nolana* species, including *N. adansonii*, *N. aticoana*, *N. humifusa*, *N. laxa*, *N. ivaniana*, *N. plicata*, *N. elegans* and *N. rupicola*; and that the germination percentage increased when adding gibberellic acid, but not with chemical treatments (H<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub>, HNO<sub>3</sub> and ethephon). Cabrera *et al.* (2015) also obtained a higher germination percentage with scarification in a specific area of the mericarp (funicular scar) and addition of gibberellic acid. After-ripening treatment, under specific conditions of temperature and relative humidity, could also have an effect on germination (Douglas and Freyre, 2006).

## Hypothesis

Similarity between species is expected in species of *Nolana* in terms of type and level of dormancy that their seeds present; the similarity would be greater in more closely related species. In addition, there should be a relationship between the level of dormancy and the range of distribution (or niche diversity) for the species of the genus, with species with a wider distribution presenting a lower dormancy than species whose distribution is more restricted.

## **Objectives**

### **a. General objective**

To characterize and relate the dormancy of seeds of twelve *Nolana* species (Solanaceae) from different populations of the coastal desert of Atacama, Chile, according to habitat diversity and phylogeny.

### **b. Specific objectives**

1. To establish the germination requirements of twelve Chilean species of the genus *Nolana* (Solanaceae) in different populations along the Atacama coastal desert.
2. To determine the existence of dormancy patterns in terms of habitat diversity and phylogeny.
3. To examine the anatomy and morphology of fruits of twelve species of the genus *Nolana*.
4. Characterize the different stages in the fruit development of a selected *Nolana* species.

## Species under study

A phylogenetic estimate for *Nolana*, using a combination of molecular markers, has been reconstructed by Dillon *et al.* (2007, 2009) and Tu *et al.* (2008). This phylogeny supports the monophyly of the genus and has identified several clades supported by geographic distributions and morphological synapomorphies (Dillon *et al.*, 2009). According to these analyses, the genus is of Chilean origin, with two different introductions to Peru and subsequent radiation (Dillon *et al.*, 2009). Of the seven clades identified, four are confined to Chile and three are mainly Peruvian with some presence in Chile (Fig. 3). A brief description of each clade, and a detail of the twelve species that were the focus of this study, will be given below.

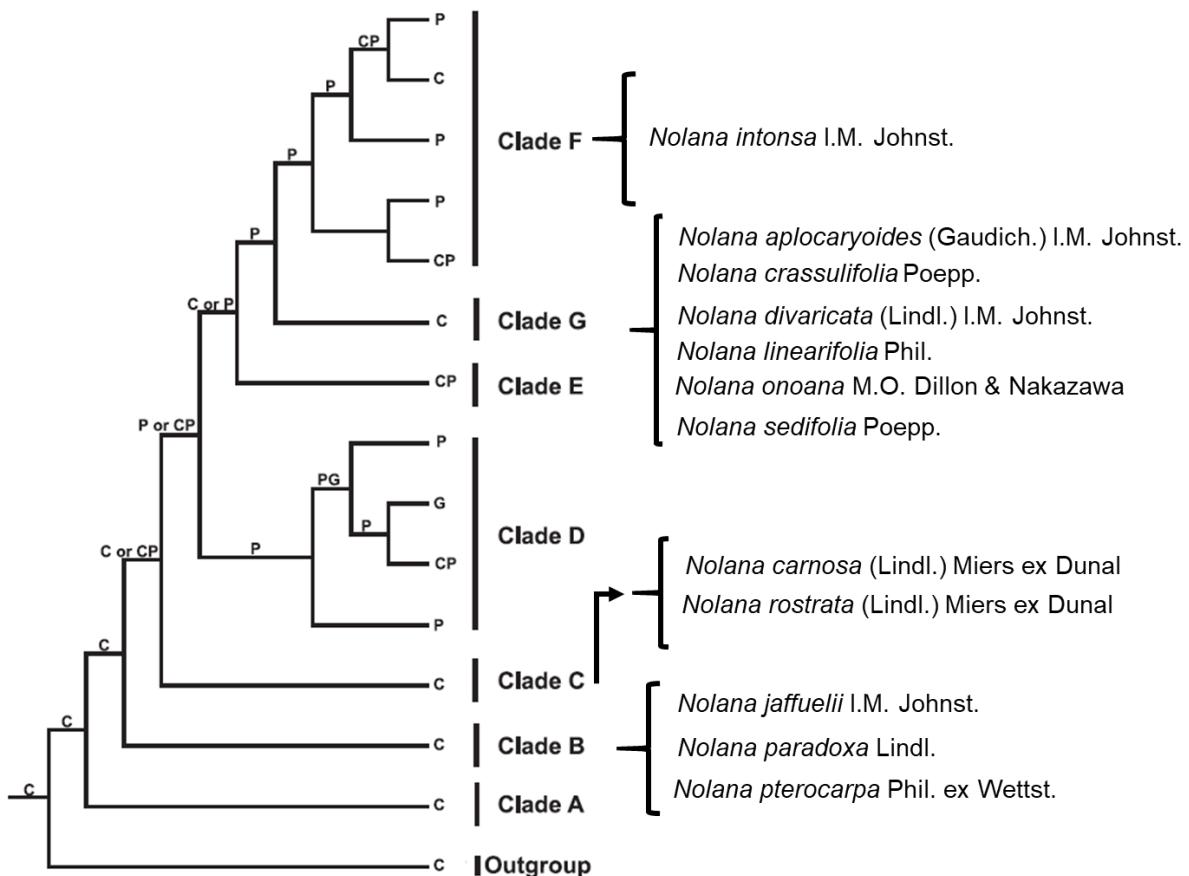


Fig. 3. Biogeographic diversification of *Nolana*, modified from Dillon *et al.* (2009), showing the clade of the 12 studied *Nolana* species. C, Chile; P, Peru; G, Islas Galápagos, Ecuador.

- Clade G (Chile): *N. aplocaryoides*, *N. crassulifolia*, *N. divaricata*, *N. linearifolia*, *N. onoana*, *N. sedifolia*.

Clade G has recovered a strictly Chilean group of 13 species represented by small to moderate shrubs (traditionally called "*Dolia*") and annuals, all with highly reduced corollas, often white or yellowish (Dillon *et al.*, 2009) but some have bluish flowers.

- *N. aplocaryoides* (Gaudich.) I.M. Johnst. (Fig. 4) is an annual herb, erect and branched, with uniseriate glandular hairs (Mesa, 1981). Endemic to northern Chile, it grows between the regions of Tarapacá and Atacama, in plains in full sun (Zuloaga et al., 2008). Its flowers are small, with an infundibuliform tubular corolla (funnel-shaped), white, lilac or light blue (Mesa, 1981; Riedemann et al., 2006).



Fig. 4. *N. aplocaryoides*. Habitat context and growth habit.

- *N. crassulifolia* Poepp. (Fig. 5) is an endemic prostrate shrub that forms mats on rocks near the sea. Abundant in the Valparaíso region, it is also found in Atacama and Coquimbo regions (Zuloaga et al., 2008). Leaves can be spatulate or slender and linear, 15-25 mm long; pedicels are normally 3-8 mm long (Johnston, 1936), corollas white.



Fig. 5. *N. crassulifolia*. Habitat context and growth habit.

- *N. divaricata* (Lindl.) I.M. Johnst. (Fig. 6) usually forms a dense succulent globose bush 1-2.5 m tall and seems to be confined to the hills near the sea (Johnston, 1936), in the regions between Antofagasta and Coquimbo (Zuloaga et al., 2008). Leaves can be broadly spatulate or clavate, quite glabrous; corollas are usually purple (Johnston, 1936).



Fig. 6. *N. divaricata*. Habitat context and growth habit.

- *N. linearifolia* Phil. (Fig. 7) is a trailing, herbaceous annual plant, although it can also behave as persistent. It is only found in the northern regions of Antofagasta and Atacama, in Chile (Zuloaga *et al.*, 2008). The delicate, bright blue tubular corollas of this species have lobes deeply notched, so it appears to be 10-lobed (Johnston, 1936).



Fig. 7. *N. linearifolia*. Habitat context and growth habit.

- *N. onoana* M.O. Dillon & Nakazawa (Fig. 8) is a robust annual tap-rooted herb to 1 m tall; stems erect to ascending, but decumbent when densely leafy. Corollas are narrowly infundibuliform, lavender to light blue (Dillon *et al.*, 2007b). Its distribution covers only the Antofagasta region (Zuloaga *et al.*, 2008).



Fig. 8. *N. onoana*. Habitat context and growth habit.

- *N. sedifolia* Poepp. (Fig. 9) is an endemic perennial shrub or sub-shrub that can measure up to 100 cm tall, with very small and succulent leaves, and small, axillary, white and tubular corolla (Dillon et al., 2007; Mesa, 1981). It has an extensive distribution between the regions of Tarapacá and Valparaíso (Zuloaga et al., 2008).



Fig. 9. *N. sedifolia*. Habitat context and growth habit.

- Clade F (Peruvian and Chilean): *N. intonsa*

Clade F is comprised of 18 Peruvian species and one Chilean species (*N. intonsa*), which shares strong morphological resemblance to southern Peruvian species, such as *N. pallida* (Dillon et al., 2009).

- *N. intonsa* I.M. Johnst (Fig. 10) is an annual or perennial plant, with pubescent leaves and whitish, long hairs. The flowers are solitary and have a whitish, lanuginous calyx, and an infundibuliform corolla 18 to 23 mm long, purple with a deep-purple throat

(Mesa *et al.*, 1998). It is another Chilean endemic, only found in the Tarapacá region (Zuloaga *et al.*, 2008).



Fig. 10. *N. intonsa*. Habitat context and growth habit.

- Clade C (Chilean): *N. carnosa*, *N. rostrata*

Clade C has recovered a monophyletic group comprised of 6 species traditionally classified as *Alona*. These are all large-flowered shrubs confined to Chile (Dillon *et al.*, 2009).

- *N. carnosa* (Lindl.) Miers ex Dunal (Fig. 11) is a compact, densely and ascendingly branched bush with large fruit and flowers. Sessile leaves hug the stem, although not completely. It is a plant of the sandy coastal plains (Johnston, 1936), growing only in Chile in the Tarapacá region and Atacama region (Zuloaga *et al.*, 2008).



Fig. 11. *N. carnosa*. Habitat context and growth habit.

- *N. rostrata* (Lindl.) Miers ex Dunal (Fig. 12) is a very slender, loosely branched, sprawling shrub. The sessile leaves also hug the stems but are less dense than in *N. carnosa*; they can be short or long. Corolla is normally blue, but there is also an albino form (Johnston, 1936). It has an extensive distribution, only in Chile, between Tarapacá and Coquimbo regions (Zuloaga *et al.*, 2008).



Fig. 12. *N. rostrata*. Habitat context and growth habit.

- Clade B (Chilean): *N. paradoxa*, *N. jaffuelii*.

Clade B, previously recognized as a segregate genus, *Sorema*, currently consists of two sub-groups: *N. pterocarpa*-*N. baccata*-*N. parviflora*, which are erect annuals with small flowers; and *N. paradoxa*-*N. rupicola*, which are rosette-forming, taprooted plants with larger flowers (Dillon *et al.*, 2009). These species are only present in Chile (Dillon *et al.*, 2009).

- *N. jaffuelii* I.M. Johnst (Fig. 13) is an annual, glabrescent (almost hairless) plant; with succulent leaves, oblanceolate, adorned by small vesicles. The corolla of the flower is infundibuliform, violet, 13-20 mm long; the calyx is 8-10 mm long (Johnston, 1936). It has been reported for Chile and Peru; in Chile, it is found between Tarapacá and Antofagasta regions (Zuloaga *et al.*, 2008).



Fig. 13. *N. jaffuelii*. Habitat context and growth habit.

- *N. paradoxa* Lindl. (Fig. 14) is an annual, succulent species with a deep fleshy tap-root; its elongate stems are prostrate and freely and loosely branched; the corolla doubles the calyx in length, and its colour is light blue with a white throat (Reiche,

1910). This is the sole member of the family south of the Valparaíso area; it ranges along the whole coast of central Chile south to Chiloé, and grows exclusively on the sandy sea-shore (Johnston, 1936).



Fig. 14. *N. paradoxa*. Habitat context and growth habit.

- *N. parviflora* (Phil.) Phil. (Fig. 15) is a decumbent annual with small inconspicuous white corollas (Johnston, 1936). Being an endemic, it is found in Chile between Antofagasta and Atacama regions (Zuloaga *et al.*, 2008).

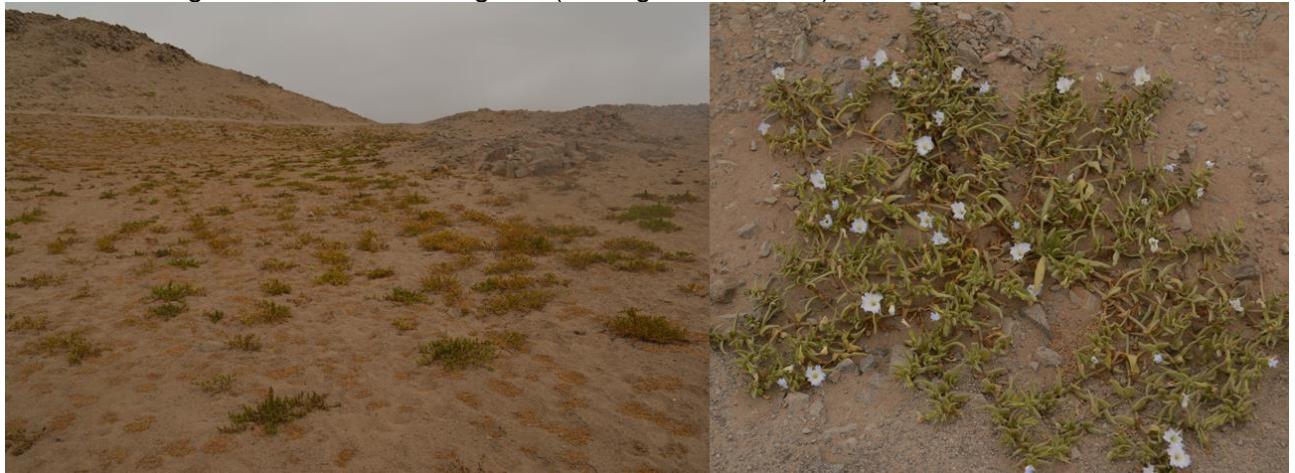


Fig. 15. *N. parviflora*. Habitat context and growth habit.

### **Characterization of seed collecting sites**

The collection sites (Fig. 16) for the twelve species studied correspond to:

- Alto Patache Fog Oasis, Tarapacá region: the cliff reaches 800 masl. The development of ephemeral vegetation begins at 250 m, and it is possible to observe small shrubs and cacti over 600 m (Pinto and Luebert, 2009). In years when there are rains (very rare), it is also possible to find plants in the inner plateau (Pliscoff *et al.*, 2017).

- Antofagasta hills, Antofagasta region: located in close vicinity to the city of Antofagasta and near La Chimba National Reserve, which presents a high diversity of species in very specific spots, and is threatened by human activities. It is possible to find here a great profusion of annual species that appear in rainy years.
- Pan de Azúcar National Park, Atacama region: it is characterized by an underdeveloped coastal plain, which gives rise to a coastal cliff with a maximum height of 800 masl, dissected by numerous ravines. The flora of the park is the typical predominant in the arid zones. In the sectors near the coast, there is a greater diversity of vegetation, due to the presence of coastal fog (CULTAM, 2014).
- Llanos de Challe National Park, Atacama region: main scenario of the blooming desert phenomenon on the coast, there are more than 220 different species, of which 206 are native (CONAF, 2019).
- Punta de Choros, Coquimbo region: located next to the Pingüino de Humboldt National Reserve, it corresponds to a coastal sector with presence of shrubs growing very close to the sea.
- Los Molles, Valparaíso region: located in the transition zone between the dominant sclerophyllous scrub in Coquimbo and the sclerophyllous forest characteristic of the vegetation of central Chile (Lund and Teillier, 2012), it is considered a biodiversity hotspot and hosts several species in threat categories (Lund and Teillier, 2012). Although it is located to the south of the area considered part of the coastal desert, it is in the Valparaíso region, which is the southern limit of distribution for some *Nolana* species (*N. crassulifolia*, *N. elegans* and *N. sedifolia*).



Fig. 16. Distribution map of seed collection showing locations (in yellow) and reference cities (in red). Map made using Google Earth.

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## **Chapter II. Characterization of seed dormancy of twelve Chilean species of *Nolana* (Solanaceae) from the coastal Atacama Desert**

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### **Abstract**

The genus *Nolana* (Solanaceae) comprises numerous species endemic to the coastal Atacama Desert of Chile and Peru, of high potential ornamental and conservation value. These species could face adverse environmental conditions in the future, so in order to support conservation efforts, it is necessary to understand their germination requirements and characterize their dormancy. Different treatments were performed on mericarps of 12 species of *Nolana*: control (intact seeds embedded in distilled water), scarification in germination plug and distilled water, and scarification in germination plug and addition of GA<sub>3</sub> (500 ppm); their permeability to water was also tested. It was determined that the species did not present physical dormancy, as had been previously reported, but rather physiological dormancy. Germination results after treatments were not homogeneous among all twelve species, indicating differences in their dormancy levels. Also, the important role of the endosperm in the prevention of germination for the studied *Nolana* species was highlighted. Regarding the relationship between the level of physiological dormancy (expressed as the percentage of germination for the most successful treatment) and the latitudinal distribution of the species or their phylogenetic closeness, it was determined that, for the studied species, their proximity in terms of clades was more relevant than their latitudinal distribution.

Keywords: mericarp, physiological dormancy, germination, endosperm

### **Introduction**

Dormancy of seeds in environments with high temporal and spatial variability, such as deserts, is often described as a bet-hedging strategy (Eberhart and Tielbörger, 2012). Not all mature seeds produced by a plant germinate immediately after dispersal, or in the following season; in many cases, as a survival strategy for the species, a significant amount

of seeds remains dormant in the soil, for a period that can last for years or even decades (Aguado *et al.*, 2012), waiting for favourable conditions for seedling establishment. The more extreme the environmental conditions, the greater the percentage of plant species that exhibit dormant seeds (Guterman, 2002).

The Atacama Desert is considered the most arid in the world; the hydrological balance (balance between the input of water to the system by precipitation, and outputs by evaporation/evapotranspiration) is the most negative when compared with that of other deserts (Weischet, 1975). Along the coast it has a temperate climate, with constant presence of fog produced by cold currents in the Pacific Ocean (Shmida, 1985). The high frequency and intensity of fog is a decisive ecological factor, to which the existence and maintenance of vegetation formations known as "fog oases" (Ellenberg, 1959, cited in Rundel *et al.*, 1991) or "lomas vegetation" (Rundel *et al.*, 1997) is attributed.

In the coastal desert of Chile and Peru, the Solanaceae family is represented by 18 genera and 128 species and is the second family with better representation in the lomas vegetation (Dillon, 2005). The genus *Nolana* L. ex L. f. (Solanaceae) currently comprises 90 species of arid and semi-arid environments in Chile and Peru, of which 46 are exclusively in Chile and another three are shared with Peru. They are annual or perennial herbaceous plants or small shrubs with leaves with different degrees of succulence and flowers of different colors and sizes, tubular and hermaphrodites (Dillon *et al.*, 2007; Tu *et al.*, 2008; Dillon *et al.*, 2009). *Nolana* is the only genus found in the whole range of lomas formations, where it stands out as the most conspicuous floristic element (Tago-Nakazawa and Dillon, 1999). Some *Nolana* species are important components of the blooming desert phenomenon, associated with ENSO (El Niño Southern Oscillation) events of short periods of heavy rainfall and relatively high temperatures (Dillon, 2005) that occur every 3 to 7 years, which lead to massive blooming episodes that replenish soil seed banks for annual and perennial plants (Tago-Nakazawa and Dillon, 1999; Dillon, 2005). Several of these species have high ornamental potential due to their foliage and flowering characteristics (Freyre *et al.*, 2005; Riedemann *et al.*, 2006; Fig. 1), and also high conservation value (Tu *et al.*, 2008, Dillon *et al.*, 2009) given that they are endemic to habitats with extreme conditions of aridity and salinity.

Dillon *et al.* (2009) identified several strongly supported clades within the *Nolana* genus, with geographic and morphological fidelity, four of which are confined to Chile and three are mainly Peruvian with some presence in Chile. The majority of the species grows preferentially in the arid and semiarid zones of coastal Chile and Peru, although some species are found in high altitude habitats (> 1,000 m a.s.l.) and a few of them, i.e. *N. paradoxa* and *N. sedifolia*, have distributions that range over 1,000 km (Johnston, 1936).

The Solanaceae family has been included among the families that present physical dormancy (Baskin *et al.*, 2000); however, Baskin *et al.* (2000) have not yet found evidence that representatives of this family present this type of dormancy. Physiological non-deep dormancy, apparently induced by the endosperm and the testa, has been detected in Solanaceae (Finch-Savage and Leubner-Metzger, 2006). For *Nolana*, Freyre *et al.* (2005) and Douglas and Freyre (2006), working in *Nolana paradoxa* and *N. aplocaryoides* among others, mention that germination occurs when opening a "funicular plug" or "germination plug" in mericarps, and that the percentage of germination increases when adding gibberellic

acid, but not with chemical treatments ( $H_2SO_4$ ,  $KNO_3$ ,  $HNO_3$  and ethephon). Cabrera *et al.* (2015) also obtained a higher percentage of germination in *N. jaffuelii* with specific scarification in the funicular scar area (which indicates the location of the germination plug), and application of gibberellic acid, reporting for the studied species dormancy of the physical and physiological type. For the other studied species, no references have been found regarding their dormancy.

A growing body of evidence indicates a decline in the lomas vegetation or fog oases of the coastal desert, which is manifested in loss of vitality of the plants, receding individual populations, and probably also loss of species (Rundel *et al.* 1997; Muñoz-Schick *et al.* 2001; Pinto *et al.* 2001; Egaña *et al.*, 2004; Pinto and Luebert, 2009; Schultz *et al.*, 2011). Since projections indicate that rainfall will continue decreasing in the arid and semi-arid area of northern Chile by the end of the century, while temperatures could rise on average (Santibañez *et al.*, 2017), the opportunity for an improvement in the development of these ecosystems is low, and conversely, they are expected to continue deteriorating (Schultz *et al.*, 2011). It is crucial to establish the germination requirements of the species, and their possible dormancy mechanisms, so that its storage in seed banks and subsequent propagation for *in situ* restoration or *ex situ* cultivation, can be successful and help preserve them and the ecosystems in which they live (León-Lobos *et al.*, 2012).

For certain species, it has been found that there is a relationship between dormancy level and habitat (*Penstemon* spp., Scrophulariaceae, in Meyer *et al.*, 1995); or between dormancy level and phylogenetic closeness (several species in Dayrell *et al.*, 2016). However, for *Nolana* such relationships have not been established.

Therefore, the objective of this study was to characterize the type of dormancy of twelve *Nolana* species present in the coastal desert of Chile, to determine their germination requirements, and to evaluate if there is any relationship between level of dormancy and geographical distribution or phylogeny of the species.

## Materials and methods

### 2.1 Plant material

Mericarps of 12 species of *Nolana* (Fig. 1) were collected between 2015 and 2016 from individual plants at maturity and in their dispersal phase at different locations (Table 1) and kept in labelled paper bags. Whenever possible, mericarps were collected randomly within the population from at least 50 different individuals, to obtain a representative sample for each species (León-Lobos *et al.*, 2003). Fruits were then kept in paper bags, partially dried (they were put inside jars with equal weight of silica gel, for 2 to 3 days) and stored at 20°C and 40% RH until used for analysis. Selection of species is the result of availability of suitable fruits (i.e. mature mericarps, black/brown in colour and with dry or senescent calyx) in the field, which explains the overrepresentation of certain clades.



Figure 1. Context, flowers and fruits of four *Nolana* species included in this study. A, B and C: *N. divaricata* (clade G); D, E and F: *N. intonsa* (clade F); G, H and I: *N. carnosa* (clade C); J, K and L: *N. jaffuelii* (clade B).

Table 1. Species of the genus *Nolana* selected in the study, latitudinal distribution in Chile, collection site and clade (Flora del Conosur, 2014 and Enciclopedia de la Flora Chilena, 2014).

<b>Species</b>	<b>Latitudinal distribution*</b>	<b>Collection site</b>	<b>Clade*</b>
1 <i>Nolana aplocaryoides</i> (Gaudich.) I.M. Johnst.	22° 4'S / 27° 4'S	Pan de Azúcar, Atacama (26°08.786'S, 70°39.943'W)	G

2	<i>Nolana crassulifolia</i> Poepp.	26° 21.054'S / 33° 55.002'S	Llanos de Challe, Atacama (28°11.086'S, 71°09.751'W) Punta de Choros, Coquimbo (29°14.786'S, 71° 28.021'W)
3	<i>Nolana divaricata</i> (Lindl.) I.M. Johnst.	23° 30.582'S / 30° 54'S	Punta de Choros, Coquimbo (29°14.601'S, 71°27.840'W) Llanos de Challe, Atacama (28°11.991'S, 71°09.524'W)
4	<i>Nolana linearifolia</i> Phil.	21° 55.98'S / 26° 1.998'S	Hills of Antofagasta, Antofagasta (23°30.133'S, 70°23.069'W)
5	<i>Nolana onoana</i> M.O. Dillon & Nakazawa	23° 12'S / 23° 51.738'S	Hills of Antofagasta, Antofagasta (23°29.903'S, 70°21.817'W)
6	<i>Nolana sedifolia</i> Poepp.	19° 55.998'S / 33° 5.922'S	Alto Patache fog oasis, Tarapacá (20°49.542'S, 70°09.392'W) Los Molles, Valparaíso (32° 14.444'S, 71°31.133'W)
7	<i>Nolana intonsa</i> I.M. Johnst.	19° 55.998'S / 21° 25.002'S	Alto Patache fog oasis, Tarapacá (20°49.309'S, 70°09.366'W) F
8	<i>Nolana rostrata</i> (Lindl.) Miers ex Dunal	18° 49.998'S / 30° 3'S	Llanos de Challe, Atacama (28°06.806'S, 71°09.257'W) C
9	<i>Nolana carnosa</i> (Lindl.) Miers ex Dunal	26° 9'S / 28° 35.094'S	Llanos de Challe, Atacama (28°02.612'S, 71°07.022'W)
10	<i>Nolana jaffuelii</i> I.M. Johnst.	20° 13.002'S / 26° 9'S	Alto Patache fog oasis, Tarapacá (20°48.903'S, 70° 09.630'W)
11	<i>Nolana paradoxa</i> Lindl.	29° 54'S / 43° 21'S	Los Molles, Valparaíso (32°14.433'S, 71°31.133'W) B
12	<i>Nolana parviflora</i> (Phil.) Phil.	27° 4.002'S / 28° 13.002'S	Hills of Antofagasta, Antofagasta (23°30.133'S, 70°23.069'W)

\*Letters for clades according to Dillon *et al.*, 2009; a brief description of clades is given in Figure 2.

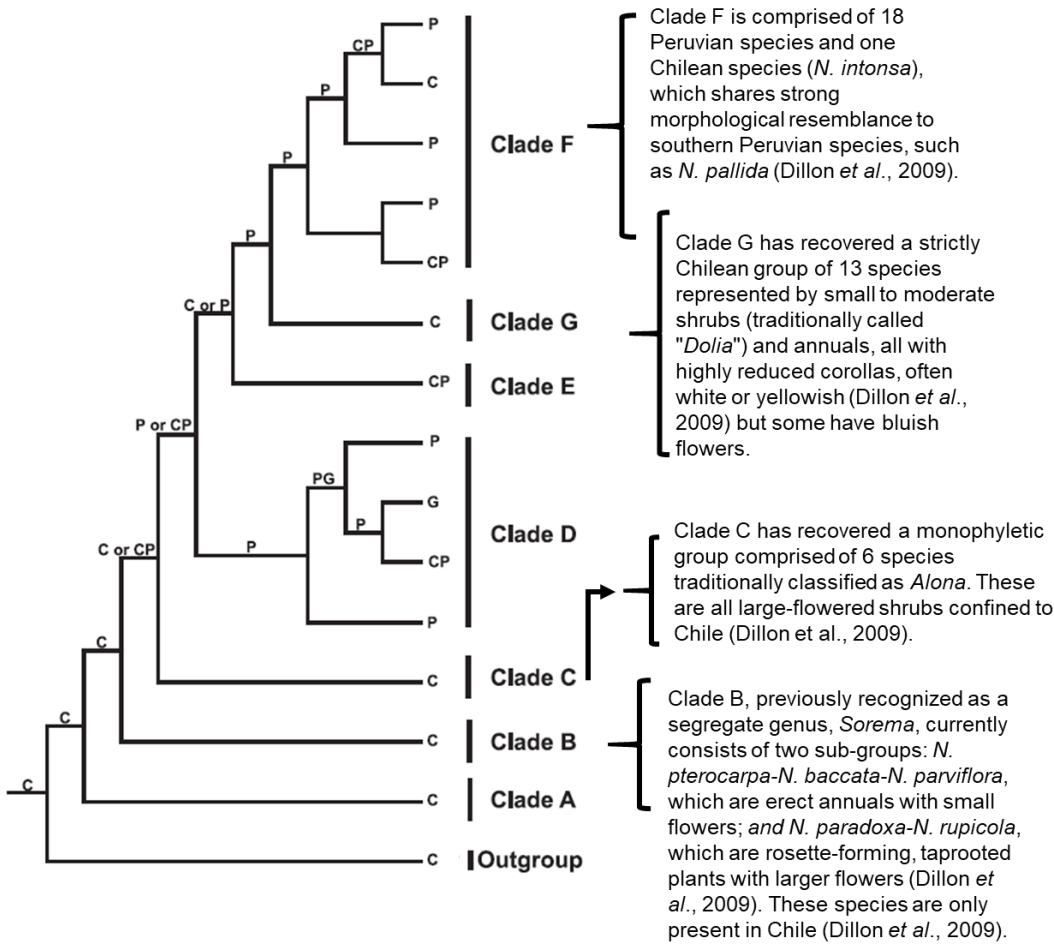


Figure 2. Biogeographic diversification of *Nolana*, modified from Dillon et al. (2009), showing the clade of the 12 studied *Nolana* species. C, Chile; P, Peru; G, Islas Galápagos, Ecuador.

### Seed viability

A tetrazolium test with 2,3,5-triphenyl-2H-tetrazolium chloride (Tz) was performed on a sample of 25 mericarps for each species, to evaluate the viability of seed lots. This was done in a separate sample of seeds, and not in the same mericarps after finishing the germination experiments, given the extension of the experiments and the possibility of fungal damage. Mericarps were scarified (germination plug was removed) and stained with 1% Tz solution for approximately 24 hours at 30° C. After that time, mericarps were cut in halves and surfaces were observed using a magnifier. Only seeds that were completely stained red (embryo and endosperm) were considered viable.

### Seed imbibition

To evaluate if water was able to penetrate and reach the embryo in intact mericarps, methylene blue (1gr/100 mL) was used. Twenty seeds of 12 species were left in metal

containers with enough dye to cover them (the largest were covered in half), and 5 were evaluated after 3, 24, 48 and 196 hours (room temperature). They were washed with distilled water and dried with paper towel, then allowed to air dry for 20 minutes at room temperature. Finally, stained seed were sectioned using a scalpel and observed under a stereoscopic magnifier Olympus SZ2-ILST (Olympus Corporation, Tokyo, Japan), making sure that the cut allowed to see the plug and half the embryo (longitudinal cut).

### Evaluation of germination and characterization of dormancy

Evaluation of germination for the twelve species was performed in three experiments. Mericarps were placed in 9 cm diameter Petri dishes, over three layers of filter paper, saturated in distilled water or a solution of gibberellic acid ( $GA_3$ ). Plates were hermetically sealed with Parafilm to prevent drying and were watered as needed. Physiological germination (radicle emergence over 2 mm) was evaluated. Results were reported as total germination percentage.

#### *Experiment 1. Role of funicular plug on germination*

In order to make a comparison regarding the type and level of dormancy between the different species, the same germination experiments were carried out on all the studied species. Germination tests were carried out during 2015 and 2016, using seeds from ten *Nolana* species collected in the previous months. Seeds were randomly assigned to one of the following treatments: (1) Control (Ct), intact mericarps imbibed in distilled water; (2) scarification (Sc), removal of germination plug and imbibition in distilled water; (3) Sc and imbibition in a solution of 500 ppm  $GA_3$  (Sc+ $GA_3$ ). A scalpel was used to dissect the pericarp at the funicular scar area avoiding radicle damage, as this proved to be the most effective treatment for the germination of mericarps of *N. jaffuelii* (Cabrera et al., 2015). Germination was evaluated three days a week during 45 days in a chamber at constant 20°C (40% RH), in four replicates of 25 seeds each.

*Experiment 2: Additional experiment (*N. linearifolia*):* a single species was selected due to the number of available mericarps, and the following germination tests were performed: (1) Control, intact mericarps imbibed in distilled water; (2) 500 ppm  $GA_3$ , intact mericarps imbibed in gibberellic acid at 500 ppm; (3) 1000 ppm  $GA_3$ , intact mericarps imbibed in gibberellic acid at 1000 ppm; (4) Plug scarification, removal of germination plug, and imbibition in distilled water; (5) Plug scarification with partial removal of endosperm, imbibed in distilled water; (6) Plug scarification + 500 ppm  $GA_3$ , scarified mericarps with partial removal of endosperm imbibed in gibberellic acid (500 ppm  $GA_3$ ); (7) 10/25, intact mericarps at alternating temperatures of 10/25°C, imbibed in distilled water; (8) 10/25 +  $GA_3$ , intact mericarps at alternating temperatures of 10/25°C, imbibed in gibberellic acid (500 ppm  $GA_3$ ); and (9) Cold stratification (5°C) of intact mericarps for 4 months. Germination was evaluated three times a week in four replicates (25 seeds each) per treatment, during 45 days in a chamber at constant 20°C (unless otherwise noted).

#### *Experiment 3. Role of endosperm on germination*

Based on observations after Exp. 1 and the further tests on *N. linearifolia*, a third set of germination tests were performed using mericarps from species that had high germination percentage, and two additional species (*N. aplocaryoides* and *N. paradoxa*) that were collected and included afterwards, in order to better understand the role of endosperm in the germination of seeds. Three treatments were defined: (1) Control (Ct), intact mericarps imbibed in distilled water; (2) scarification (Sc), removal of germination plug and imbibition in distilled water, as done for Exp. 1; and (3), Sc and partial removal of endosperm, and imbibition in distilled water (Sc+en). Germination was evaluated three times a week in four replicates (25 seeds each) per treatment, during 45 days in a chamber at constant 20°C.

The constant temperature of 20°C was chosen for several reasons: (1) it was necessary to have the same set of experiments to apply to all species; (2) the moderate temperatures of the Chilean coast, on average, are around 20°C for the months in which germination occurs (Fick & Hijmans, 2017); and (3) the constant temperature of 20°C was effective in the treatments performed by Cabrera *et al.* (2015).

## Statistical analysis

The effects of treatments on germination percentages were analyzed statistically using the general linear model (GLM) procedure of the SAS program (SAS Institute, Cary, NC, USA). An analysis of variance (ANOVA) was used to determine the existence of significant differences between treatments. When significant differences were detected ( $p < 0.05$ ), Least Significant Difference (LSD;  $\alpha=0.05$ ) test was used to detect significant differences in the comparison between pairs of treatments. Before the analysis, germination percentages were transformed to the arcsin of the square root of the fraction value, but untransformed data are presented in the results and used for discussion.

## Results and discussion

### 3.1 Methylene blue staining

In *Nolana*, dispersal units are fruits called mericarps, which can vary in number between species from 2 to 30 per schizocarp and from laterally united and multi-seeded to completely free and single-seeded (Tago-Nakazawa and Dillon, 1999; Knapp, 2002). Seeds within the mericarp remain in independent chambers (Saunders, 1936) and are firmly embedded within the fruit. To facilitate analysis, in this study we consider only one seed per mericarp, so “seed” will be the unit in which results are expressed.

The testa of the seed, and also certain tissues of the fruit (such as the pericarp), can prevent germination for a considerable period of time by being impervious to water and preventing its passage to the embryo (Baskin *et al.*, 2000; Finch-Savage and Leubner-Metzger, 2006). The impermeability may be caused by one or more layers of palisade cells in the testa, or by sclereids with lignified secondary walls present in fruit tissues (Bewley *et al.*, 2013). A thick layer of sclereids was identified by Cabrera *et al.* (2015) in *Nolana jaffuelii* mericarps; however, when embedding intact mericarps of different *Nolana* species in methylene blue,

we found that the water was able to enter until reaching the embryo for all the mericarps and species, usually within 48 hr since imbibition started (Fig. 3) and confirmed that the entrance route of the water is the germination plug. Therefore, there would be no impermeability to water and therefore, it does not correspond to physical dormancy, since this type of dormancy is defined as impermeability of the fruit or seed to water (Baskin and Baskin, 2004, 2014).

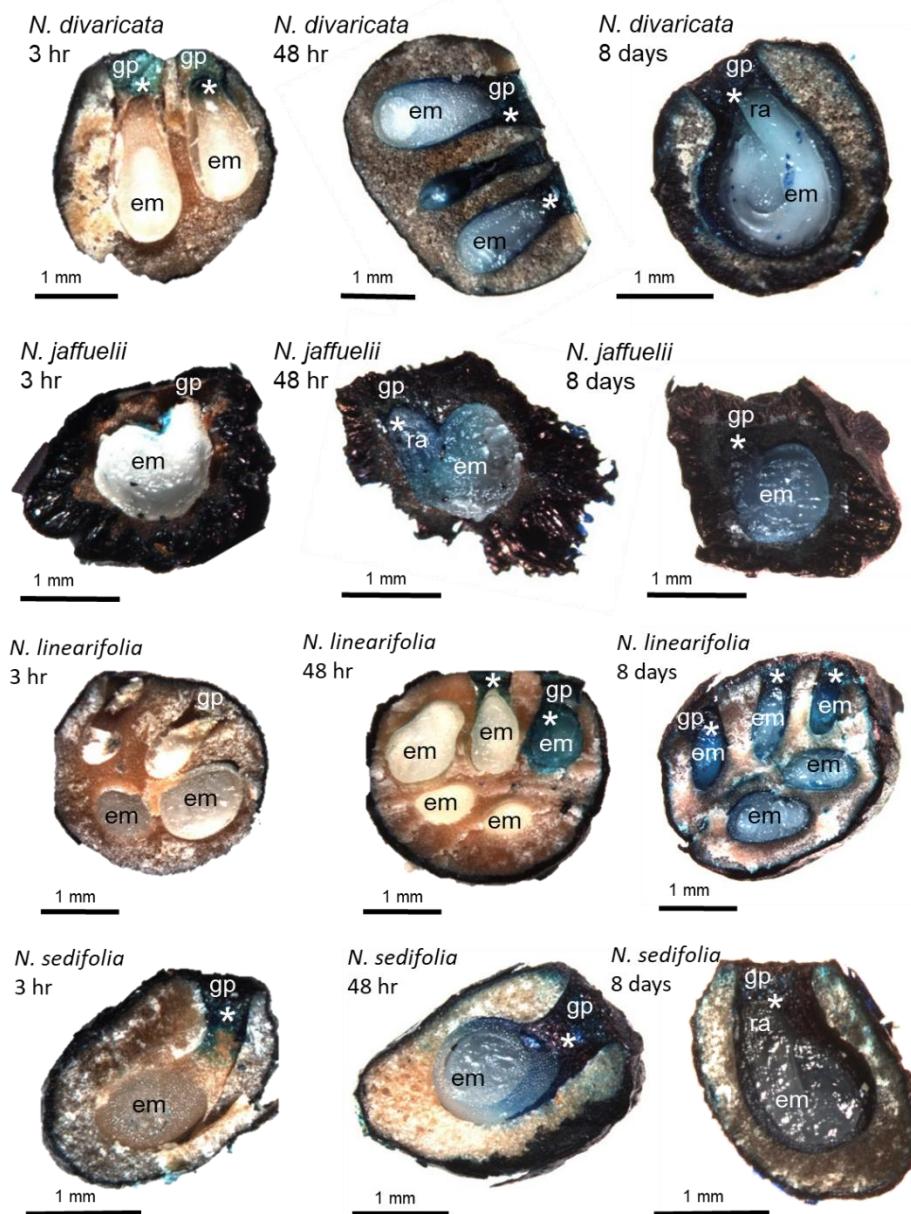


Figure 3. Longitudinal sections of intact mericarps (with germination plug) of *N. divaricata*, *N. jaffuelii*, *N. linearifolia* and *N. sedifolia* at 3 hours, 24 hours, 48 hours and 8 days after imbibition in methylene blue. Abbreviations: em: embryo; gp: germination plug; ra, radicle; \* indicates regions which have imbibed.

### 3.2 Evaluation of germination and characterization of dormancy

#### *Role of funicular plug on germination*

Seeds with physiological dormancy (PD) are permeable to water and possess a physiological inhibiting mechanism that prevents radicle emergence (Baskin and Baskin, 2014), which could also be associated to structures surrounding the embryo (endosperm, testa or fruit walls) (Baskin and Baskin, 2004). In hot semideserts and deserts, PD is the most common type of seed dormancy for shrubs, perennial succulents, herbaceous perennials and annuals (Baskin and Baskin, 2014). Three levels of PD (non-deep, intermediate and deep) have been distinguished according to strength of the physiological inhibiting mechanism, response to gibberellic acid and dormancy-breaking requirements (Baskin and Baskin, 2014).

Our results indicate that seeds of the studied species present physiological dormancy, following the definition by Baskin and Baskin (2004, 2014). Germination results after the first experiment (expressed in different germination percentages in response to treatments) indicate significant differences on percentages between control, scarification (removal of germination plug) and scarification+GA<sub>3</sub> treatments (*p* value <.0001) for each of the species, indicating that the germination plug indeed plays a role on germination of *Nolana* species. Therefore, it was confirmed that the best treatment for all species corresponds to a cut in the germination plug and the addition of gibberellic acid. The only exception was *N. intonsa*, which did not present germination with any of the treatments applied (Table 2).

Species also showed varying levels/degrees of depth in terms of their seed dormancy. This has also been reported by other studies on seeds of species belonging to one same genus (Van Assche *et al.*, 2002 in *Rumex* (Polygonaceae); Barreto *et al.*, 2016 in *Stachytarpheta* (Verbenaceae), Giorni *et al.*, 2018 in *Xyris* (Xyridaceae)). In our case, differences among species ranged from 0 to 62% (Table 2). Species that in general had higher germination percentages were those belonging to Clade G, then those of Clade B, and finally those of Clade C. Species of Clade F (*N. intonsa*) did not register germination.

It is important to take into account the seed viability data (Table 2) given by the tetrazolium test to correct some germination percentages. In general, the percentages remain relatively stable for the species, except for *N. crassulifolia*, which increases significantly, as for *N. divaricata* and *N. sedifolia*.

Table 2. Germination percentages of mericarps from 13 accessions of 10 *Nolana* species in response to three treatments: (1) Control (Ct), intact mericarps imbibed in distilled water; (2) scarification (Sc), removal of germination plug and imbibition in distilled water; (3) scarification and imbibition in a solution of 500 ppm GA<sub>3</sub> (Sc+ GA<sub>3</sub>). Percentage of seed viability was determined by tetrazolium test (1%) of 25 mericarps per species.

Species	Clade	Collection site	Ct <sup>1</sup> (%)	Sc <sup>1</sup> (%)	Sc+GA <sub>3</sub> <sup>1</sup> (%)	Viable seeds (%)	p value <sup>2</sup>
<i>N. crassulifolia</i>	G	Punta de Choros	16 b	16 b	34 a	60	0.0683
<i>N. crassulifolia*</i>	G	Llanos de Challe	1 b	40 a	62 a	65	0.0038
<i>N. divaricata</i>	G	Llanos de Challe	0 b	23 a	35 a	50	<0.0001
<i>N. divaricata*</i>	G	Punta de Choros	0 b	4 b	37 a	91	0.0004
<i>N. linearifolia</i>	G	Antofagasta hills	3 b	3 b	55 a	96	<0.0001
<i>N. onoana</i>	G	Antofagasta hills	15 b	25 b	54 a	75	0.0012
<i>N. sedifolia</i>	G	Los Molles	0 b	24 a	30 a	38	0.0008
<i>N. sedifolia*</i>	G	Alto Patache	0 c	25 b	53 a	61	<0.0001
<i>N. intonsa</i>	F	Alto Patache	0	0	0	40	0.4053
<i>N. carnosa</i>	C	Llanos de Challe	0 b	2 ab	13 a	96	0.0457
<i>N. rostrata</i>	C	Llanos de Challe	0 b	0 b	17 a	76	<0.0001
<i>N. jaffuelii</i>	B	Alto Patache	0 c	5 b	27 a	88	<0.0001
<i>N. parviflora</i>	B	Antofagasta hills	0 b	19 a	25 a	84	0.0011

<sup>1</sup>For each species and collections, values with different letters are significantly different according to a LSD test ( $\alpha=0.05$ )

<sup>2</sup>P value from an analysis of variance.

\* For *N. crassulifolia*, *N. divaricata* and *N. sedifolia*, data from two different populations are presented.

It was not possible to ascribe a level of physiological dormancy to each species, since the experiments did not focus on determining the level of each but rather establishing comparisons between species. However, it is possible to associate a less deep level of PD in the case of some species which showed germination after imbibition in distilled water (control treatment), such as *N. onoana* and *N. crassulifolia*; others with a more intermediate level of PD (*N. divaricata*, *N. linearifolia*, *N. sedifolia*, *N. jaffuelii*, *N. parviflora*), which showed germination after scarification and the addition of gibberellic acid; and finally, some that hardly germinate with any of the treatments, which would correspond to a deeper dormancy (*N. carnosa*, *N. intonsa*, *N. rostrata*) (Baskin and Baskin, 2004). Further experiments, taking into account varying periods of cold stratification, need to be performed in order to establish more accurate degrees or levels of PD.

To add to the analysis, for those species for which it was possible to do so (*N. crassulifolia*, *N. divaricata* and *N. sedifolia*), germination data from different populations was compared for the same species (Table 2). Results showed differences between populations in the case of *N. crassulifolia* (Punta de Choros and Llanos de Challe) and *N. divaricata* (Punta de Choros and Llanos de Challe), but not for *N. sedifolia*, even when populations were very far apart (Alto Patache and Los Molles). It would therefore seem that the dormancy trait in

two of these species is modified by the environment in which they grow, but this conclusion could not be extended to all species, since for *N. sedifolia*, dormancy is determined more by genotype than by the environment.

### 3.3 Additional experiments with *Nolana linearifolia*

The additional experiments carried out with *N. linearifolia* allowed us to confirm the type of treatments that are most successful (Fig. 4), which related to scarification at the funicular scar area, independent of the addition of gibberellic acid. In several other treatments, mericarps were left intact (control, addition of different concentrations of GA<sub>3</sub>, cold stratification and alternate temperatures) as a way to simulate what would happen in nature and to understand how the germination plug is released; however, they yielded very low results (<10%).

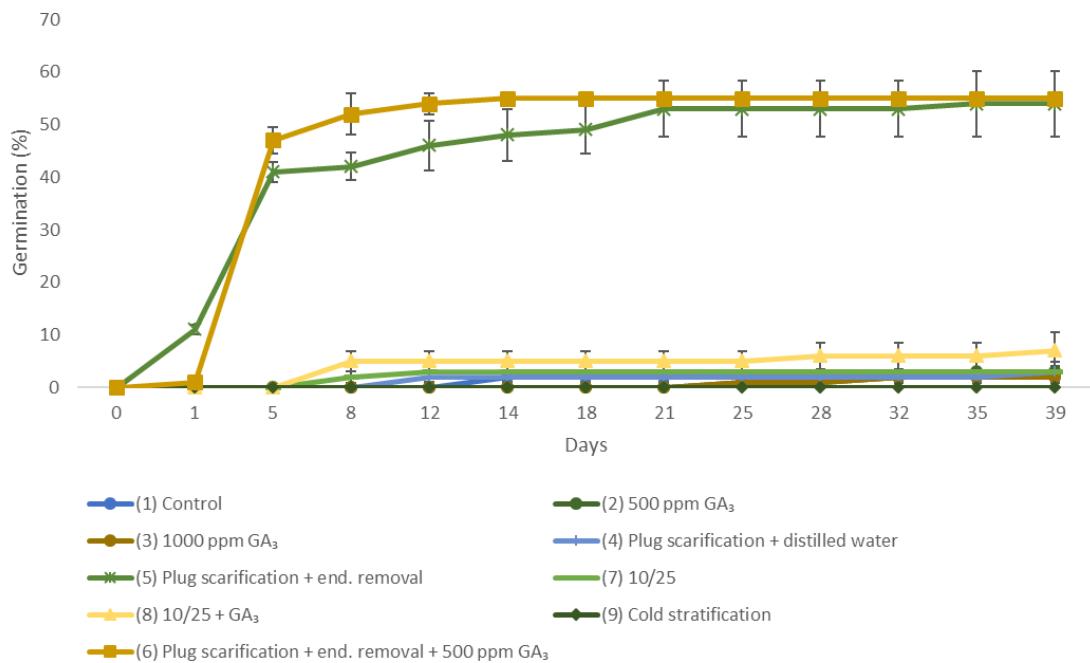


Fig. 4. Cumulative germination of *Nolana linearifolia* mericarps after eight treatments (T): (1) Control: intact mericarps imbibed in distilled water; (2) 500 ppm GA<sub>3</sub>: intact mericarps imbibed in gibberellic acid at 500 ppm; (3) 1000 ppm GA<sub>3</sub>: intact mericarps imbibed in gibberellic acid at 1000 ppm; (4) Plug scarification, i.e., removal of germination plug, imbibed in distilled water; (5) Plug scarification with partial removal of endosperm, imbibed in distilled water; (6) Plug scarification + 500 ppm GA<sub>3</sub>: scarified mericarps with partial removal of endosperm imbibed in gibberellic acid (500 ppm GA<sub>3</sub>); (7) 10/25: intact mericarps at alternating temperatures of 10/25°C, imbibed in distilled water; (8) 10/25 + GA<sub>3</sub>: intact mericarps at alternating temperatures of 10/25°C, imbibed in gibberellic acid (500 ppm GA<sub>3</sub>); and (9) Cold stratification (5°C) of intact mericarps for 4 months. Data used is an average ± standard error of four replicates.

### 3.4 Role of endosperm on germination

The PD may be caused by structures that cover the embryo, including endosperm (or perisperm), seed coats and indehiscent fruit walls, among others, which can restrict radicle emergence, especially in freshly matured seeds (Baskin and Baskin, 2014). In seeds of type species of the family Solanaceae (pepper, tomato, tobacco), the diploid embryo is surrounded by two layers: the endosperm (triploid) and the testa, of maternal origin (mostly dead cells). The micropylar endosperm (of several layers of cells), which covers the tip of the radicle, has been identified as a limiting factor for germination in species of the Solanaceae family, as was reported in *Datura* (Sánchez et al., 1990) and *Solanum* (Groot and Karssen, 1987). This micropylar endosperm acts as a mechanical barrier for germination and participates in the regulation of the restriction of the embryo, by affecting the balance of hormones (ABA / gibberellins) and sensitivity to them (Finch-Savage and Leubner-Metzger, 2006).

After the experiments carried out for *N. linearifolia*, and having finished the first set of experiments in the other species, a different set of treatments was tested to elucidate the role of the endosperm in *Nolana* germination; this was only done for species that had high germination percentages after removal of germination plug and addition of gibberellic acid (Exp. 1), the majority of which belong to clade G. Results indicate that there was an evident relationship between the partial removal of the endosperm and germination of these species (Table 4), comparable to the addition of gibberellic acid. The extraction of the germination plug, therefore, although it is necessary because it is the point from which the radicle emerges, is not as decisive as the removal of the layer immediately below; that is, the endosperm.

Table 4. Germination percentages of mericarps from 6 *Nolana* species in response to three treatments: (1) Control (Ct), intact mericarps imbibed in distilled water; (2) scarification (Sc), removal of germination plug and imbibition in distilled water; (3) scarification and partial removal of endosperm (Sc+en) and imbibition in distilled water. Percentage of seed viability was determined by tetrazolium test (1%) of 25 mericarps per species.

Species	Clade	Collection site	Ct <sup>1</sup> (%)	Sc (%)	Sc+en <sup>*</sup> (%)	Viable seeds (%)	p value <sup>2</sup>
<i>N. aplocaryoides</i>	G	Pan de Azúcar	0 c	23 b	63 a	96	<0.0001
<i>N. crassulifolia</i>	G	Los Molles Llanos de Challe	6 c	22 b	55 a	48	<0.0001
<i>N. divaricata</i>	G	Antofagasta hills	0 c	23 b	40 a	84	<0.0001
<i>N. linearifolia</i>	G		3 b	3 b	54 a	96	<0.0001
<i>N. paradoxa</i>	B	Los Molles	2 b	16 b	54 a	84	0.0088
<i>N. sedifolia</i>	G	Los Molles	1 c	22 b	40 a	60	<0.0001

<sup>1</sup>For each column, values with different letters are significantly different according to a LSD test ( $\alpha=0.05$ )

<sup>2</sup>P value from an analysis of variance.

\* Scarification plus partial removal of endosperm

The weakening of this barrier seems to be a prerequisite for the protrusion of the radicle during germination in the species that present it (Finch-Savage and Leubner-Metzger, 2006), particularly in the micropylar portion (Sánchez *et al.*, 1990). While gibberellins promote weakening -which may be due to enzymes that the same tissue synthesizes-, ABA would be inhibiting it (Finch-Savage and Leubner-Metzger, 2006). The removal of the endosperm and testa layers opposite the radicle tip may also assist this process to permit radicle protrusion (Groot and Karssen, 1987).

### *3.5 Relationship between dormancy and geographical distribution of species and clades*

Similar traits and life cycles are expected for species within a genus; different germination requirements of the species may reflect specific adaptations to the occupied habitat (Van Assche *et al.*, 2002). Barreto *et al.* (2016) studied the viability and germination of seeds of 12 species of *Stachytarpheta* (Verbenaceae), and their results showed differences in germination among species that appeared to be related to their natural habitats and geographic distributions. Conversely, Giorni *et al.* (2018) found that, for the *Xyris* species they studied (Xyridaceae), the effects of temperature on seed germination did not explain the patterns of geographic distribution nor the endemism seen among the species examined. In an extensive study on species from campo rupestre grasslands in Brazil, Dayrell *et al.* (2016) found that phylogeny was better correlated than ecology with seed dormancy categories, indicating that geological and climatic history had a more important role in driving seed dormancy than contemporary factors (Dayrell *et al.*, 2016).

When germination results of the studied *Nolana* species (corrected by the percentage of seed viability) were correlated with the latitudinal distribution of each species (Fig. 5), no evident relationship was obtained. Some species with a very limited distribution had a high percentage of germination (*N. onoana*), whereas those with a wide latitudinal distribution (i.e. *N. rostrata*), contrary to what was expected, had lower germination percentages.

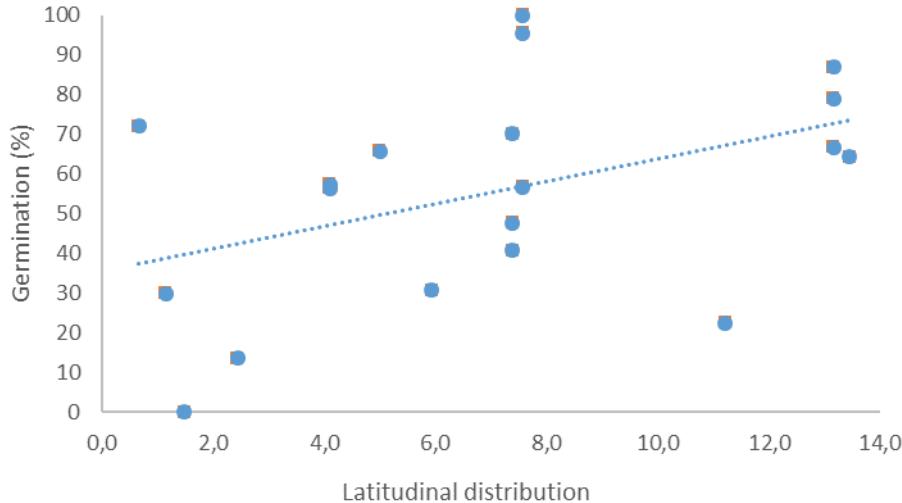


Fig. 5. Correlation between germination percentage of 19 accessions of 12 species of *Nolana* for the best treatment (removal of germination plug and imbibition in a solution of 500 ppm GA<sub>3</sub>, or removal of germination plug and partial removal of endosperm) and latitudinal distribution, expressed as the difference between the northernmost and the southernmost latitudinal distribution point (decimal degrees). R = 0,439 (p value = 0,060).

Regarding the relation between the level of physiological dormancy (expressed inversely as the percentage of germination for the most successful treatment, i.e. scarification + GA<sub>3</sub>) and phylogeny, it could be determined that, for the studied species, their proximity in terms of clades (Fig. 2) was more relevant than their latitudinal distribution. Species that belong to Clade G showed, as a whole, higher percentages of germination (i.e. least deep dormancy) for the most successful treatment than the species of the other clades, but Clade B also had high germination percentages (Fig. 6).

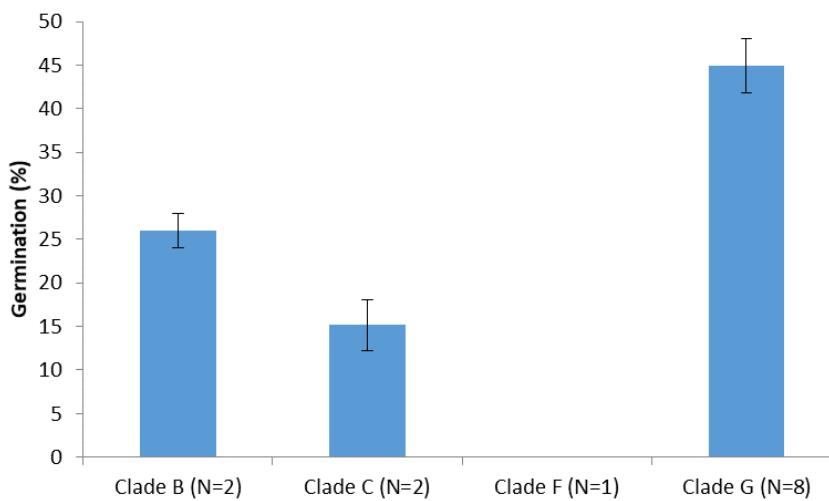


Fig. 6. Germination percentage of *Nolana* species for the Sc+GA<sub>3</sub> treatment (removal of germination plug and imbibition in giberrellic acid), grouped into clades B, C, F, G. The number of

species/populations included for each clade is noted. Data used is an average  $\pm$  standard error of four replicates.

The species that showed higher germination percentages were *N. crassulifolia* (clade G), a prostrate shrub that forms mats or draping rocks near the sea (Johnston, 1936), with a distribution between 26° and 33°S; and *N. aplocaryoides* (clade G), an erect and branched annual (Mesa, 1981), extending from Antofagasta to Atacama (22° to 27°S). Both have wide distributions, but in different environments, since *N. crassulifolia* reaches to central Chile (semi-desert), while *N. aplocaryoides* is only found in the desert. *N. onoana* (clade G), however, which also presented a relatively high germination percentage, is only found at a few locations in Antofagasta region. A particular case is *N. paradoxa* (clade B), with the most extensive distribution from the center to the south of coastal Chile (33°S - 42°30'S), where the climate is temperate humid; *N. paradoxa* had high germination percentages, but not higher than the other species mentioned.

Clade B includes two subclades: rosette-forming, taprooted plants with larger flowers, and erect annuals with slightly smaller flowers (Dillon *et al.*, 2009), while Clade G is composed of small to moderate shrubs and annuals, all with highly reduced corollas, often white or yellowish (Dillon *et al.*, 2009). These two clades have similar germination results, although their growth habits are very different, and their mericarps (see Hepp *et al.*, in press) are also highly dissimilar. Altogether, this last clade (G) was found to have the higher germination percentage, i.e. the least deep physiological dormancy. We would have expected a higher level of dormancy in species with annual habits, since they depend more on seedling survival and/or formation of a seed bank for persistence (Dayrell *et al.*, 2016; Venable, 2007), which was partly the case of *N. paradoxa*; but as mentioned before, its germination percentage was not higher than those of most Clade G species. Dayrell *et al.* (2016) also found no significant correlations between seed dormancy and life history traits, such as growth habit.

Although other studies have also found no evidence of a correlation between dormancy and geographic distribution (Giorni *et al.*, 2018; Dayrell *et al.*, 2016), for *Nolana* it remains to be studied if species that occupy a greater diversity of ecological niches present a greater plasticity in their dormancy levels (lower levels of PD). Our analysis in this case focused on the variation in conditions that may exist between different latitudes along the coast but did not include the habitat or niche occupation for each of the species. Further studies are needed to determine if there is such a pattern.

The highest germination percentage reached by the species studied was > 60%, and some of them reached much lower percentages, even 0 for the most successful treatment in other species; in fact, for most species there was almost no germination when the germination plug was not removed (control treatment), that is, when local conditions were simulated. This would indicate a very "cautious" germination strategy, as suggested by Guterman (1995), with higher thresholds of response to rainfall to begin germination, or deeper levels of dormancy. It is important to consider that some of these species inhabit places where rainfall occurs every 15 years or more, as is the case of *N. intonsa*, *N. jaffuelii* and *N. sedifolia* in the fog oasis of Alto Patache in the North of Chile (Orellana *et al.*, 2017; Pliscoff *et al.*, 2017).

## **Conclusions**

Our results indicate that seeds of the studied species present physiological dormancy with varying levels/degrees of depth. However, they do not present physical dormancy, as has been reported previously, since water was able to enter the mericarp and reach the embryo in all the species studied. Furthermore, the role of the endosperm in the prevention of germination for the *Nolana* species studied is emphasized; the weakening (or removal) of this barrier facilitates germination in the species that present it, particularly in the micropylar portion. For the species studied, the results show a relationship between the percentage of germination (or level of dormancy) of the species and their proximity in terms of clades, but not with respect to their latitudinal distribution. Hence, our hypothesis of a greater similarity in terms of type and level of dormancy among more closely related species was corroborated; but we found no evidence of a relationship between level of dormancy and range of distribution (latitudinal) for the species of the genus.

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### **Chapter III. Current and future patterns of distribution of *Nolana* species (Solanaceae) from the coast of Chile and their relation to seed dormancy**

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#### **Abstract**

The genus *Nolana* (Solanaceae) comprises numerous species endemic to the arid and semi-arid environments in the coastal area of Chile and Peru. Seed dormancy of the physiological type with varying levels of depth has been reported for certain *Nolana* species. The objective of this study is to establish the current and future distribution patterns of the genus for Chilean species, to analyse if there is a relation between distribution (expressed as niche breadth) and level of seed dormancy of a set of eight species, and how that may influence survival of species under future climate change scenarios. Results for the current distribution pattern confirm the two reported centres of diversity of *Nolana*, in Arequipa (Peru) and between Antofagasta and Atacama (Chile); in the future, a contraction towards the coast is projected. As for the change the eight studied species will present in future climatic scenarios, there is a diversity of responses according to the species. Species that have lower dormancy values are also those whose distribution range will remain stable or expand, and conversely, distribution range will contract in the case of species with high dormancy values. We found no evidence of a relation between distribution and dormancy; that is, dormancy level is not explained by niche breadth.

Keywords: niche breadth, Atacama desert, climate change, mericarp

#### **Introduction**

The genus *Nolana* L. ex L. f. (Solanaceae) currently comprises 90 species of annual or perennial herbaceous plants or small shrubs with leaves with different degrees of succulence and flowers of different colors and sizes (Dillon *et al.*, 2007; Tu *et al.*, 2008; Dillon *et al.*, 2009). The majority of species grows preferentially in the arid and semiarid zones of coastal Chile and Peru, although some species are found in high altitude habitats (> 1,000 m a.s.l.) and a few of them, i.e. *N. paradoxa* and *N. sedifolia*, have distributions that

range over 1,000 km (Johnston, 1936). Dillon *et al.* (2009) have identified several strongly supported clades within the *Nolana* genus, with geographic and morphological fidelity, four of which are confined to Chile and three that are mainly Peruvian with some presence in Chile.

According to Rundel *et al.* (1991) and Schultz *et al.* (2011), the coastal desert of Atacama extends between 18°S and 30°S. The climate here is moderate in terms of temperature, which in general is quite stable in the latitudinal range along the coast (average temperature of 15-18° C from Valparaíso to Tarapacá; Fick & Hijmans, 2017). Atmospheric humidity is high (annual average of 65-80%), and annual rainfall ranges from less than 1 mm in the northernmost stations to around 80 mm in La Serena (Schultz *et al.*, 2011; Chilean Meteorological Office, Integrated Territorial Information System (SIIT)). In the area north of 21°30'S, the vegetation is concentrated in small patches in isolated sites that receive fog, usually on slopes with south or southwest exposure (Schultz *et al.*, 2011); the high frequency and intensity of fog is a decisive ecological factor, to which is attributed the existence and maintenance of these vegetation formations known as "fog oases" (Rundel *et al.*, 1991) or "Lomas vegetation" (Rundel *et al.*, 1997). Continuing south, up to around 26°S, vegetation increases but remains dispersed. From there towards 30°S, the fog oases extend in a relatively closed formation along the windward slope of the coastal mountain range (Rundel *et al.*, 2007, cited in Schultz *et al.*, 2011).

In environments with high temporal variability, such as deserts, seed dormancy is often described as a bet-hedging strategy (Eberhart and Tielbörger, 2012). Not all mature seeds produced by a plant germinate immediately after dispersal, or in the following season; in many cases, as a strategy of survival, a significant amount of seeds remains dormant in the soil, for a period that can last for years or even decades, forming what is known as a seed bank (Aguado *et al.*, 2012).

Dormancy can be defined as "an innate property of the seed that defines the environmental conditions in which the seed is capable of germination" (Finch-Savage and Leubner-Metzger, 2006), and its induction is controlled both at the genetic level and by the environment. Genetic control can be associated with both the embryo genotype and that of the mother plant (Contreras and Rojas, 2010); the state of dormancy is influenced by the environment in which the seed develops (maternal environment), but also by the external environment when dispersed, so it can change over time (Finch-Savage and Leubner-Metzger, 2006). According to Baskin and Baskin (1998, 2004), there are five types of seed dormancy, the most frequent being physiological dormancy (PD), which has three levels of depth (not deep, intermediate and deep). The dormancy status of a batch of seeds can be established based on embryo morphology, the permeability of the seed coat to water, and the capacity of fresh seeds to germinate within one month (Baskin and Baskin, 2004; Schwienbacher *et al.*, 2011).

In a study on seed dormancy of 12 *Nolana* species, Hepp *et al.* (in preparation) found that seeds presented PD with varying levels of depth, emphasizing the role of the micropylar portion of the endosperm in the prevention of germination for the *Nolana* species studied. Freyre *et al.* (2005) and Douglas and Freyre (2006) also reported PD for another nine *Nolana* species, both from Chile and Peru. This property would allow the species to defer

germination in time; to this is added the fibrous hard fruits called mericarps that *Nolana* species present, a character that is unique in the Solanaceae family (Knapp, 2002, Dillon *et al.*, 2009). The pericarp of mericarps is constituted by sclereids, which give strength and rigidity to the fruit (Cabrera *et al.*, 2015) and may provide protection to the embryo, as it would allow the mericarps to remain relatively intact in the soil seed bank for many years (Hepp *et al.*, 2019, *in press*).

Although other studies have found no evidence of a correlation between dormancy and geographic distribution (Giorni *et al.*, 2018; Dayrell *et al.*, 2016), in *Nolana* this has not been studied. Hepp *et al.* (*in preparation*) focused in the latitudinal variation (expressed as the difference between the northernmost and southernmost latitudinal distribution point in decimal degrees), which showed no pattern, but did not include the habitat or niche occupation for each of the species. The presence of the genus in the whole range of “lomas formations”, where it stands out as the most conspicuous floristic element (Tago-Nakazawa and Dillon, 1999), makes it interesting for this type of study.

The objective of this study was to establish the current and future distribution pattern of the genus for Chilean species, and to determine which variables define their presence. A second objective was to analyse if there is a relationship between distribution pattern and the level of dormancy presented by seeds of a set of species. Also, if such a relationship exists, given the projections of less rainfall and increasing temperatures in the arid and semi-arid area of northern Chile by 2050 (Santibañez *et al.*, 2017), how will it change in the future?

## Materials and methods

### Species occurrences database

A presence occurrences database of 1,637 unique records was compiled for 45 species of the genus *Nolana* (50% of total number of known species) of Chile and Peru (Fig. 1); only species with at least 10 unique occurrences were selected for modelling. The complete list of species and number of occurrences by database is presented in Annex 1. The source of species occurrences where the following Herbaria: SGO (National Museum of Natural History, Santiago, Chile); CONC (Botany Department, Universidad de Concepción, Concepción, Chile); Global Biodiversity Information Facility (GBIF) and Michael Dillon personal database.

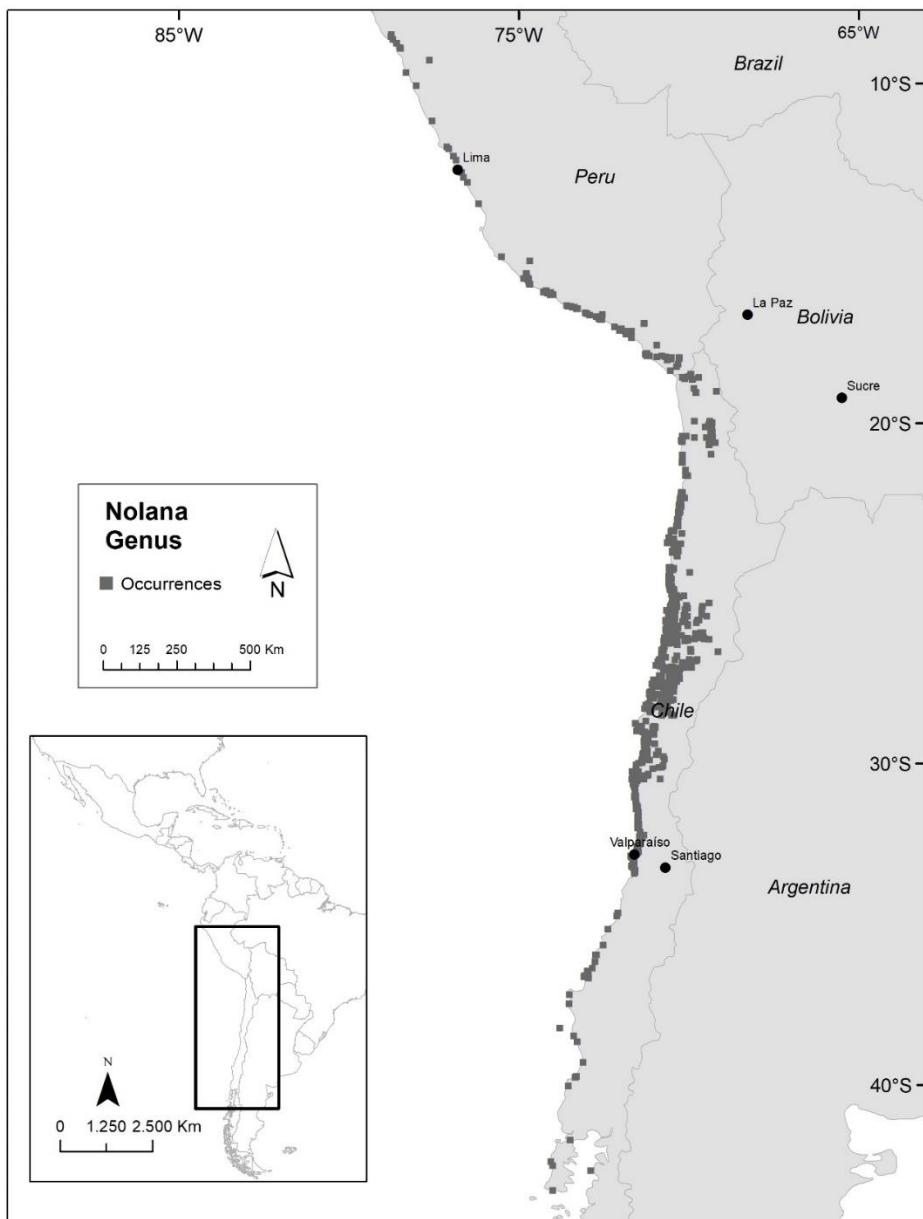


Fig. 1. Study area with all occurrences included for the *Nolana* genus.

### Environmental variables

Current climatic surfaces (1950-2000) for the study area were obtained from an improved climatic surface dataset presented in Pliscoff *et al.* (2014) and available for download in the Spatial Ecology Group webpage (<http://www.unil.ch/ecospat/home/menuguid/tools--data/data.html>). Future projections for each monthly climatic variable were performed according to the delta method (Ramirez-Villegas and Jarvis, 2010) using current climatic surfaces modelled as the baseline; for future values, the most extreme climate change scenarios of the IPCC Fifth assessment report (RCP 8.5) were selected and the Global Circulation Models (GCM) scenario Hadgem2\_ES at 2070-2100 time period. Future GCM scenarios were downloaded from CIAT-GCM (Centro Internacional de Agricultura Tropical-

Global Climate Model <http://ccafs-climate.org/>). Future monthly climatic surfaces were used to derive 19 bioclimatic variables (Nix, 1986; Busby, 1991), which are widely used in species distribution modelling (Synes and Osborne, 2011), bioclimatic dataset are available for download for each GCM for the year 2080 (RCP 8.5) in the Spatial Ecology Group webpage.

A variable selection step was applied in which pairwise Pearson correlations were calculated between all variables, and in cases when correlation values were greater than 0.8 (Correlation matrix are presented in Annex 2), one of the two variables was excluded (Elith *et al.*, 2010) in order to avoid strongly correlated variables in the set of predictors (Kozak and Wiens, 2006). This assessment resulted in five variables:

- Annual Mean Temperature (BIO1)
- Mean Diurnal Range (BIO2)
- Annual Precipitation (BIO12)
- Precipitation Seasonality (BIO15)
- Precipitation of the Coldest Quarter (BIO19)

In addition, a cloud cover variable was added to the final bioclimatic dataset for the characteristics of coastal desert environment where *Nolana* species inhabit. The Global 1-km cloud cover dataset was used (Wilson and Jetz, 2016) which presents a monthly cloud cover data derived from MODIS satellite images. The annual mean cloud cover extracted in the study area extent was also used. Final six variables (five bioclimate plus cloud cover) were used to calibrate the current models and project them to future conditions.

### **Species distribution modelling**

MaxEnt software (Phillips *et al.*, 2016) was used to define suitability areas (a habitat's potential to support a particular species) of *Nolana* species under current and future conditions. MaxEnt looks for the species distribution suitability of probabilities closest to uniform (maximum entropy), constrained to the fact that feature values match their empirical average (Phillips and Dudík, 2004), MaxEnt performs very well with relatively small sample sizes of presence-only data (Elith *et al.*, 2006; Wisz *et al.*, 2008). Also, many studies have found strong support for high temporal transferability of MaxEnt models (Rapacciulo *et al.*, 2012). MaxEnt was ran using default parameters and the logistic output as an estimate of environmental suitability of species was used. Presence records of each species were randomly split into two subsets: 20% for training and 80% for testing. The accuracy of models was evaluated with the area under the receiver operating characteristic curve (AUC) (Lobo *et al.*, 2008). After modelling the current distribution of *Nolana* species, the environmental suitability of species was projected in one future climate scenario (HadGem2\_ES rcp 8.5 for 2070-2100 period). MaxEnt predictions were transformed into binary outputs using the 10% percentile of training presences. Future changes in suitability were evaluated using SDMtools (Brown, 2014) under ArcGis 10.6 software (ESRI 2017). For each species, expansion, contraction and stability were calculated under future scenarios, and also direction of change following centroids comparison between current and future models.

## Germination tests

In order to establish the relation between habitat suitability and seed dormancy of a set of species, the results of germination experiments and seed viability reported in Hepp *et al.* (in preparation) were used for 10 species of *Nolana* (Table 1, Fig. 2). These 10 species belong to 3 clades within the genus, as described in Dillon *et al.* (2009) and can be summarized as follows:

- Clade G has recovered a strictly Chilean group of 13 species represented by small to moderate shrubs (traditionally called "*Dolia*") and annuals, all with highly reduced corollas, often white or yellowish (Dillon *et al.*, 2009) but some have bluish flowers.
- Clade C has recovered a monophyletic group comprised of 6 species traditionally classified as *Alona*. These are all large-flowered shrubs confined to Chile (Dillon *et al.*, 2009).
- Clade B, previously recognized as a segregate genus, *Sorema*, currently consists of two sub-groups: *N. pterocarpa*-*N. baccata*-*N. parviflora*, which are erect annuals with small flowers; and *N. paradoxa*-*N. rupicola*, which are rosette-forming, taprooted plants with larger flowers (Dillon *et al.*, 2009). These species are only present in Chile (Dillon *et al.*, 2009).

Experiments considered for this study correspond to the most successful treatment for all species, based on Cabrera *et al.* (2015) and Hepp *et al.* (in preparation): removal of germination plug and addition of gibberellic acid (GA<sub>3</sub> 500 ppm); or removal of germination plug and partial removal of endosperm. Germination tests were carried out during 2015 and 2016, using fruits from *Nolana* species collected in the previous months. Germination was evaluated three days a week during 45 days in a chamber at constant 20°C and light (40% RH), in four replicates of 25 seeds each. Physiological germination (radicle emergence over 2 mm) was evaluated. Results were reported as total germination percentage.

Table 1. Selected species of the genus *Nolana*, collection site, results of germination experiments (Ct and BT; Hepp *et al.*, in preparation), and clade (based in Dillon *et al.*, 2009). Treatments are as follows: (Ct) control, intact mericarps imbibed in distilled water; (BT) best treatment, scarification (removal of germination plug) and imbibition in a solution of 500 ppm GA<sub>3</sub>. Percentage of seed viability was determined by tetrazolium test (1%) of 25 mericarps per species.

Species	Collection site	Ct (%)	BT (%)	Viable seeds (%)	Clade*
<i>Nolana aplocaryoides</i> (Gaudich.) I.M. Johnst.	Pan de Azúcar, Atacama (26°08.786'S, 70°39.943'W)	0	63	96	
<i>Nolana crassulifolia</i> Poepp.	Llanos de Challe, Atacama (28°11.086'S, 71°09.751'W)	1	62	65	G
	Punta de Choros, Coquimbo (29°14.786'S, 71° 28.021'W)	16	34	60	

<i>Nolana divaricata</i> (Lindl.) I.M. Johnst.	Punta de Choros, Coquimbo (29°14.601'S, 71°27.840'W)	0	37	91	
	Llanos de Challe, Atacama (28°11.991'S, 71°09.524'W)	0	35	50	
<i>Nolana linearifolia</i> Phil.	Hills of Antofagasta, Antofagasta (23°30.133'S, 70°23.069'W)	3	55	96	
<i>Nolana sedifolia</i> Poepp.	Alto Patache fog oasis, Tarapacá (20°49.542'S, 70°09.392'W)	0	53	61	
	Los Molles, Valparaíso (32° 14.444'S, 71°31.133'W)	0	30	38	
<i>Nolana rostrata</i> (Lindl.) Miers ex Dunal	Llanos de Challe, Atacama (28°06.806'S, 71°09.257'W)	0	17	76	C
<i>Nolana carnosa</i> (Lindl.) Miers ex Dunal	Llanos de Challe, Atacama (28°02.612'S, 71°07.022'W)	0	13	96	
<i>Nolana jaffuelii</i> I.M. Johnst.	Alto Patache fog oasis, Tarapacá (20°48.903'S, 70° 09.630'W)	0	27	88	
<i>Nolana paradoxa</i> Lindl.	Los Molles, Valparaíso (32°14.433'S, 71°31.133'W)	2	54	84	B
<i>Nolana parviflora</i> (Phil.) Phil.	Hills of Antofagasta, Antofagasta (23°30.133'S, 70°23.069'W)	0	25	84	



Figure 2. Context, flowers and fruits of four *Nolana* species included in this study. A, B and C: *N. aplocaryoides* (clade G); D, E and F: *N. sedifolia* (clade G); G, H and I: *N. paradoxa* (clade B); J, K and L: *N. rostrata* (clade C).

### Dormancy Index (DI)

With the germination results of the experiments described above, a dormancy index was calculated. This Dormancy index was modified from Offord *et al.* (2004); percentage of germinated seeds for the best treatment (BT; Hepp *et al.*, in preparation) and control treatment (Ct) were both included. Viability of seeds was tested using tetrazolium (2,3,5-

triphenyl-2H-tetrazolium chloride, or Tz) on a sample of 25 fruits for each species (staining for 24 hours at 30° C, 1% Tz solution, all fruits without germination plug).

A first step was to calculate the initial Dormancy Index (Dli) taking into account only the percentage of seeds germinated in the best treatment, as not all treatments were equally successful, and the percentage of viable seeds. The percentage of seeds germinated in Ct was then incorporated on a second step, resulting on a final Dormancy Index (Dlf).

The higher the value of the Index, the more likely that the seed lot was dormant:

$$Dli = 1 - (\text{seed germinated in BT \%} / \text{viable seeds \%})$$

$$Dlf = (Dli * 100) - (\text{seed germinated in Ct \%} / Dli)$$

### Niche overlap and breadth

To compare the potential distribution between *Nolana* species modelled, we calculated niche overlap and breadth indexes. The niche overlap Schoener's Index shows the similarity between the distributions of *Nolana* niche models where low values indicate low overlap and values near 1 reflects a high overlap between potential distribution modeled. Also, Levin's niche breadth index was calculated, using inverse concentration and uncertainty values to express distribution breadth, values close to 1 indicate broader niche. All calculations were done using the ENMTools 1.3 package in R.

The maximum adequacy value from the location point (collection site) for each species (probability of presence) was extracted.

## Results and discussion

### Current and future distribution

After the analysis of all occurrences for *Nolana*, information for the genus was updated: there is a total of 90 species, 49 of which are present in Chile and 43 are found in Peru; both countries only share three species (*N. adansonii*, *N. gracillima* and *N. lycioides*). *N. jaffuelii*, which was previously reported for Peru, has no modern records and therefore is considered a Chilean endemic. Dillon *et al.* (2009) mention there appears to be a barrier to dispersal along the coast between 18° and 20°S (Dillon *et al.*, 2009), between Tacna (Peru) and the southern limit of the Arica region (Chile).

In terms of the current distribution of *Nolana* species, as mentioned in Dillon *et al.* (2007), there are two concentration areas or centres of greater species diversity: one in the southern Peruvian Department of Arequipa (14°38'S / 70°50'W to 17°17'S / 75°05'W) where 28 species are recorded (Rosado, 2019), and another in the northern Chile from Antofagasta (21°26'S / 69°18'W to 25°56'S / 69°37'W) to Atacama region (25°56'S / 69°37'W to 29°24'S / 70°20'W, with 31 species (Fig. 3A).

In the future, a contraction towards the coast is projected (Fig. 3B), although both centres of diversity are maintained (highlighted in boxes, Fig. 3A and 3B); in addition, there would be an increase in the number of *Nolana* species that would be distributed in these areas. Direction of change (Fig. 4) indicates the loss of species in certain areas of higher altitude, outside of the areas of greater concentration of species. The calculated expansion, contraction and stability for all species under future scenarios is found in Annex 3.

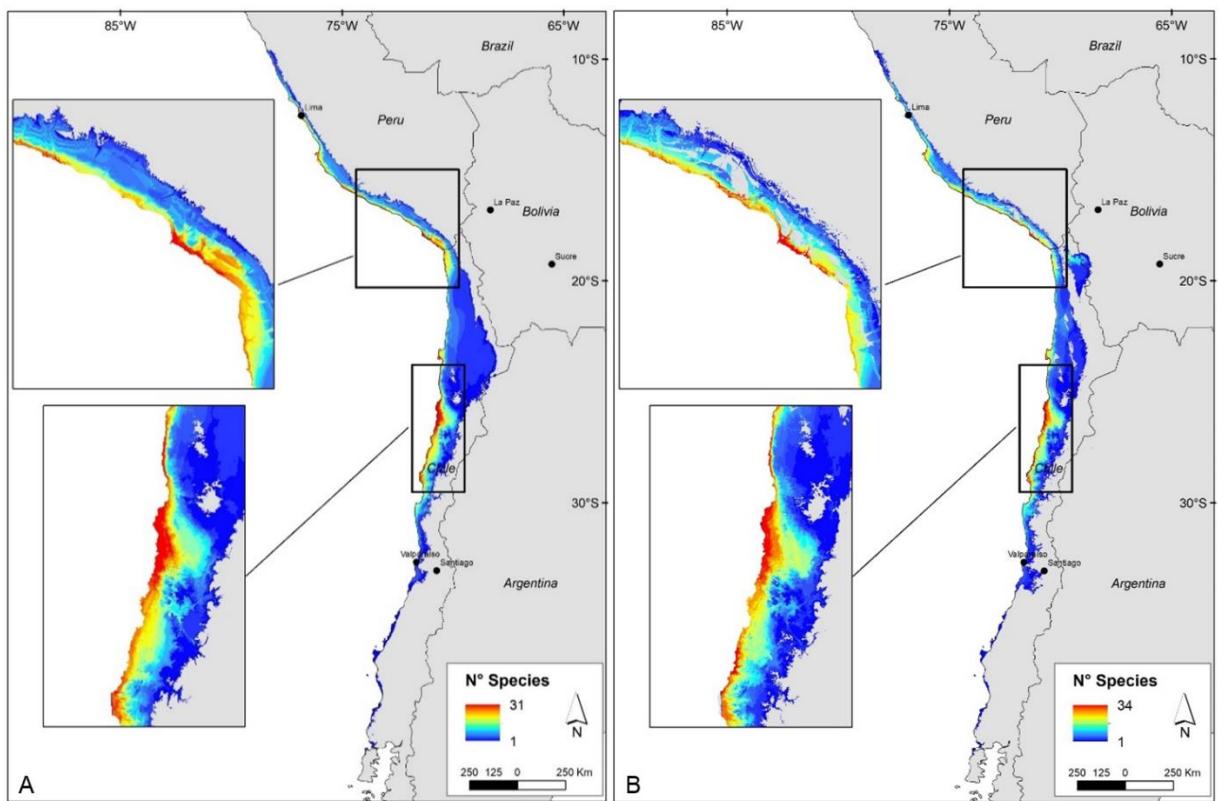


Fig 3. Current (A) and future (B) distribution of *Nolana* species in Chile and Peru. B: Environmental suitability of species was projected in one future climate scenario (HadGem2\_ES rcp 8.5 for 2070-2100 period).

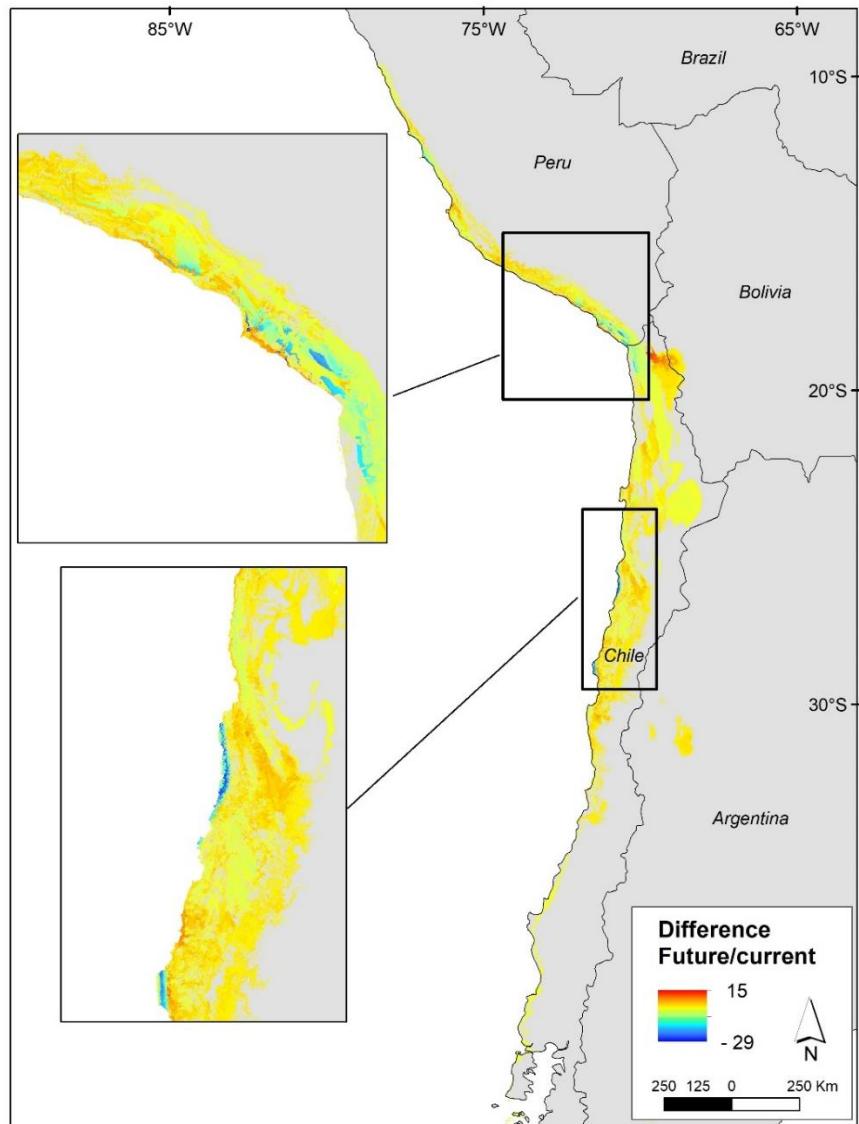


Fig. 4. Direction of change following centroids comparison between current and future models.

### Species distribution modelling

The variable that best defines the presence for most species is annual precipitation (Annex 4). In the case of *N. crassulifolia*, a species with distribution between Atacama ( $26^{\circ} 21.054'S$ ) and Valparaíso ( $33^{\circ} 55.002'S$ ), it is the precipitation seasonality; and in the case of *N. paradoxa*, with a very extensive and fundamentally coastal distribution, it is the mean diurnal temperature range.

In terms of their spatial distribution, the species that most coincide, both in terms of overlap (how their habitat suitability overlap in space) and similarity (how similar they are in shape of habitat suitability), are *N. rostrata* (clade C) and *N. divaricata* (clade G). These are two similar species in terms of growth habit, although they belong to two different clades. *N. divaricata* usually forms a dense succulent globose bush 1-2.5 m tall and seems to be confined to the hills near the sea (Johnston, 1936), in the regions between Antofagasta and

Coquimbo (Zuloaga *et al.*, 2008). *N. rostrata* is a very slender, loosely branched, sprawling shrub with an extensive distribution, only in Chile, between Tarapacá and Coquimbo regions (Zuloaga *et al.*, 2008).

*N. aplocaryoides* and *N. sedifolia* also have high values for both indexes; in this case, these are two species with different forms of growth, but corresponding to the same clade (G). *N. sedifolia* is an endemic perennial shrub or sub-shrub that can measure up to 100 cm tall; it has an extensive distribution between the regions of Tarapacá and Valparaíso (Zuloaga *et al.*, 2008). *N. aplocaryoides* is an annual herb, erect and branched; endemic to northern Chile, it grows between the regions of Tarapacá and Atacama, in plains in full sun (Zuloaga *et al.*, 2008).

Table 2. Overlap Index. The numbers highlighted in bold correspond to high values.

Species	Nsed	Nap	Ncar	Ncra	Ndiv	Nlin	Npa	Nro
<i>N. sedifolia</i> (Nsed)		<b>0.621</b>	0.342	0.378	<b>0.536</b>	0.473	0.177	0.343
<i>N. aplocaryoides</i> (Nap)			0.276	0.233	<b>0.519</b>	<b>0.607</b>	0.093	0.283
<i>N. carnosa</i> (Ncar)				0.391	<b>0.544</b>	0.135	0.116	<b>0.591</b>
<i>N. crassulifolia</i> (Ncra)					0.350	0.105	0.389	0.275
<i>N. divaricata</i> (Ndiv)						0.271	0.132	<b>0.645</b>
<i>N. linearifolia</i> (Nlin)							0.038	0.121
<i>N. paradoxa</i> (Npa)								0.093
<i>N. rostrata</i> (Nro)								

Table 3. Similarity Index. The numbers highlighted in bold correspond to high values.

Species	Nsed	Nap	Ncar	Ncra	Ndiv	Nlin	Npa	Nro
<i>N. sedifolia</i> (Nsed)		<b>0.878</b>	0.643	0.688	<b>0.811</b>	<b>0.780</b>	0.338	0.618
<i>N. aplocaryoides</i> (Nap)			0.568	0.509	<b>0.787</b>	<b>0.878</b>	0.252	0.551
<i>N. carnosa</i> (Ncar)				0.613	<b>0.824</b>	0.355	0.235	<b>0.837</b>
<i>N. crassulifolia</i> (Ncra)					0.632	0.315	0.610	0.515
<i>N. divaricata</i> (Ndiv)						0.525	0.263	<b>0.883</b>
<i>N. linearifolia</i> (Nlin)							0.159	0.305
<i>N. paradoxa</i> (Npa)								0.189
<i>N. rostrata</i> (Nro)								

As for the change that the eight species will present in future climatic scenarios, there is a diversity of responses according to the species (Fig. 5). Some will remain stable and will even increase their range (*N. crassulifolia*, *N. divaricata*, *N. sedifolia*), but others will be more affected and their range will contract (for example, *N. aplocaryoides*, *N. carnosa*, *N. linearifolia*, *N. paradoxa*, *N. rostrata*; see also Annex 4).

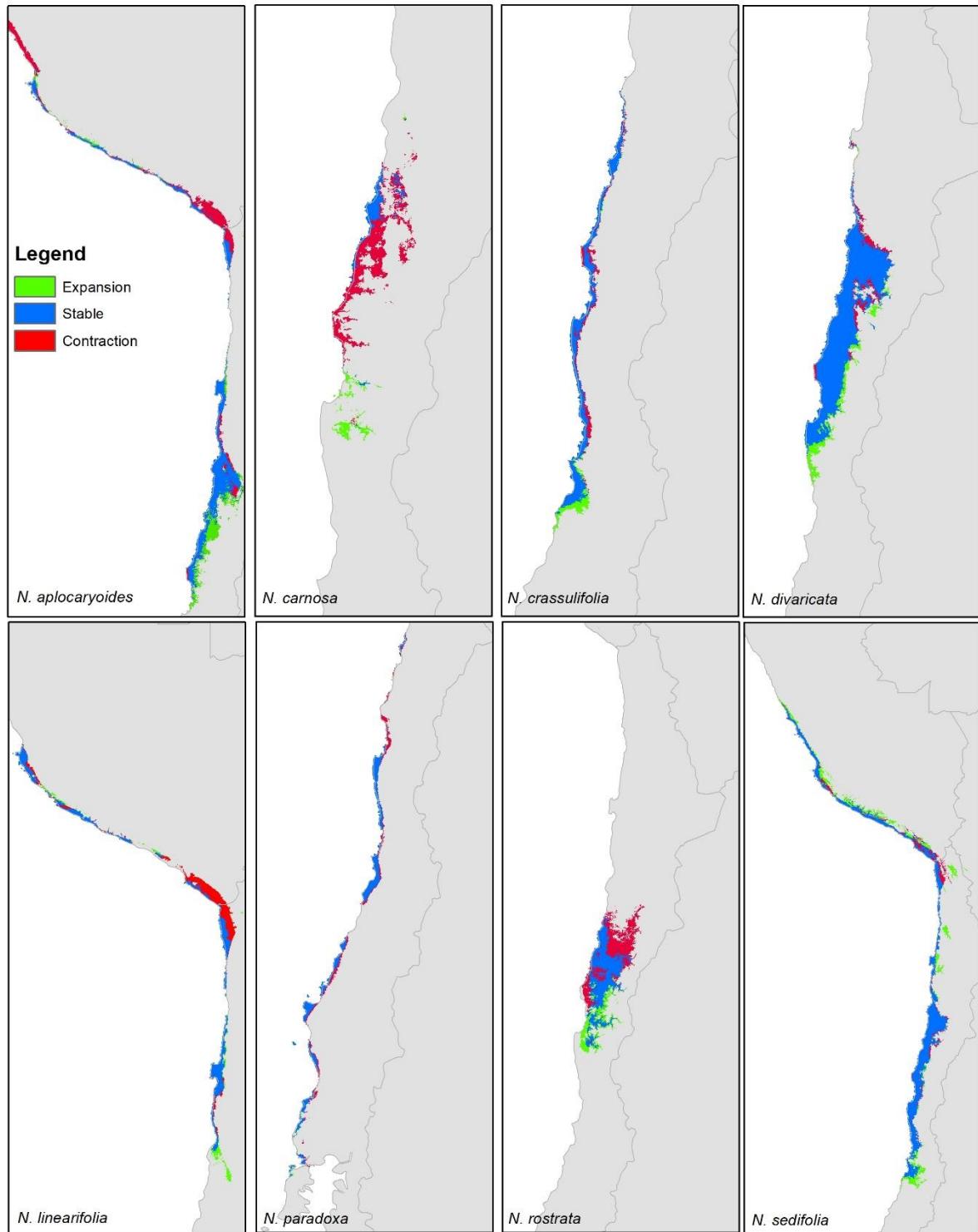


Fig 5. Spatial patterns of change under future climate scenarios for 8 species: *N. aplocaryoides*, *N. carnosia*, *N. crassulifolia*, *N. divaricata*, *N. linearifolia*, *N. paradoxa*, *N. rostrata* and *N. sedifolia*. Habitat suitability of species was projected in one future climate scenario (HadGem2\_ES rcp 8.5 for 2070-2100 period).

### **Relation between distribution and dormancy**

According to previous studies by Hepp *et al.* (in preparation), species of clade G present the less deep seed dormancy, adding to *N. paradoxa* (clade B); species belonging to clade C are the most dormant. Table 4 presents the dormancy index (initial and final) for the 8 species studied plus another two (*N. jaffuelii* and *N. parviflora*) that could not be modelled, where this information is confirmed.

Through species distribution modelling, we found that the potential areas of *N. aplocaryoides*, *N. carnosa*, *N. linearifolia*, *N. paradoxa* and *N. rostrata* would be reduced under future global climate change scenarios. *N. carnosa* and *N. rostrata* have the higher dormancy values of all studied species, while the rest present low dormancy values (Table 4).

Among the species that will remain stable or even increase their range of distribution is *N. crassulifolia*, which is the one with the lowest dormancy value. *N. sedifolia* also has a low value, while for *N. divaricata* the value is intermediate (Table 4).

Plasticity in the seed regeneration trait may counteract the projected narrowing of the species distribution range, as mentioned in Peng *et al.* (2019) for *Saussurea* (Asteraceae) species; however, in this case, the species that have lower dormancy values are also those whose distribution range will not be reduced, according to the models used in this study; and conversely, distribution range will contract in the case of species with high values for the dormancy index.

Table 4. Dormancy Index (initial, D<sub>li</sub>, and final, D<sub>lf</sub>) for 10 species of *Nolana*, ordered by lower to higher dormancy level. HS corresponds to the highest suitability value for each collection point.

<b>Species</b>	<b>Clade</b>	<b>D<sub>li</sub></b>	<b>D<sub>lf</sub></b>	<b>HS</b>
<i>N. crassulifolia</i>	G	0.433	6.41	0.930851
<i>N. paradoxa</i>	B	0.357	30.11	0.852613
<i>N. sedifolia</i>	G	0.333	30.33	0.977771
<i>N. aplocaryoides</i>	G	0.344	34.38	0.988483
<i>N. linearifolia</i>	G	0.438	36.89	0.723844
<i>N. divaricata</i>	G	0.593	59.34	0.995523
<i>N. jaffuelii</i>	B	0.693	69.32	Not modelled

<i>N. parviflora</i>	B	0.702	70.24	Not modelled
<i>N. rostrata</i>	C	0.776	77.63	0.877566
<i>N. carnosa</i>	C	0.865	86.46	0.983326

To add to the analysis, Table 5 presents the niche breadth, expressed as the inverse concentration for each species. It can be observed that *N. sedifolia* is the species with greater niche breadth; not *N. paradoxa*. This last species stands out for having a wide distribution between 29°S and 43°S, the only species of the genus that reaches this latitude, but inhabits mainly on the coast, very close to the sea (Fig. 3, *N. paradoxa*). *N. sedifolia* also has a very wide distribution (between 19°S and 33°S) and is also capable of inhabiting inland environments (Fig. 3, *N. sedifolia*). The species with the lowest niche breadth would be *N. carnosa*.

Table 5. Niche breadth expressed as inverse concentration for each of the 8 modelled *Nolana* species.

Species	Inverse concentration	Uncertainty
<i>Nolana aplocaryoides</i>	0.8101277	0.03394854
<i>Nolana carnosa</i>	0.7468854	0.0135538
<i>Nolana crassulifolia</i>	0.7496458	0.01372035
<i>Nolana divaricata</i>	0.8188687	0.03815467
<i>Nolana linearifolia</i>	0.7820484	0.02815799
<i>Nolana paradoxa</i>	0.7887218	0.02333149
<i>Nolana rostrata</i>	0.7894775	0.02724888
<i>Nolana sedifolia</i>	<b>0.8601784</b>	0.07315427

However, seed dormancy was not correlated with niche breadth (Fig. 6); that is, species with a greater niche amplitude, capable of occupying a greater variety of habitats, is not necessarily the one with the lowest dormancy index. At the same time, species with high dormancy rates also have intermediate levels of niche occupation.

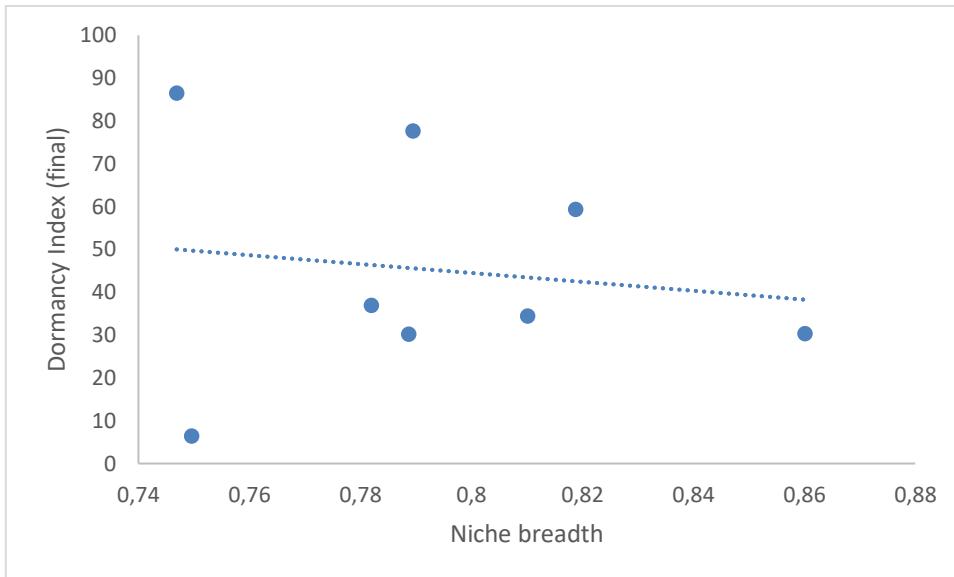


Fig. 6. Correlation between germination percentage and niche breadth for eight species of the *Nolana* genus. The value of  $R^2$  for the trendline is given.  $R = -0,142$  ( $p$  value = 0,736).

In an extensive study on species from campo rupestre grasslands in Brazil, Dayrell *et al.* (2016) found that phylogeny was better correlated than ecology with seed dormancy categories, indicating that geological and climatic history had a more important role in driving seed dormancy than contemporary factors (Dayrell *et al.*, 2016). This may also be the case for the genus *Nolana*; Hepp *et al.* (in preparation) reported that the proximity of *Nolana* species in terms of clades seemed to be more relevant than their latitudinal distribution (expressed as the difference between the northernmost and the southernmost latitudinal distribution point).

Clade C species (*N. carnosa* and *N. rostrata*) should have priority in conservation programs, since they have the higher dormancy levels and a contraction of their potential habitat is expected in the future. In particular, *N. carnosa* also has a low niche breadth. Seeds of both species should be candidates for long-term storage in Seed Banks. On the other hand, live collections of these plants should be kept in botanical gardens, in order to have more material for propagation and research activities. In general, clade G species are of less concern, given the wide niche they present and their low dormancy levels; however, some populations of these species are more susceptible to the threats of climate change, especially in the northernmost locations. In the case of *N. paradoxa* of clade B, although its extensive distribution will contract significantly in the future, it is a species with high germination levels (low dormancy value), whose detached leafy stems readily root and it also has floating mericarps that can be dispersed over long distances (Johnston, 1936).

## Conclusions

The current pattern of distribution for the genus *Nolana*, that confirms two centres of species diversity, could be established; in the future, there will be a contraction towards the coast,

but some species will keep their distribution area stable, and some will even expand. However, it is worrisome that the species whose seeds have higher values according to the dormancy index, are those that have a smaller niche breadth and also, whose distribution will contract in the future. Special efforts should be directed to include these species in an integrated conservation programme, including both *in situ* and *ex situ* initiatives.

On the other hand, no evidence was found of a relationship between the niche breadth of the species and their dormancy level; this means that the response to germination experiments (or level of physiological dormancy) cannot be predicted according to the distribution presented by the species. It is likely that the dormancy trait has a greater genetic influence than the maternal environment (environment under which the seed develops).

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## Annexes

1. Complete list of species and number of occurrences.

Species	Records
<i>Nolana acuminata</i>	75
<i>Nolana adansonii</i>	13
<i>Nolana albescens</i>	52
<i>Nolana aplocaryoides</i>	65
<i>Nolana baccata</i>	65
<i>Nolana balsamiflua</i>	10
<i>Nolana carnosa</i>	22
<i>Nolana cerrateana</i>	10
<i>Nolana chancoana</i>	11
<i>Nolana clivicola</i>	18
<i>Nolana crassulifolia</i>	62
<i>Nolana coelestis</i>	35
<i>Nolana confinis</i>	11
<i>Nolana diffusa</i>	20
<i>Nolana divaricata</i>	44
<i>Nolana elegans</i>	57
<i>Nolana filifolia</i>	73
<i>Nolana flaccida</i>	32
<i>Nolana glauca</i>	13
<i>Nolana gracillima</i>	10
<i>Nolana humifusa</i>	18
<i>Nolana incana</i>	48
<i>Nolana inconspicua</i>	18
<i>Nolana inflata</i>	12
<i>Nolana leptophylla</i>	107
<i>Nolana linearifolia</i>	31
<i>Nolana lycioides</i>	11
<i>Nolana mollis</i>	39
<i>Nolana pallida</i>	10
<i>Nolana pallidula</i>	10
<i>Nolana paradoxa</i>	45
<i>Nolana parviflora</i>	17
<i>Nolana patula</i>	16
<i>Nolana peruviana</i>	93
<i>Nolana ramosissima</i>	42
<i>Nolana rostrata</i>	70
<i>Nolana rupicola</i>	64
<i>Nolana salsolooides</i>	62
<i>Nolana sedifolia</i>	52

<i>Nolana spathulata</i>	20
<i>Nolana sphaerophylla</i>	18
<i>Nolana stenophylla</i>	26
<i>Nolana tarapacana</i>	18
<i>Nolana villosa</i>	68
<i>Nolana werdermannii</i>	11

## 2. Correlation matrix for all bioclimatic variables plus cloud cover.

3. Expansion, stability and contraction under future scenarios for all *Nolana* modelled species.  
 Bold number correspond to the 8 species for which germination data is available.

Species	Expansion	Stability	Contraction
<i>Nolana acuminata</i>	12819	27526	1861
<i>Nolana adansonii</i>	8062	26631	4251
<i>Nolana albescens</i>	6227	5910	13456
<i>Nolana aplocaryoides</i>	<b>10707</b>	<b>26819</b>	<b>15344</b>
<i>Nolana baccata</i>	5114	8665	11438
<i>Nolana balsamiflua</i>	1505	8678	8457
<i>Nolana carnosa</i>	<b>7172</b>	<b>1837</b>	<b>6729</b>
<i>Nolana cerrateana</i>	2253	15215	2831
<i>Nolana chancoana</i>	1896	7148	2625
<i>Nolana clivicola</i>	2629	13748	10439
<i>Nolana coelestis</i>	4846	4658	6066
<i>Nolana confinis</i>	20653	76132	16603
<i>Nolana crassulifolia</i>	<b>6692</b>	<b>9475</b>	<b>2175</b>
<i>Nolana diffusa</i>	5724	9918	8370
<i>Nolana divaricata</i>	<b>17456</b>	<b>41539</b>	<b>4471</b>
<i>Nolana elegans</i>	6326	20441	4166
<i>Nolana filifolia</i>	8629	14234	9360
<i>Nolana flaccida</i>	1983	30192	168
<i>Nolana glauca</i>	11846	4723	354
<i>Nolana gracilima</i>	15413	96298	59339
<i>Nolana humifusa</i>	371	7034	11133
<i>Nolana incana</i>	4314	4533	2006
<i>Nolana inconspicua</i>	7049	14483	7071
<i>Nolana inflata</i>	1214	1743	681
<i>Nolana leptophylla</i>	11986	36307	9277
<i>Nolana linearifolia</i>	<b>2726</b>	<b>14974</b>	<b>9485</b>
<i>Nolana lycioides</i>	13712	78799	23169
<i>Nolana mollis</i>	4287	4809	4854
<i>Nolana pallida</i>	2405	11686	4579
<i>Nolana pallidula</i>	9808	104386	27693
<i>Nolana paradoxa</i>	<b>446</b>	<b>10465</b>	<b>39547</b>
<i>Nolana parviflora</i>	35169	35674	9837
<i>Nolana patula</i>	13008	6391	75
<i>Nolana peruviana</i>	3988	11653	11131
<i>Nolana ramosissima</i>	3346	1988	2127
<i>Nolana rostrata</i>	<b>7226</b>	<b>17071</b>	<b>13670</b>
<i>Nolana rupicola</i>	3860	8867	496
<i>Nolana salsolooides</i>	7471	21511	3349

<i>Nolana sedifolia</i>	<b>22502</b>	<b>76609</b>	<b>10788</b>
<i>Nolana spathuata</i>	2669	5792	8005
<i>Nolana sphaerophylla</i>	1620	3535	469
<i>Nolana stenophylla</i>	10336	14167	11962
<i>Nolana tarapacana</i>	16855	47871	80320
<i>Nolana villosa</i>	12792	22584	8860
<i>Nolana werdernannii</i>	26893	13968	2133

4. Final six variables (five bioclimate plus cloud cover) used for current and future models.

Species	Annual Mean Temp	Mean Diurnal Range	Annual Pp	Pp Seasonality	Pp of Coldest Quarter	Cloud Cover
<i>Nolana aplocaryoides</i>	0.5	29.6	<b>56.9</b>	1.3	9.4	2.3
<i>Nolana carnosa</i>	8.3	10.2	<b>33.3</b>	28.5	18.1	1.6
<i>Nolana crassulifolia</i>	2.2	30.8	1	<b>45.4</b>	16.6	4.1
<i>Nolana divaricata</i>	1.4	15.8	<b>48</b>	18.6	15	1.2
<i>Nolana linearifolia</i>	0.1	25.8	<b>71.1</b>	0	1.8	1.1
<i>Nolana paradoxa</i>	10.6	<b>47.9</b>	0	10.1	17.1	14.3
<i>Nolana rostrata</i>	1.8	0.2	<b>32.3</b>	25.1	24	16.7
<i>Nolana sedifolia</i>	8.1	38.3	<b>41.8</b>	3.6	5.5	2.6

## **Chapter IV. Comparative fruit anatomy and morphology of twelve species of *Nolana* (Solanaceae) of the coastal Atacama Desert, Chile**

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### **Abstract**

The genus *Nolana* (Solanaceae) comprises numerous species endemic to the arid and semi-arid environments in the coastal area of Chile and Peru and is distinguished within the Solanaceae family by the penta-carpelar ovary that develops fibrous fruits called mericarps. The objective of this study was to assess the anatomical and morphological differences or similarities among mericarps in 12 Chilean species of *Nolana* in order to contribute to the identification of species and examine possible phylogenetic relationships. Cross sections were stained using safranin and fast green and prepared for microscopic examination. Mericarps were also analysed using a scanning electron microscope and a stereoscopic magnifier. Three different tissues were identified: an external one (exocarp), corresponding to a mono-stratified epidermis; a middle one (mesocarp), of variable thickness, occupying the bulk of the pericarp and the one that presents more variation between species; and an inner one (endocarp), constituted by one layer of very dense sclereids, which was not always easily distinguishable. It was not possible to determine a clear relationship between the anatomy and morphology of the mericarps and the position of the species within clades of the genus. However, trends were observed, and the new information generated could be of use for the identification of species. The relationship between mericarp structure, and the seed germination and survival of these species is also discussed.

Keywords: mericarp, mesocarp, seed, arid environments

### **Introduction**

Species of the genus *Nolana* L. ex L. f. (Solanaceae-Nolaneae) are mainly restricted to the Atacama and Peruvian Deserts along the western coast of South America, in Chile and Peru. There are 90 species in total, of which 49 are present in Chile; 46 are endemic to this country and just three species are shared with Peru. Around 70 species are endemic to the lomas formations of both countries, generally located in the coastal Atacama and Peruvian desert; the other 13 occur in habitats of higher altitude (> 1,000 masl) (Dillon, 2005), and two of them, *N. paradoxa* and *N. sedifolia*, have extensive distributions along the coast of Chile (over 1,500 and 1,400 km, respectively). According to Dillon *et al.* (2009), *Nolana* is the only genus found throughout the entire range of lomas formations, and it stands out as the most conspicuous floristic element there at the species level. Although the taxonomy of this genus at species level is still not completely resolved, as new species continue to be

discovered (Hepp and Dillon, 2018) and doubts persist regarding the identity of many others (Dillon, pers. com.), a phylogenetic estimate for *Nolana*, using a combination of molecular markers, has been reconstructed by Dillon *et al.* (2007, 2009) and Tu *et al.* (2008). This phylogeny supports the monophyly of the genus and has identified several clades supported by geographic distributions and morphological synapomorphies (Dillon *et al.*, 2009). According to these analyses the genus is of Chilean origin, with two different introductions to Peru and subsequent radiation (Dillon *et al.*, 2009). Of the seven clades identified, four are confined to Chile and three are mainly Peruvian with some presence in Chile.

*Nolana* species have been acknowledged to have high ornamental potential due to their succulent foliage and flowering characteristics (Freyre *et al.*, 2005; Riedemann *et al.*, 2006), but there are other reasons which make the study of this genus particularly interesting. It has a high degree of endemism and presence in habitats with extreme conditions of aridity and salinity, which gives it a high conservation value (Tu *et al.*, 2008, Dillon *et al.*, 2009); also, some species present compounds with fungicidal activity in fungi of agricultural importance (*N. sedifolia*, Vio-Michaelis *et al.*, 2012). Additionally, the genus belongs to the Solanaceae family and to the Solanoideae sub-family (Olmstead *et al.*, 2008), where tomato and pepper also belong. This makes it a possible source of genes of interest for the development of cultivars that are better adapted to conditions of aridity and/or salinity; these intergeneric hybridizations have been studied and produced in other families such as Brassicaceae (Kaneko and Woo Bang, 2014). However, *Nolana* species have been poorly studied and there is limited information regarding their ecology, reproductive biology and establishment strategies, knowledge that is essential for their conservation and potential use (but see Freyre *et al.*, 2005; Douglas and Freyre, 2010; Jewell *et al.*, 2012; Cabrera *et al.*, 2015).

It is likely that the success of *Nolana* species in these arid environments is due to the development of anatomical characters such as foliar succulence, salt glands and trichomes that capture moisture and restrict transpiration, and also to the development of fibrous hard fruits, a character that is unique in the Solanaceae family (Knapp, 2002, Dillon *et al.*, 2009). These fruits correspond to schizocarps (indehiscent fruit originated by a gynoecium of two or more concrescent carpels; Font Quer, 2000), which split up into mericarps when ripe. Therefore, in *Nolana*, dispersal units are not seeds but mericarps, which can vary in number between species from 2 to 30 per schizocarp and from laterally united and multi-seeded to completely free and single-seeded (Tago-Nakazawa and Dillon, 1999; Knapp, 2002). The pericarp is rich in sclereids, which give strength and rigidity to the fruit (Cabrera *et al.*, 2015). Although variable in size, shape and degree of fusion between species, the mericarp is considered an autapomorphy of *Nolana* (Knapp, 2002).

The shape and structure of seeds or propagation units can provide important information to identify species or make phylogenetic inferences in many plant families (Wada and Reed, 2008, Gontcharova *et al.*, 2009, Wada and Reed, 2010, Camelia, 2011), although according to Knapp (2002), this may sometimes be problematic since fruit type has proven to be highly homoplasious. However, anatomical and morphological information provide valuable information to help elucidate taxonomic uncertainties within a specific group (Liu *et al.*, 2006; Camelia, 2011). Additionally, when studying the structure of tissues it is possible to infer

their function (eg. resistance, defence against insects, etc.), as many studies have done (Charini & Barboza, 2007; Gehan-Jayasuriya *et al.*, 2007; Ruiz Sanchez *et al.*, 2017).

The objectives of the present work are to identify morpho-anatomical differences across the mericarps of 12 Chilean species of *Nolana* belonging to four different clades , in order to: 1) better understand the relationship between fruit structure and the survival and germination of the species, and 2) provide species specific characters useful for taxonomical purposes. A greater similarity is expected in species that are closer according to clades, both in both in morphology and anatomy of the mericarps.

## **Materials and methods**

### **2.1 Plant material**

Mericarps (dispersal units) of 12 species of *Nolana* were collected from individual plants before dispersal at different locations in Chile (Table 1). Selection of species is the result of availability of suitable fruits (i.e. mature mericarps, black/brown in colour and with dry or senescent calyx) in the field (Fig. 1), which explains the overrepresentation of certain clades (Fig. 2).

Collected mericarps were stored in an incubator at 20°C (18-24°C) and 40% RH (35-45%) until evaluation. Identification of the species was possible with the help of Dr. Michael Dillon, consulting herbarium specimens at the Natural History Museum of Santiago de Chile (SGO) and using the available bibliography.



Figure 1. Flowers (above) and fruits (below) of six *Nolana* species included in this study. A and G: *N. crassulifolia* (clade G); B and H: *N. divaricata* (clade G); C and I: *N. sedifolia* (clade G); D and J. *N. carnosa* (clade C); E and K: *N. intonsa* (clade F); F and L: *N. paradoxa* (clade B).

Table 1. Coordinates of collection site and clade description of the 12 *Nolana* species of this study.

	<b>Species</b>	<b>Coordinates</b>	<b>Clade*</b>	<b>Description of clade*</b>
1	<i>Nolana aplocaryoides</i> (Gaudich.) I.M. Johnst.	26°08.786'S, 70°39.943'W	G	Clade G has recovered a strictly Chilean group of 13 species represented by small

2	<i>Nolana crassulifolia</i> Poepp.	32°14.446'S, 71°31.177'W	to moderate shrubs (traditionally called "Dolia") and annuals, all with highly reduced corollas, often white or yellowish (Dillon <i>et al.</i> , 2009) but some have bluish flowers.
3	<i>Nolana divaricata</i> (Lindl.) I.M. Johnst.	29°14.601'S, 71°27.840'W	
4	<i>Nolana linearifolia</i> Phil.	23°30.133'S, 70°23.069'W	
5	<i>Nolana onoana</i> M.O. Dillon & Nakazawa	23°29.903'S, 70°21.817'W	
6	<i>Nolana sedifolia</i> Poepp.	20°49.542'S, 70°09.392'W	
7	<i>Nolana intonsa</i> I.M. Johnst.	20°49.309'S, 70°09.366'W	F Clade F is comprised of 18 Peruvian species and one Chilean species ( <i>N. intonsa</i> ), which shares strong morphological resemblance to southern Peruvian species, such as <i>N. pallida</i> (Dillon <i>et al.</i> , 2009).
8	<i>Nolana carnosa</i> (Lindl.) Miers ex Dunal	28°02.612'S, 71°07.022'W	C Clade C has recovered a monophyletic group comprised of 6 species traditionally classified as <i>Alona</i> . These are all large-flowered shrubs confined to Chile (Dillon <i>et al.</i> , 2009).
9	<i>Nolana rostrata</i> (Lindl.) Miers ex Dunal	28°06.806'S, 71°09.257'W	
10	<i>Nolana jaffuelii</i> I.M. Johnst.	20°48.903'S, 70°09.630'W	B Clade B, previously recognized as a segregate genus, <i>Sorema</i> , currently consists of two sub-groups: <i>N. pterocarpa</i> - <i>N. baccata</i> - <i>N. parviflora</i> , which are erect annuals with small flowers; and <i>N. paradoxa</i> - <i>N. rupicola</i> , which are rosette-forming, taprooted plants with larger flowers (Dillon <i>et al.</i> , 2009). These species are only present in Chile (Dillon <i>et al.</i> , 2009).
11	<i>Nolana paradoxa</i> Lindl.	30°11.734'S, 71°25.377'W	
12	<i>Nolana parviflora</i> (Phil.) Phil.	23°30.133'S, 70°23.069'W	

\*Letters for clades and description according to Dillon *et al.*, 2009.

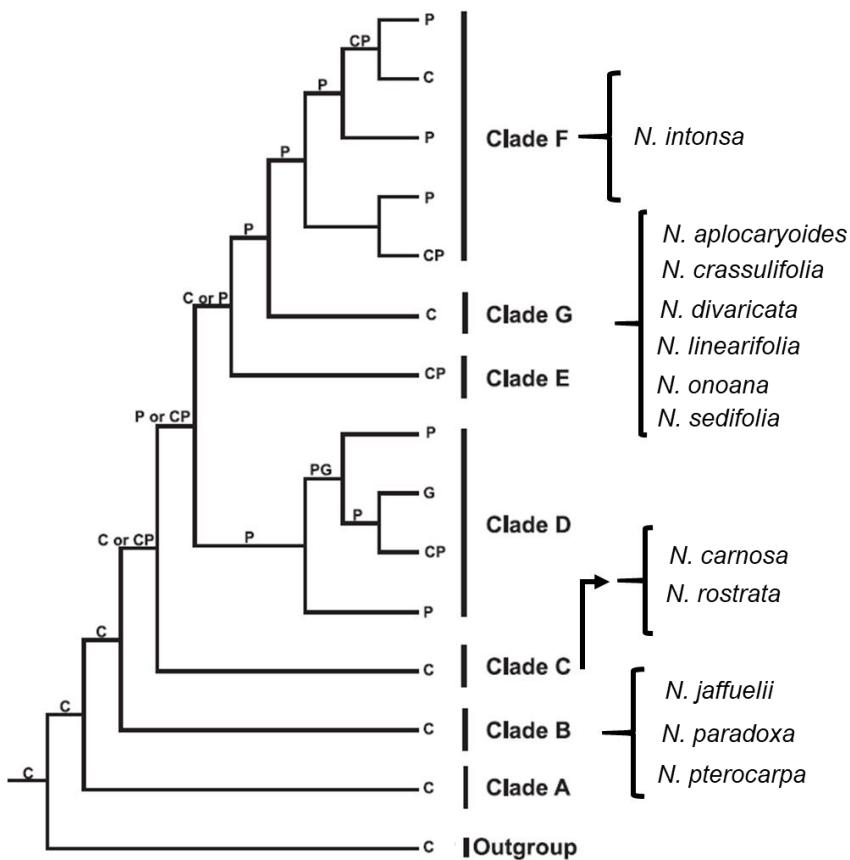


Figure 2. Biogeographic diversification of *Nolana*, modified from Dillon *et al.* (2009), showing the clade of the 12 studied *Nolana* species. C, Chile; P, Peru; G, Islas Galápagos, Ecuador.

## 2.2 Stereoscopic magnifier

The colour, size, presence of dispersal structures, and external appearance of the dispersion unit were recorded. A sample of each species was photographed using a stereoscopic magnifier (Olympus SZ2-ILST).

## 2.3 Scanning electron microscope

Scanning electron microscopy (SEM) was used to examine the morphology of the fruits, the surface of the funicular scar and the longitudinally sectioned mericarps. Samples of 12 species were mounted on aluminium SEM stubs and sputtercoated with a platinum-gold alloy using a Quorum Sputter Coater Q150T. Mericarps were examined and photographed using a HITACHI S-4700 SEM at an accelerating voltage of 2.0 kV and working distance of 12.0 mm. Images were saved as tagged image file format (TIFF).

## 2.4 Histological analysis

Five mericarps per species were randomly selected and fixed in formol acetic alcohol (FAA), then dehydrated and preserved in paraffin in preparation for sectioning. Cross and

longitudinal histological sections were made through the ripe mericarps using a microtome (to a thickness of 16 µm) and stained with safranin-fastgreen solution. Sections were observed under a light microscope and photographed using a Moticam 5 camera and measured with Motic Images Plus 2.0 ML.

## Results and discussion

### 3.1 Fruit anatomy

In the anatomical analysis with SEM images, the presence of one to several seed or embryonic chambers within mericarps, with evident differences between species, was confirmed; species such as *N. intonsa*, *N. linearifolia* or *N. rostrata* have multiple embryonic chambers, as opposed to *N. aplocaryoides*, *N. jaffuelii* or *N. onoana*, which usually bear one to two embryonic chambers per mericarp (Fig. 3). According to Johnston (1936), the fruit of *Nolana* would have evolved from a multiseminated berry in which the carpellar and placental tissue have united and hardened forming a firm and lobed structure, morphologically a schizocarp, divided at maturity into a variable number of mericarps (Saunders, 1936). These mericarps are very particular since each embryo is divided into a separate embryonic chamber, formed by parenchymal tissue (Bondeson, 1986).

Also evident in most of the mericarps is a slightly depressed, elliptical area which corresponds to the funicular scar (Fig. 3A-3C; Fig. 3G-3I) and indicates the location of each embryonic chamber (Fig. 3D-3F; Fig. 3J-3L). This structure was described in Bondeson (1981) as a “germination plug”, since the protruding radicle pushes the plug out during germination. It corresponds to the funicular tissue that goes through the pericarp; it can be detached mechanically and appears to influence germination, as fruits scarified in this region obtained higher percentages of germination than did intact fruits (Cabrera *et al.*, 2015). Since each plug separates independently, it is possible to assume that there would be more than one opportunity for germination for those mericarps with more than one embryonic chamber (Fig. 3J, 3K and 3L). No differences were observed between species in terms of structure, size or position of the germination plug.

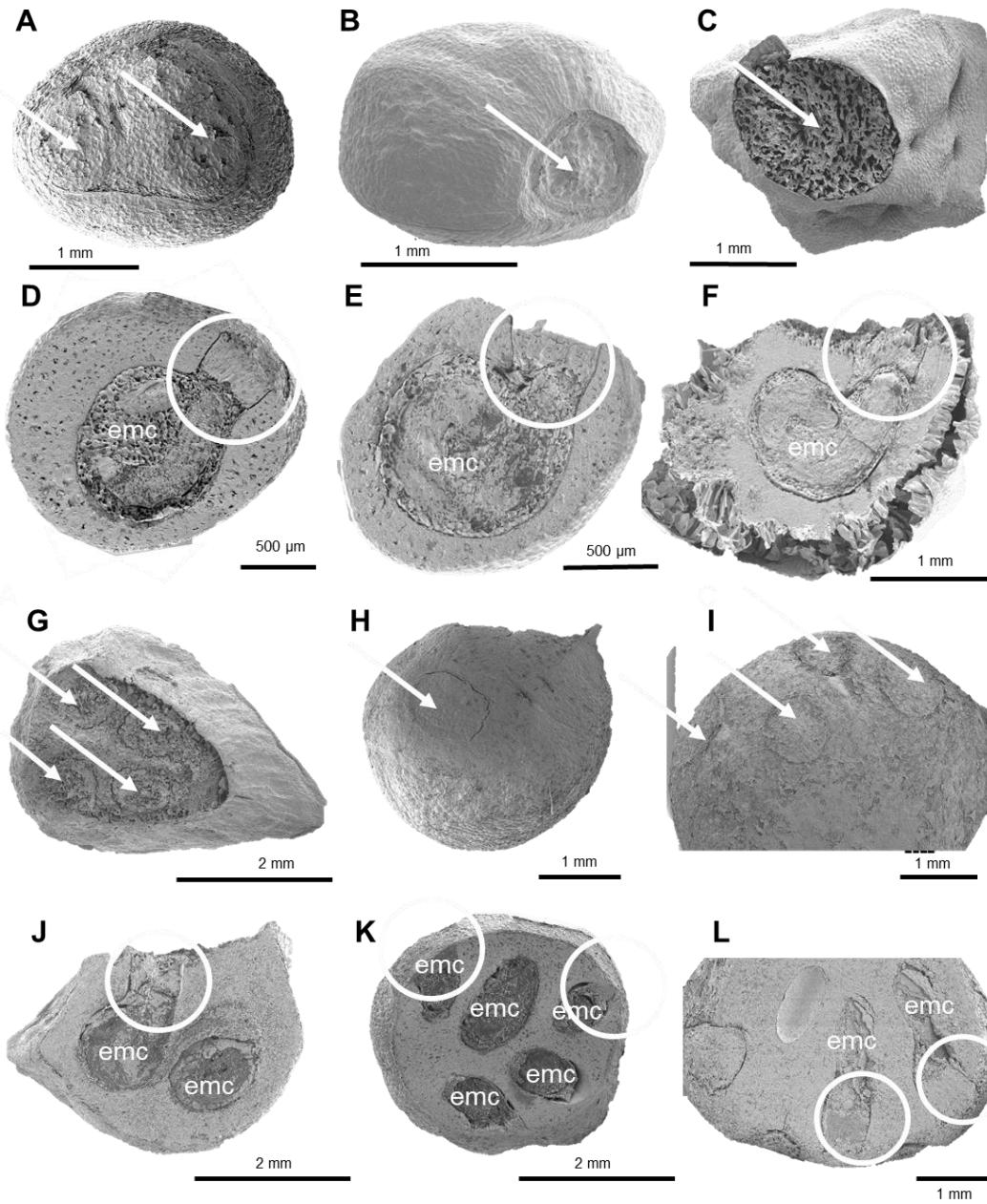


Figure 3. Scanning electron microscopy (SEM) images for mature (dispersed) mericarps of six *Nolana* species: intact (A) and longitudinally cut (D) mericarps of *N. aplocaryoides*; intact (B) and longitudinally cut (E) mericarps of *N. onoana*; intact (C) and longitudinally cut (F) mericarps of *N. jaffuelii*; intact (G) and longitudinally cut (J) mericarps of *N. intonsa*; intact (H) and longitudinally cut (K) mericarps of *N. linearifolia*; intact (I) and longitudinally cut (L) mericarps of *N. rostrata*. Arrows indicate the location of funicular scar; circles enclose the structure that corresponds to the germination plug. Bars indicate mm. abbreviations: emc, embryonic chamber.

The histological study performed on mericarps of these twelve *Nolana* species reveals the presence of three layers. Following the definition of Richard (1819, quoted in Pabón-Mora & Litt, 2011), we consider an external layer (exocarp), corresponding to a mono-stratified epidermis whose cells contain vacuoles with tannins and a thin protective cuticle; an inner

layer, the endocarp, constituted by only one layer of very dense sclereids; and in the middle, the mesocarp, of variable thickness, generally occupying the bulk of the pericarp, and the one that presents more variation between species (Fig. 4).

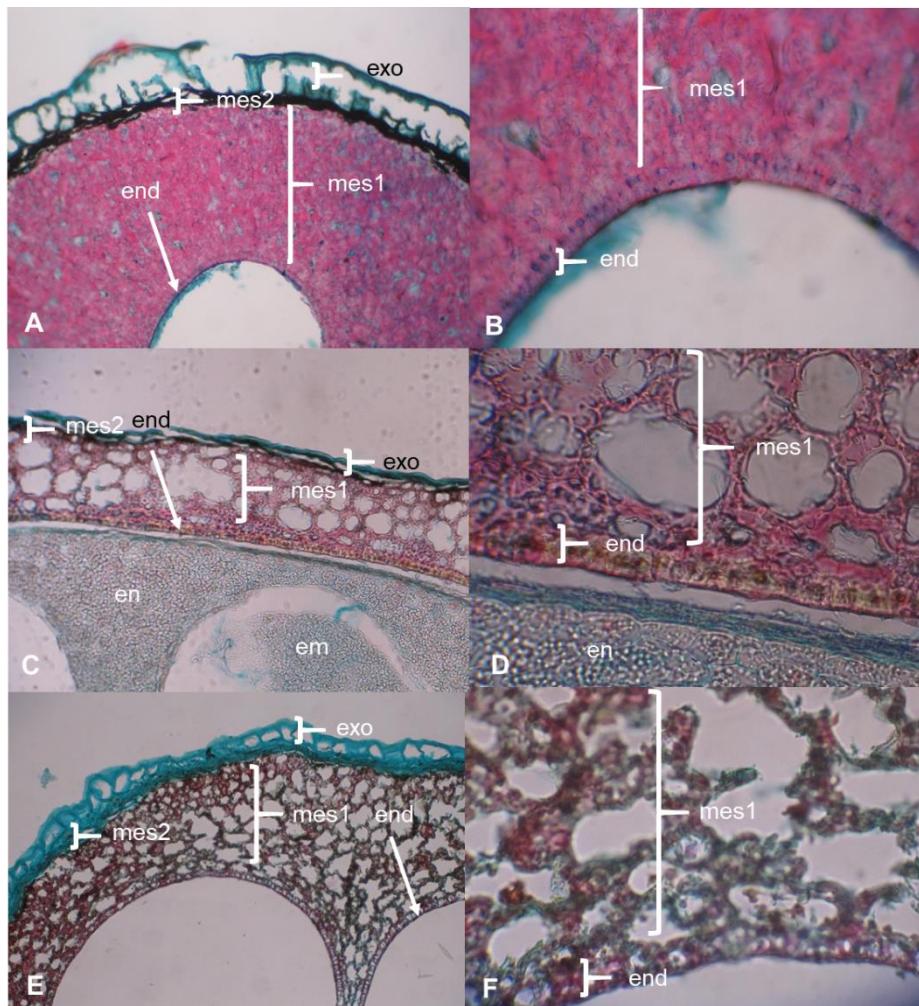


Figure 4. Pericarp of three *Nolana* species at two magnifications (40x, left images; and 400x, right images). A and B: *N. crassulifolia* (clade G); C and D: *N. paradoxa* (clade B); E and F: *N. linearifolia* (clade G). Abbreviations: en, endosperm; end, endocarp; em, embryo; exo, exocarp; mes, mesocarp.

The exocarp is variable in terms of size of the cells, but it was generally presented as a monostratified layer (Fig. 6 and 7, Table 2).

The mesocarp consists of two histologically differentiated zones, as has been described for other Solanaceae species (*Solanum* spp.; Chiarini and Barboza, 2007). There is an internal zone constituted by sclereids that can either be very dense, or present empty cells with undulated but lignified walls, and an external one, formed by radially elongated cells with suberized walls in some species (*N. carnosa* and *N. jaffuelii*; Fig. 5, Table 2), or by parenchymatic cells that may be collapsed, in others (*N. aplocaryoides*, *N. crassulifolia*, *N. divaricata*, *N. intonsa*, *N. rostrata* and *N. sedifolia*; Fig. 6 and 7, Table 2). The determination of these two zones was possible due to the study of the fruit development of *N. paradoxa* in different stages; in early stages, it was possible to visualize the origin of these two zones

within the mesocarp and to clearly distinguish the monolayer of the endocarp (Hepp *et al.*, in preparation), although previous studies concluded that the lignified and extended portion of the mericarp corresponded to the endocarp (Bondeson, 1986; Cabrera *et al.*, 2015).

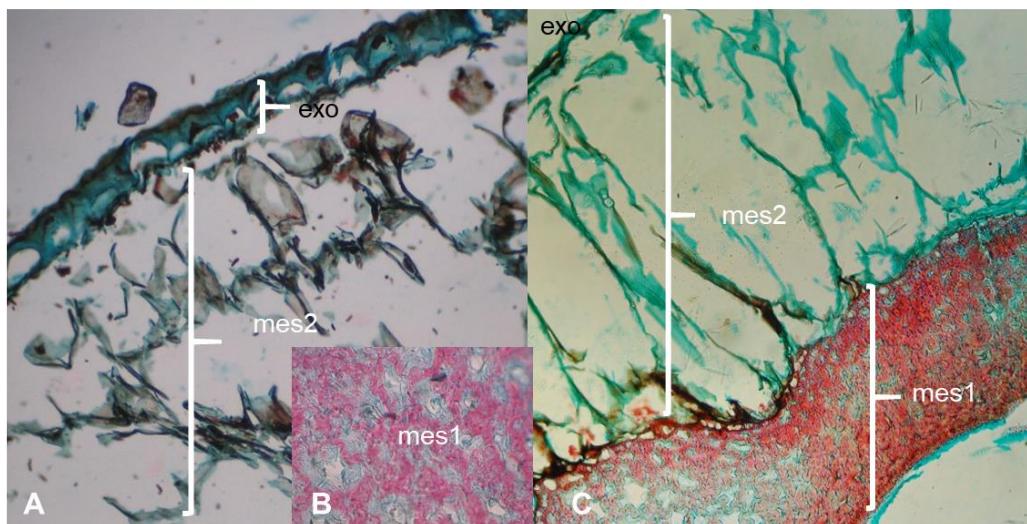


Figure 5. Pericarp of two *Nolana* species. A and B: *N. carnosa* (clade C); C: *N. jaffuelii* (clade B). Abbreviations: exo, exocarp; mes1, internal zone of the mesocarp; mes2, external zone of the mesocarp.

The several layers of sclereids of the mesocarp provide hardness and rigidity to the fruit and would also allow the mericarps to remain relatively intact in the soil seed bank for many years, despite the extreme conditions prevalent in the Atacama Desert. To this is added the external mesocarp with suberized cells in the case of *N. carnosa* and *N. jaffuelii* (Fig. 5), which could help isolate the embryo from the external environment surrounding the fruit. An additional protection may be given by the presence of chemical compounds in the mericarp cover; for example, an antifungal activity on *Botrytis cinerea* was discovered for *N. sedifolia* (Vio-Michaelis *et al.*, 2012). The hard layer of sclereids, along with other compounds such as tannins and cuticle present in the exocarp and suberized cells of the outer mesocarp, may confer water impermeability to the mericarp, which could be related to physical dormancy of the seeds, as was suggested by Cabrera *et al.* (2015).

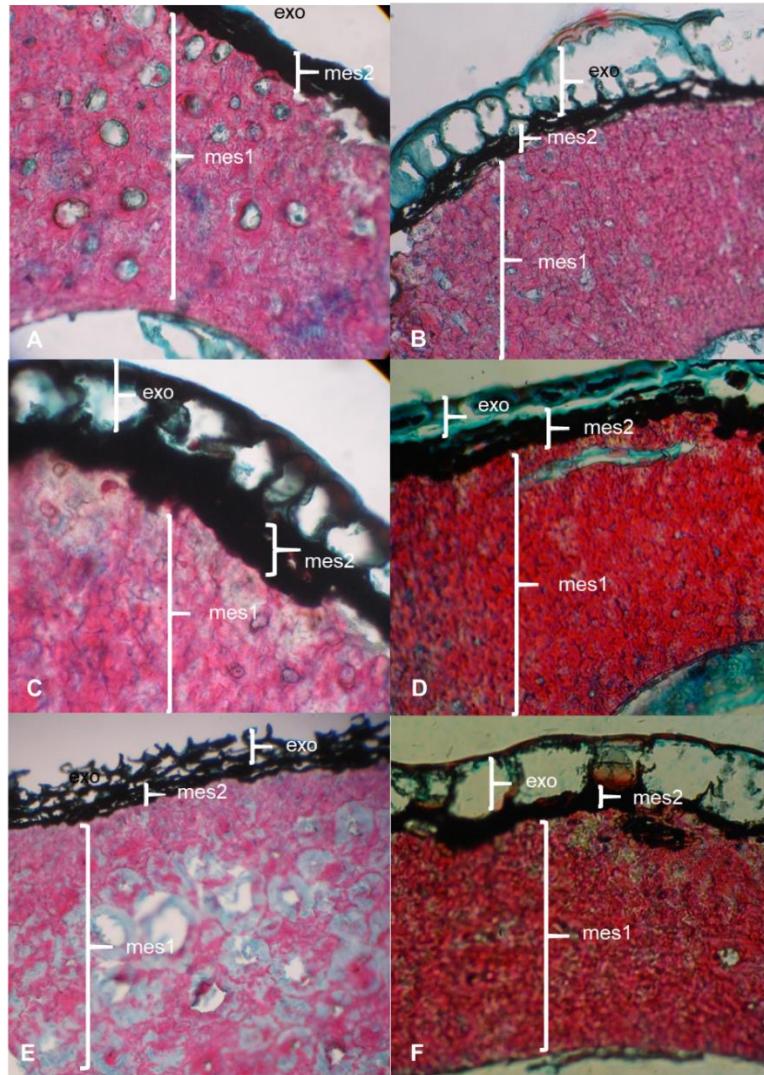


Figure 6. Pericarp of six *Nolana* species: A: *N. aplocaryoides* (clade G); B: *N. crassulifolia* (clade G); C: *N. divaricata* (clade G); D: *N. intonsa* (clade F); E: *N. rostrata* (clade C); F: *N. sedifolia* (clade G). Abbreviations: exo, exocarp; mes1, internal zone of the mesocarp; mes2, external zone of the mesocarp.

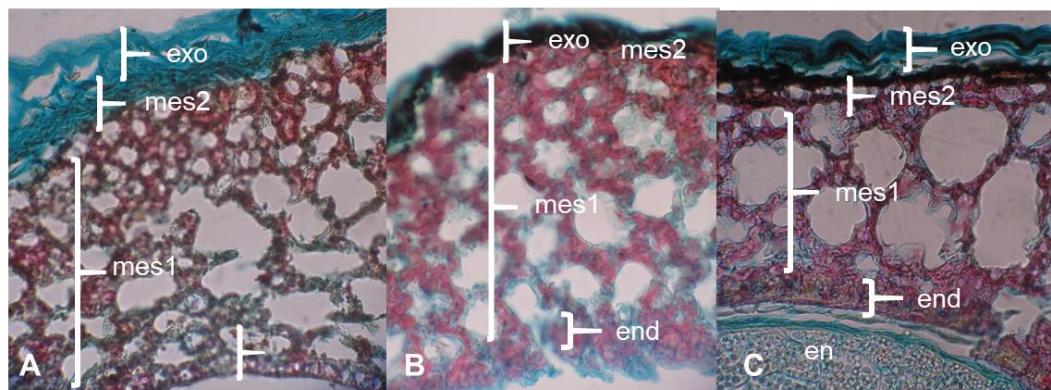


Figure 7. Pericarp of three *Nolana* species. A: *N. linearifolia* (clade G); B: *N. onoana* (clade G); C: *N. paradoxa* (clade B). Abbreviations: en, endosperm; end, endocarp; exo, exocarp; mes1, internal zone of the mesocarp; mes2, external zone of the mesocarp.

It is possible to classify the twelve *Nolana* species into three groups according to the composition of the mesocarp: (1) those with two well differentiated zones, one external with several layers of large cells with suberized walls and one internal of dense sclereids (Fig. 5), as in *N. jaffuelii* and *N. carnosa*; (2) those with a large (internal) zone of very dense sclereids and an external layer that is collapsed or difficult to distinguish (Fig. 6), as in *N. aplocaryoides*, *N. crassulifolia*, *N. divaricata*, *N. intonsa*, *N. parviflora*, *N. rostrata* and *N. sedifolia*; and (3) those with a wide internal zone of large or medium sized cells, empty or with large vacuoles (giving them a “porous” appearance), undulated lignified cell walls, and a collapsed external layer that is difficult to distinguish (Fig. 7), as in *N. linearifolia*, *N. onoana* and *N. paradoxa* (Table 2).

Table 2. Anatomical mericarp features of the 12 species of *Nolana* studied. Clades correspond to phylogenetic study of *Nolana* by Dillon et al. (2007, 2009) and Tu et al. (2008).

Species	Clade	Mericarp size (sample)	Exocarp	Mesocarp	
				External zone	Internal zone
“Two-zones” group – Fig. 5					
<i>N. jaffuelii</i>	B	1.8 mm diameter	Unistratate, isodiametric cells about 0.04 mm diameter	1-4 layers of large elongated cells with suberized walls, irregular thickness in whole (0.13 thinnest section - 0.43 mm thickest section)	Layer of irregular thickness (0.12 thinnest section - 0.48 mm thickest section), constituted by very dense isodiametric sclereids
<i>N. carnosa</i>	C	6 mm long, 5.5 mm wide	Unistratate, isodiametric cells, 0.05 mm average width	1.2-1.7 mm width, several (8-10) layers of large elongated cells with suberized walls	0.5 mm average width, layer of very dense isodiametric sclereids
“Dense” group – Fig. 6					
<i>N. aplocaryoides</i>	G	1.5 mm diameter	Indistinct, thin unistratate layer	0.03 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	0.15 to 0.45 mm width, layer of very dense isodiametric sclereids, some intracellular spaces noticeable
<i>N. crassulifolia</i>	G	2 mm long, 1.5 mm wide	Unistratate, isodiametric cells about 0.07 mm diameter	0.03 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	0.35 to 0.8 mm width, layer of very dense isodiametric sclereids
<i>N. divaricata</i>	G	2 mm long	Unistratate, isodiametric cells about 0.05 mm diameter	0.04 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	0.5 mm average width, layer of very dense isodiametric sclereids, some intracellular spaces noticeable

<i>N. intonsa</i>	F	3.5 mm long	Unistrate, isodiametric cells, 0.04 mm average width	0.05 mm average width, 1 layer of round or elongated cells with suberized walls, with crushed appearance at maturity	0.1-0.3 mm width (area surrounding the embryonic chambers), very dense isodiametric sclereids, some intracellular spaces noticeable
<i>N. parviflora</i>	B	5 mm long	Indistinct, thin unistrate layer	0.04 mm average width, 1 layer of round or elongated cells with suberized walls	0.07 mm average width, layer of very dense isodiametric sclereids. Slightly thicker at point of union with wing
<i>N. rostrata</i>	C	6 mm long	Indistinct, thin unistrate layer	0.06 mm average width, 1 to 3 layers of isodiametric cells, with crushed appearance at maturity	1 to 1.8 mm width, layer of very dense isodiametric sclereids
<i>N. sedifolia</i>	G	2 mm long, 1.5 mm wide	Unistrate, isodiametric cells about 0.05 mm diameter	0.02 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	0.1 to 0.35 mm width, layer of very dense isodiametric sclereids

"Porous" group – Fig. 7

<i>N. linearifolia</i>	G	2.3 mm diameter	Unistrate, isodiametric cells about 0.035 mm diameter	0.025 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	Medium sized, apparently empty cells with undulated but sclerified walls (area surrounding the embryonic chambers: 0.15 thinnest section - 0.4 mm thickest section)
<i>N. onoana</i>	G	1.5 mm diameter	Indistinct, thin unistrate layer	0.02 mm average width, 1 apparent layer of isodiametric cells, with crushed appearance at maturity	Medium sized, apparently empty cells with undulated but sclerified walls (0.11 thinnest section - 0.6 mm thickest section)
<i>N. paradoxa</i>	B	2.6 mm long	Unistrate, isodiametric or rectangular cells, 0.02 mm average width	1 to 3 layers of medium sized elongated cells, crushed appearance at maturity, irregular thickness in whole (0.02 thinnest section - 0.1 mm thickest section)	Medium to large, almost empty cells with undulated but sclerified walls, forming a spongy tissue in appearance (0.11 thinnest section - 0.85 mm thickest section)

It was not possible to identify an evident relationship between the anatomy of the mericarps and the location of the species within the clades. However, a tendency was observed, since most of the mericarps of species belonging to Clade G have an internal zone of the mesocarp with very dense sclereids, and an external zone that is barely distinguishable, very thin and with a crushed appearance. This feature was also presented by *N. intonsa* of Clade F and *N. rostrata* of Clade C. Two species of Clade G also had an internal zone of the mesocarp with large and empty cells, with undulated walls, which gave them a porous

or aerated appearance. This feature was more evident in *N. paradoxa* (Clade B) than in *N. linearifolia* and *N. onoana* (both Clade G), as was also revealed in the SEM analysis (Fig. 3), where these last two species appear to have a pericarp very similar in density to the rest of Clade G species (*N. divaricata*, *N. crassulifolia*, *N. aplocaryoides* and *N. sedifolia*). Thus, we can conclude that there is a gradient in terms of size and content of cells of the internal zone of the mesocarp for the 12 species studied. Although this is not enough by itself to identify species, it is a new tool which may assist identification, and that can be complemented with other analyses for this purpose.

### **3.2 Fruit morphology**

According to Dillon *et al.* (2009), although the mericarp is a unique character and distinguishes *Nolana* from other members of the Solanaceae family, its morphology, and their number and size, are of limited use in circumscribing internal groups. However, certain trends can be observed.

Clade B is the only one that presents winged mericarps (Fig. 8), but just one of its two subclades includes species that display this feature: the *N. pterocarpa-N. baccata-N. parviflora* subgroup, which are erect annuals with small flowers and angular mericarps, at times prolonged into wings (Dillon *et al.*, 2009) (Fig. 8, Table 3). The other subclade is *N. paradoxa-N. rupicola*, rosette-forming, taprooted plants with larger flowers, and more spherical but still angular mericarps lacking prolonged wings (Dillon *et al.*, 2009) (Fig. 8). These winged mericarps could be a diagnostic character for this subclade (M. Dillon, pers. com.), although in the case of *N. jaffuelii*, which has plants that are similar to those in this first subclade, mericarps are rounded and angular, without wings (Fig. 8). All members of Clade B present numerous mericarps (10-20, up to 30 per schizocarp), unlike members of other clades as described by Dillon *et al.* (2009), which present smaller numbers (often 3 to 6, up to 8 mericarps). Species of this clade thus have a different dispersal strategy, releasing multiple mericarps that accumulate under the mother plants, and are therefore important components of the blooming desert phenomenon, at which time these species can be seen blooming profusely in the coastal Atacama Desert and inland valleys.

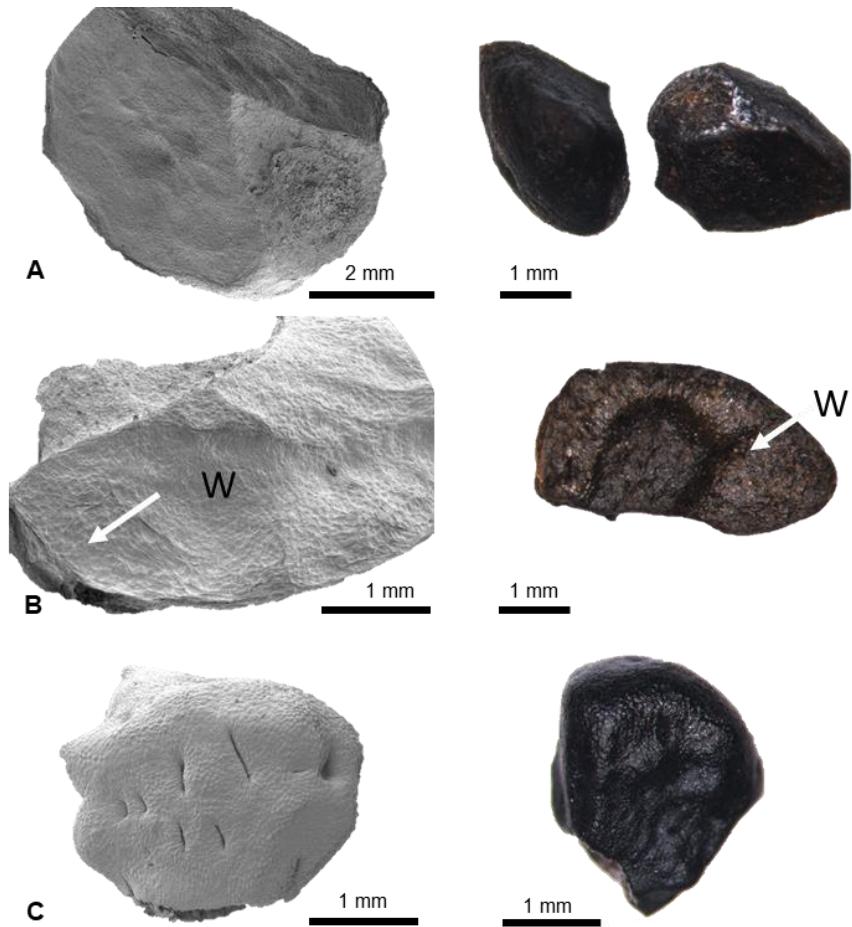


Figure 8. Morphological features of mericarps of species that belong to Clade B, SEM and stereoscopic magnifier images. A: *N. paradoxa*; B: *N. parviflora* (portion of mericarp); C: *N. jaffuelii*. W: wing. Bar (right) indicates mm.

Mericarps of clade C are relatively large (Fig. 9, Table 3) compared to all other mericarps of *Nolana* species, and in both *N. carnosa* and *N. rostrata*, they have a thin layer (exocarp plus part of the mesocarp immediately below) that covers each mericarp completely or partially. This layer can easily be detached and could be a distinguishing feature for members of this clade. Their fruits are schizocarpic, rounded or merely 5-sulcate, composed of 5 multispermous sections which are broadly joined to one another laterally (Johnston, 1936).

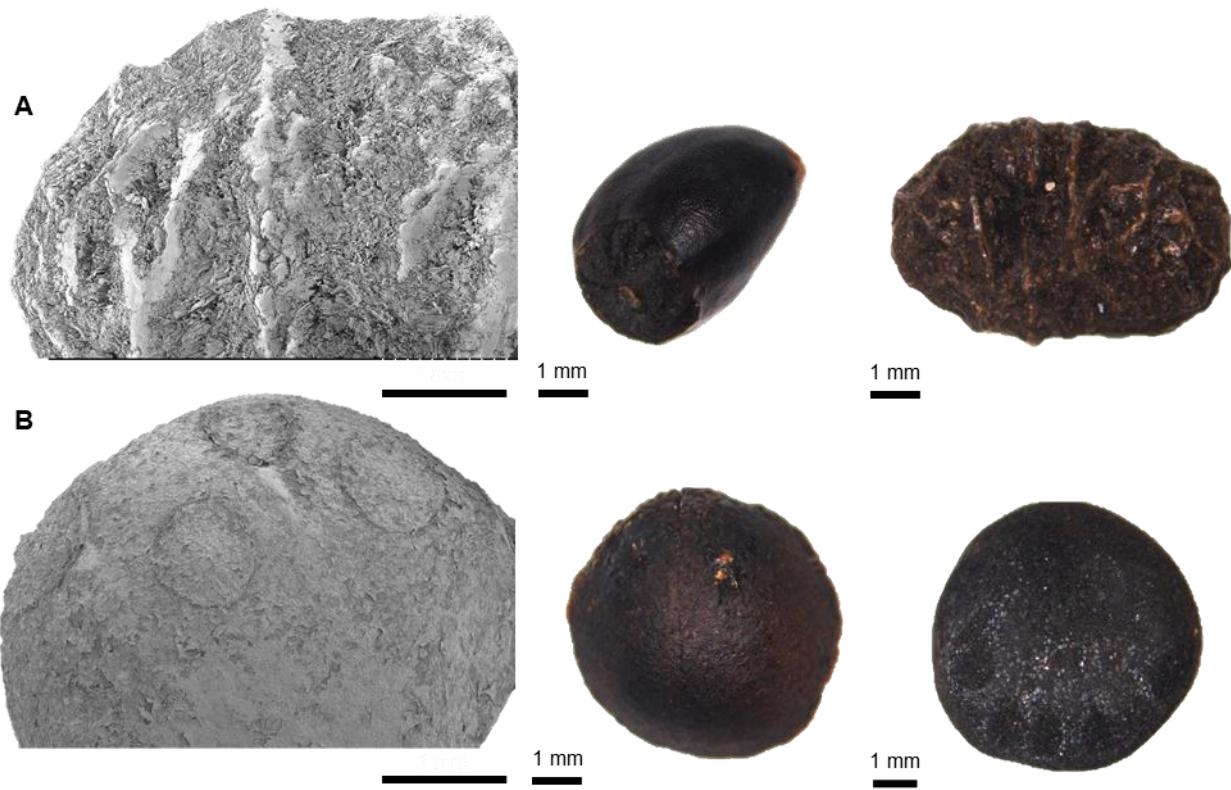


Figure 9. Morphological features of mericarps of species that belong to Clade C, SEM and stereoscopic magnifier images. A: *N. carmosa*, SEM image of portion of mericarp without cover, and photograph of mericarp with and without thin covering layer; B: *N. rostrata*, SEM image of portion of mericarp, and photograph of mericarp with and without thin covering layer. It is possible to see the funicular scar in one region of the mericarp of *N. rostrata*.

*N. intonsa* is the only Chilean species belonging to clade F; its mericarps are easily recognizable, shaped like a comma (Fig. 10, Table 3) with a rough surface of bright black colour when freshly dispersed. *N. intonsa* presents a large number of embryonic chambers within some of its mericarps (up to 8), the highest number for all studied species (Table 3).

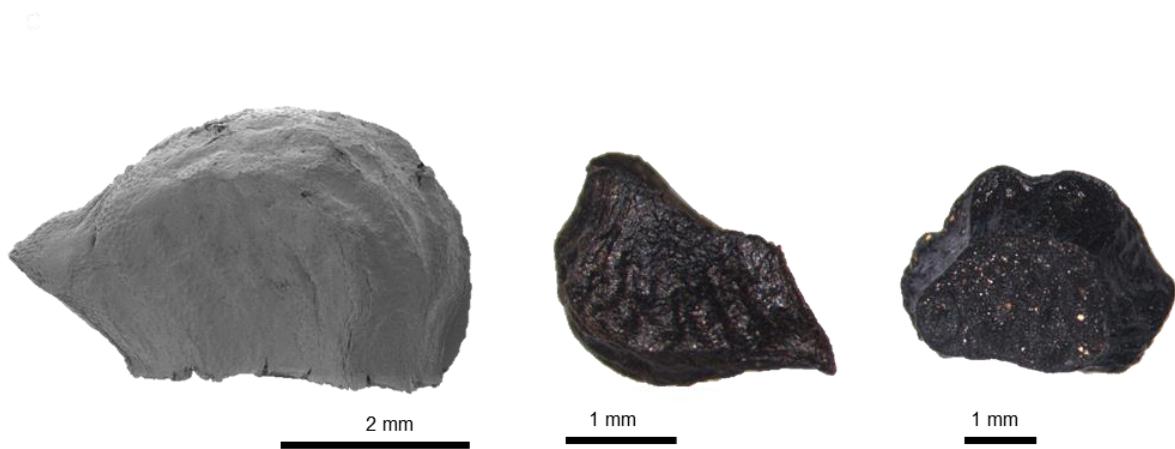


Figure 10. Morphological features of mericarps of *Nolana intonsa* (Clade F), SEM and stereoscopic magnifier images.

In clade G, mericarps of all its species have relatively different sizes, and differ in terms of texture and gloss, but they are all rounded or spherical (Fig. 11) and usually bear 1 to 3 embryonic chambers, with exception of *N. linearifolia* which has larger mericarps and can bear up to 5 chambers in one mericarp (Table 3). Johnston (1936) mentions that although the species is aberrant in corolla, the fruit agrees closely with that prevailing among the shrubby members of its genus (Clade G; Fig. 11, Table 3).

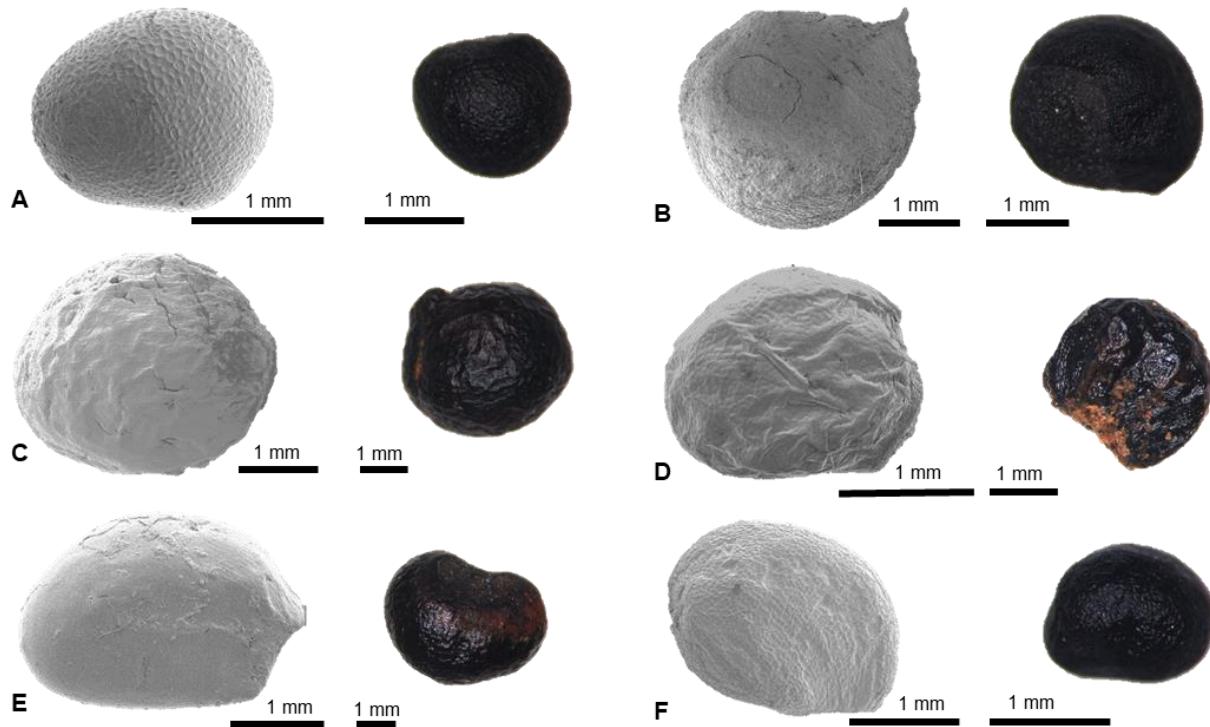


Figure 11. Morphological features of mericarps of species that belong to Clade G, SEM and stereoscopic magnifying glass images. A: *N. aplocaryoides*; B: *N. crassulifolia*; C: *N. divaricata*; D: *N. linearifolia*; E: *N. sedifolia*; F: *N. onoana*.

It is important to mention that for the recently described *Nolana patachensis* (Hepp and Dillon, 2018), unique characteristics of its mericarps were determinant to identify it as a new species for Chile. As the authors describe, its mericarps are elliptic or oval with finely bullate surfaces, which distinguishes *N. patachensis* from its congeners in northern Chile.

Table 3. Morphological mericarp features of the 12 species of *Nolana* studied. Clades correspond to phylogenetic study of *Nolana* by Dillon *et al.* (2007, 2009) and Tu *et al.* (2008).

<b>Species</b>	<b>Clade</b>	<b>Number of mericarps</b>	<b>Mericarp size</b>	<b>Mericarp shape</b>	<b>Number of seeds per mericarp</b>
<i>N. jaffuelii</i>	B	numerous (>15)	1-3 mm diameter	angular	1(-2)
<i>N. paradoxa</i>	B	numerous (>15-20)	3-6 mm long	rounded angles (polyhedral)	1-2(-3)
<i>N. parviflora</i>	B	8-15	4-6 mm long, 2-3 mm wide	2 types of mericarps, major nutlets and thinner, winged ones. Both laterally compressed	1 (winged) to 5 (only larger fruits)
<i>N. carnosa</i>	C	4-5	5-8 mm long, 4-5 mm wide	irregular, globose, extended	1 to 5
<i>N. rostrata</i>	C	5-7-8	4-6 mm diameter	rounded	2-7
<i>N. intonsa</i>	F	7-8	3.5 mm long - 2.5 mm wide	angular; unequal	1 to 8
<i>N. aplocaryoides</i>	G	3-6	1-2 mm diameter	spherical	1-2-3
<i>N. crassulifolia</i>	G	3-8	2-3 mm diameter	rounded; unequal	1-2
<i>N. divaricata</i>	G	(3)-5	2-3 mm diameter	globose; ovoid, unequal	1-2
<i>N. linearifolia</i>	G	3-6	2-3 mm diameter	spherical	(1)2 to 5
<i>N. onoana</i>	G	6-7	2-3 diameter	ovoid	1(-2)
<i>N. sedifolia</i>	G	3-8	2-3 mm long, 1-2 mm wide	oval; unequal	1-3

From: Dillon *et al.*, 2007; Mesa *et al.*, 1998; Mesa, 1981; Johnston, 1936; Reiche, 1910; and personal observations

## **Conclusions**

There are shared fruit characteristics among the twelve Chilean *Nolana* species studied, but there is no clear pattern that would allow them to be identified based only on internal anatomy of the mericarps. It was possible to classify the twelve species into 3 groups according to the composition of the mesocarp, but this was not strictly related to clades. The inner layer of the mesocarp, composed of dense sclereids, may provide protection to the embryo, as it would allow the mericarps to remain relatively intact in the soil seed bank for many years. Additionally, for species with mericarps containing more than one embryonic chamber, there would be more than one opportunity for germination, since each plug is detached independently. Therefore, the particular structure of the mericarps would be relevant in the survival of these species in desert environments, both in terms of dispersion and reproduction.

The identification of species can also be supported by the morphology or external structure of the mericarps, since within each clade it is possible to find trends presented by their fruits, particularly in terms of size and shape.

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## **Chapter V. A new endemic species of *Nolana* (Solanaceae-Nolaneae) from near Iquique, Chile**

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### **Abstract**

In connection with studies on the fog oasis or *lomas* formations at Alto Patache near Iquique, Chile, the first author encountered several *Nolana* species, including one new to science described here, *N. patachensis* J. Hepp & M.O. Dillon (Solanaceae-Nolaneae). The new species is diagnosed, described, illustrated with photographs, and compared to nearest geographic neighbors in northern Chile. To an aid in recognition, a key to *Nolana* species reported from Región of Tarapacá is provided. Putative relationships between the various *Nolana* species encountered at the type locality are discussed. Conservation efforts at the type locality are highlighted, including its unique environmental conditions, biota, and potential threats.

**Key Words:** *Nolana*, Nolaneae, endemics, *lomas* formations, new species, Región of Tarapacá, Chile, conservation, Solanaceae

### **Resumen**

En relación a los estudios sobre el oasis de niebla o las formaciones de lomas en Alto Patache cerca a Iquique, Chile; el primer autor recolectó varias especies de *Nolana*, incluida una nueva especie para la ciencia que aquí se describe, *N. patachensis* J. Hepp & M.O. Dillon (Solanaceae-Nolaneae). Además de la descripción, se realiza la diagnosis, se ilustra con fotografías y se compara con las especies vecinas más cercanas del norte de Chile. Para ayudar al reconocimiento, se proporciona una clave para las especies de *Nolana* reportadas para la Región de Tarapacá. También se discuten las relaciones putativas entre las diversas especies de *Nolana* encontradas en la localidad del tipo; así mismo, se resaltan los esfuerzos de conservación en la localidad tipo, incluyendo sus condiciones ambientales únicas, biota y amenazas potenciales.

Palabras clave: *Nolana*, Nolaneae, endémicas, formaciones de lomas, especie nueva, Región de Tarapacá, Chile, conservación, Solanaceae

## Introduction

*Nolana* L. ex L.f. (Solanaceae-Nolaneae) is a genus consisting of 90 species, including the one described here (Dillon, 2005, 2016). With the addition of this species, it brings the total number to 48 Chilean species, including 45 endemic species, and three species with disjunct distributions ranging from southern Peru (i.e., *Nolana adansonii* (Roem. & Schult.) I.M. Johnst., *N. gracillima* (I.M. Johnst.) I.M. Johnst., and *N. lycioides* I.M. Johnst.). Of this number, 12-13 *Nolana* species have been verified from Región de Tarapacá (see Table 1).

In Chile, greatest species diversity in *Nolana* is confined to near-ocean localities termed *lomas* formations, between 50--800 m elevation and along the foot of the Coastal Cordillera, usually within 20 kms of the Pacific Ocean (Rundel *et al.*, 1991; Dillon & Hoffmann, 1997). Only a few species are distributed above 2000 m and/or at a distances of 50-500 kms inland from the coast, e.g., *Nolana leptophylla*, *N. sessiliflora*.

Most Chilean species are narrow endemics, with small, restricted geographic ranges and specific ecological requirements, but a few species have larger geographic distributions and occupy several vegetation formations (Dillon, 2005).

## Materials and Methods

Descriptions were made from dried herbarium specimens deposited in SGO. All acronyms follow those in Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>). Conservation status was assigned using IUCN criteria (2017) combined with field observations and geographic distribution based on herbarium specimens. Scanning electron microscopy (SEM) was used to examine the structure of the mericarps. Samples were mounted on aluminium SEM stubs and sputter coated with a platinum-gold alloy, using a Quorum Sputter Coater Q150T. Mericarps were examined and photographed using a HITACHI S-4700 SEM at an accelerating voltage of 2.0 kV and working distance of 12.0 mm. Images were saved as TIFF (Tagged Image File Format). Sample were photographed using a stereoscopic magnifying glass.

We utilize the "morphological cluster" concept in recognition of species in *Nolana* (see Mallet, 1995), defined as "assemblages of individuals with morphological features in common and separate from other assemblages by correlated morphological discontinuities in a number of features". In addition to the diagnoses provided for the new species, specific characters useful in recognition of species are detailed in the **Key to Species of Región de Tarapacá, Chile**.

## Taxonomic treatment

***Nolana patachensis* J.Hepp & M.O. Dillon, sp. nov.**

Figure 1, 2, 3, 4, 5

TYPE: CHILE. **Región de Tarapacá**: Prov. Iquique, Alto Punta Patache, 20°49'S, 70°09'W, 1 Nov 1997, W. Sielfeld 32 (holotype: SGO-143057).

**Diagnosis**

*Nolana patachensis* is most similar to *N. onoana*, sharing similar leaf and floral morphology (Fig. 7C); however, it differs from the latter species in its spreading, prostrate habit, and erect, terete leaves. Further, its elliptic or oval mericarps with (1-)2-3(-4) seeds and finely bullate surfaces (Fig. 5A) are essentially unique within its congeners in northern Chile. The mericarps in *N. patachensis* also differ from mericarps found in *N. onoana* which are round or spherical, with a finely reticulate or alveolate surfaces. Other annual, tap-rooted species, e.g., *N. aplocaryoides*, have with much wider leaves with long, villous trichomes (Fig. 7A), and round or spherical mericarps; and *N. gracillima* with glabrescent to pilose pubescence leaves, shorter corollas (Fig. 7B), and pyriform mericarps.

**Description**

Taprooted annual **herbs** to 50 cm in diameter, 10-20 cm tall, basally-branched; stems branched, prostrate to decumbent, to 15 cm long, densely stipitate-glandular. **Leaves** alternate, sessile, the blades linear-oblong, 10-20 mm long, 3-4 mm wide, terete, erect or perpendicular orientation, succulent, densely pubescence with stout, stipitate-glandular trichomes, entire, apically rounded, the bases rounded. **Inflorescences** of solitary flowers in upper leaf axils; pedicels cylindrical, densely pubescent, 2-7 (-10) mm long. **Flowers** 5-merous; calyx narrowly campanulate, 5-7 mm wide at anthesis, densely covered with stipitate-glandular trichomes, 5-lobed, the tube ca. 3-5 mm long, ca. 5 mm wide, the lobes lanceolate, unequal, 2-3 mm long, ca. 1 mm wide, the apices obtuse or rounded; corollas zygomorphic, infundibuliform, 18-24 mm long, 8-12 mm wide at anthesis, distally lavender to light blue, the throat clear, externally and internally glabrous, the lobes obtuse; stamens 5, included, the filaments inserted on lower third of corolla, unequal, 3 long, 2 short, anthers dithecal, purple, the thecae ca. 1.2 mm long, ca. 1 mm wide, glabrous; ovary glabrous, basal nectary ca. 1 mm wide, the carpels 5, the style included, the stigma green. **Fruits** mericarps, 5, 1-seriate, oval to elliptic, black, 2-3 mm long, 1.5-2 mm wide, adaxial surfaces minutely bullate; seeds (1-)2-3(-4) per mericarp.

**Phenology:** Annual, tap-rooted annual that responds to sufficient moisture for germination and flowering during November, 1997, and more recently November-December, 2015.

**Etymology:** The species epithet is the latinisation of the geographic locality of the type collection, Alto Patache fog oasis or *lomas* formation located in Región of Tarapacá of northern Chile. As with many place names, the origins remain obscure, but it may have its origins in *Pukina*, a language distantly related to *Quechua*. In any event, it is not associated directly with either *Quechua* or *Aymara*. Further details on the locality are to be found below.

**Distribution and ecology:** *Nolana patachensis* has been recorded from two adjacent localities in Región de Tarapacá (Fig. 6), Alto Patache fog oasis or *lomas* formation ( $20^{\circ}48'S$ ,  $70^{\circ}09'W$ ) and Alto Punta Lobos ( $21^{\circ}02'W$ ,  $70^{\circ}09'W$ ). Both of these locality exhibit southwest oriented slopes that are constantly exposed to fog (Muñoz-Schick *et al.*, 2001; Calderón *et al.*, 2010; Osses *et al.*, 2017). Alto Patache fog oasis has been recognized as a priority area for conservation and is currently protected in a concession granted by the Ministry of National Assets for 25 years (since 2007) to Pontificia Universidad Católica de Chile, through the Atacama Desert Center. Its isolation has provided conditions for development of a particular biota. For example, it is the only known locality for *Santessonnia cervicornis* (Follmann) Follmann (an endemic Critically Endangered lichen species; Vargas *et al.*, 2017) and several other lichens common to the Atacama Desert. The vascular flora at Alto Patache has been estimated at approximately 42 species of vascular plants (Pliscoff *et al.*, 2017), including one of the few populations of *Alstroemeria lutea* Muñoz-Schick (Muñoz-Schick 2000). The arthropod fauna includes two endemic Coleoptera, *Scotobius patachensis* and *Scotobius larraini* (Sagredo *et al.*, 2002), and two bees, *Penapis larraini* (Hymenoptera: Halictidae: Rophitinae) (Packer, 2012) and *Neofidelia submersa* (Hymenoptera: Apoidea: Megachilidae) (Dumesh & Packer, 2013). Lastly, this locality has been implicated in the first record of a noctuid moth, *Hemieuxoa polymorpha* Forbes, for Chile, when adults were collected at Alto Patache in 1999 (Angulo & Olivares, 2005). Alto Punta Lobos is located approximately 25 kms south of Alto Patache, and like that formation, it has a compliment of perhaps 20 endemics in a flora of ca. 40 species (Muñoz-Schick *et al.*, 2001).

**Putative relationships:** *Nolana patachensis* is distinctive among its congeners in Chile with a combination of characters not encountered in any other described species. While DNA results are not available for this species, overall morphological similarity suggests relationships with *Nolana onoana*, a member of Clade G (Dillon *et al.*, 2009).

Clade G is a strictly Chilean clade including *Nolana aplocaryoides* as the sister taxon to the remainder of the clade (Dillon *et al.*, 2009). This group is represented by small to large shrubs and annuals, all with small leaves, (1-)10-20(-40) mm long and (1-)2-5(-7) mm wide, all with smaller corollas when contrasted with those in Clade B (e.g., *N. jaffuelii*) and some members with only white or yellowish corollas. The leaf pubescence is extremely variable from glabrous to densely canescent with stellate or dendritic, or arachnoid, stipitate, or simple trichomes, and rarely with superficial salt glands.

**Conservation status:** Critically Endangered (CR); overall distribution at two localities, each with  $<10$  km $^2$  (CR) and perhaps  $<250$  individuals. See IUCN (2017) for explanation of measurements. The threats to these habitats are posed by ever-expanding human pressure from mining and other industrial processes that can contaminate these fragile environments. Efforts at studying, protecting and preserving the region are underway.

**Additional specimens examined:** CHILE. Región de Tarapacá: Prov. Iquique, Alto Patache, E. Belmonte 97770 (CONC-143484), Alto Punta Patache,  $20^{\circ}49'W$ ,  $70^{\circ}09'W$ , 800 m, 8 Nov 1997, R. Pinto s.n. (SGO-142975); Alto Punta Lobos,  $21^{\circ}02'S$ ,  $70^{\circ}09'W$ , 800 m, 17 Jan 1998, R. Pinto s.n. (SGO-142976).

A series of *Nolana* collections from further south of the currently known range of *N. patachensis* need to be evaluated in the light of recent discoveries; these are from Región de Antofagasta, Prov. Tocopilla, camino a Mina Mantos de La Luna, *M. Quezada & E. Ruiz* 16 (CONC-121070), *M. Quezada & E. Ruiz* 17 (CONC-121207), and *M. Quezada & E. Ruiz* 19 (CONC-121076; SGO-127878). Another collection that should be scrutinized further; Prov. Tocopilla, Quebrada Mamilla, *F. Schlegel* 7693 (CONC-115627).

**Notes:** When herbarium material of this plant was first encountered by MOD in 2009, they had been determined as *Nolana aplocaryoides*, another tap-rooted annual species typically recorded further to the south. That species has quite different leaves and pubescence, but with similar corollas (Fig. 7A). At that time, MOD determined the sheets as *N. gracillima*, a species originally described from southern Peru (Fig. 7B) but with populations reaching northern Chile. However, upon examination of photographs of living plants with flowers taken by JH on a field trip to Alto Patache in 2015, after the abundant August rains in the sector (Figs. 2, 3), MOD recognized the plants as distinct, realized his error, and became convinced that the taxon was new to science. While close to *N. onoana* (Fig. 7C) in its floral morphology, that species has a very different growth habit and its leaves are more sulcate on the abaxial surfaces, but most distinctive are the differences in the mericarps.

**Key to *Nolana* species recorded from Región de Tarapacá, Chile.**

1	Annual, tap-rooted herbs.	2
	Perennial herbs, subshrubs or shrubs.	6
2	Leaves clearly petiolate, the bases auriculate, occasionally connate, the blades cordiform, rarely reniform or elliptic, glabrous, the surfaces with salt glands, attracting atmospheric moisture and causing the look and feel of oil	<b><i>N. adansonii</i></b>
-	Leaves, if petiolate, without auriculate bases nor connate, the blades spatulate to oblanceolate, variously pubescent, but never oily	3
3	Basal leaves with entire blades, the flowering shoots with sessile oval to ovate bracts subtending flowers, the corollas 30-40 mm wide; mericarps more than 20, 3-seriate	<b><i>N. jaffuelii</i></b>
-	Basal leaves lacking, the caulin leaves linear-lanceolate to linear-spatulate, the corollas less than 10 mm wide; mericarps 2-5, 1-seriate	4
4	Leaves linear-spatulate, the blades 7-30 mm long, apically acute, the calyx 8-10 mm long, the mericarps 2-5, 2 largest, ca. 3 mm long.	<b><i>N. tarapacana</i></b>
-	Leaves linear to linear-lanceolate, the blades 8-25 mm long, apically obtuse to rounded, the calyx 3-6 mm long, the mericarps typically 2-5, the largest 1.5-2 mm long.	5
5	Leaves 8-25 mm long, finely pilose to glabrescent, the mericarps pyriform, 1.5-2 mm long	<b><i>N. gracillima</i></b>
-	Leaves 10-20 mm long, densely stipitate-glandular pubescent, the mericarps oval to elliptic, 2-3 mm long.	<b><i>N. patachensis</i></b>
6	Leaves clavate to globular-obovate, the corollas 6-9 mm long	7
-	Leaves oblong to linear-lanceolate to linear-spatulate.	8
7	Leaves broadly clavate to globular-obovate, 5-10 mm long, densely pubescent with stellate or dendritic trichomes, the corollas suberect, white to yellowish.	<b><i>N. peruviana</i></b>
-	Leaves clavate to globular, 1-5 mm long, densely pubescent with arachnoid-tomentose pubescence, the corollas hypocrateriformis, white or rarely bluish	<b><i>N. sedifolia</i></b>
8	Leaves narrowly linear-spatulate to linear-lanceolate, 20-30 mm long, 10-20 mm wide	9
-	Leaves 10 mm long or less, linear-oblong to oblong, 1-4 mm wide.	10
9	Leaves narrowly linear-spatulate, conspicuously shaggy villous, the corollas lavender with deep purple guides in the throat.	<b><i>N. intonsa</i></b>
-	Leaves linear-lanceolate to linear-spatulate, 10-25 mm long, ca. 1 mm wide, stipitate-glandular, the corollas blue, obvious guides absent.	<b><i>N. lycioides</i></b>
10	Leaves linear-oblong, 4-5 mm long, ca. 1 mm wide, hispidulous with capitate-glandular trichomes, 3-4(-5) mericarps	<b><i>N. leptophylla</i></b>
-	Leaves linear or oblong, 2-10 mm long, pubescence of elongate trichomes, not of capitate-glandular trichomes.	11
11	Leaves oblong, 2-6 mm long, 0.7-1.5 mm wide, oblong, tomentose to villous with flaccid-elongate trichomes, mericarps 5.	<b><i>N. tocopillensis</i></b>
-	Leaves linear, 10 mm long, 3-4 mm wide, pubescent with simple trichomes, mericarps 2(-3).	<b><i>N. foliosa</i></b>

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## Annexes



Fig. 1. *Nolana patachensis* J. Hepp & M.O. Dillon. Holotype: *Sielfeld* 32 (SGO-143057). Colección Museo Nacional de Historia Natural, Chile (SGO).

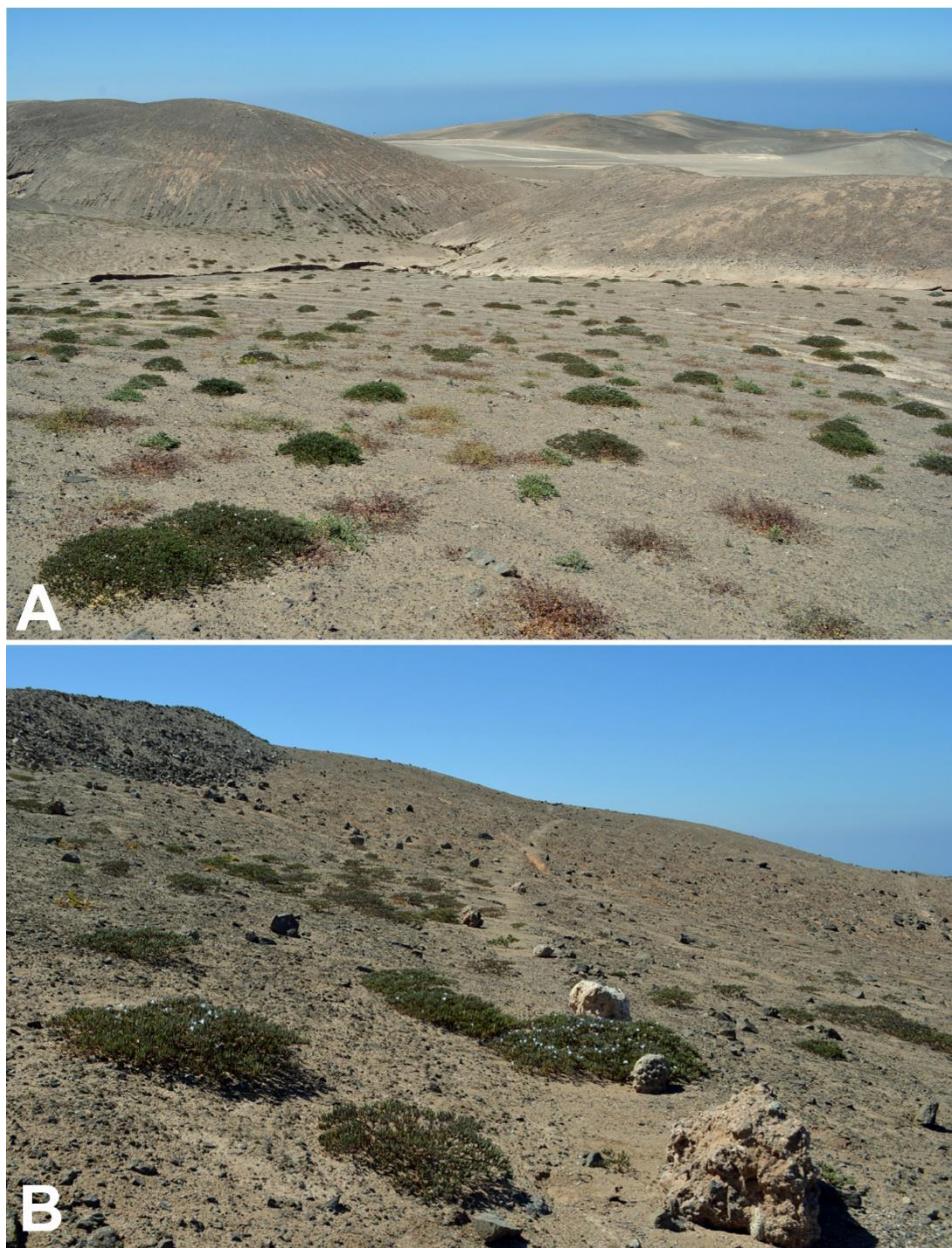


Fig. 2. *Nolana patachensis* habitats. A: Individuals of *N. patachensis* growing together with *N. jaffuelii*, *N. intonsa* and *Cristaria molinae* in December 2015, at the hills and inner plateau of Alto Patache oasis, which in dry years are devoid of plants. B: Prostrate growth habit of *N. patachensis*.

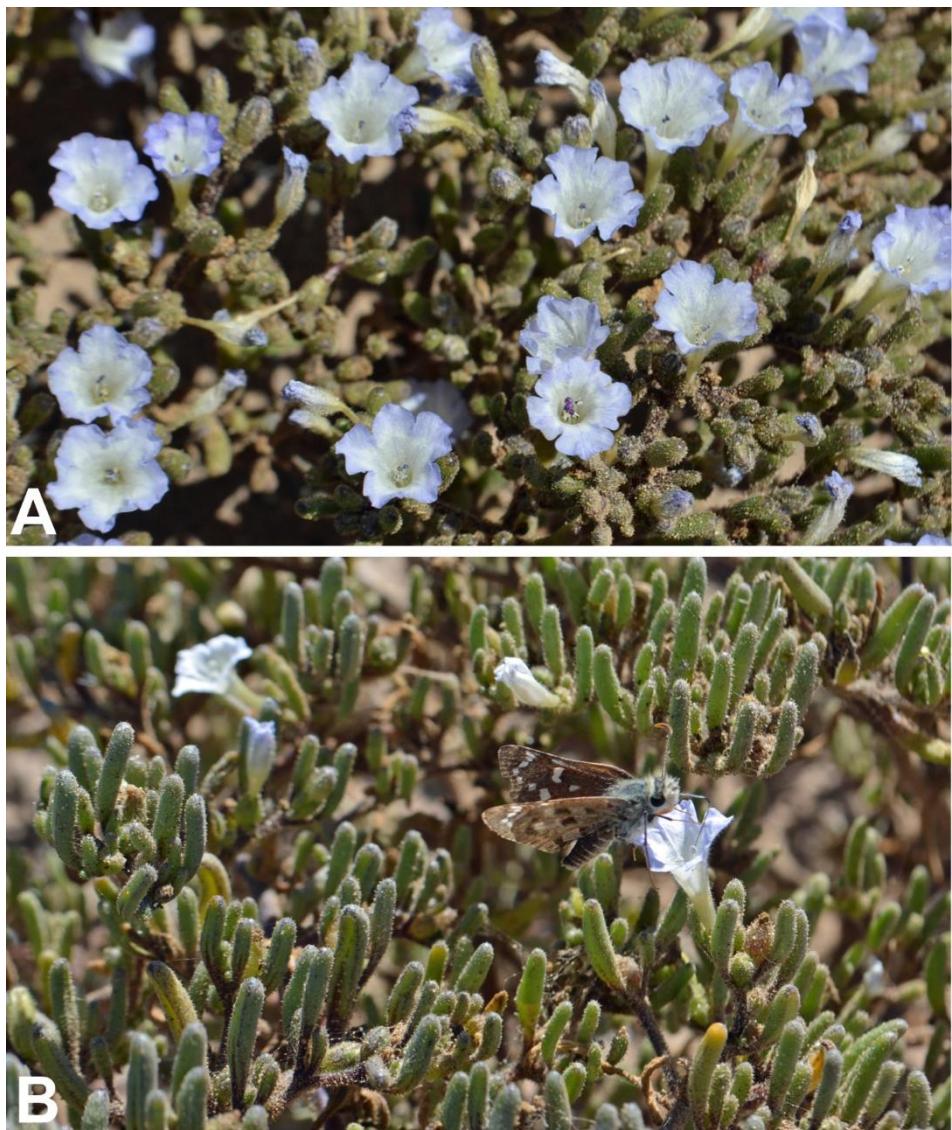


Fig. 3. *Nolana patachensis*. A. Close-up of flowers showing light blue color on the edge of the corolla. B. Lepidoptera (Family Hesperiidae - Skippers) pollinating the flowers available in the oasis with rains, December 2015. It is possible to see the trichomes on the typical erect leaves.

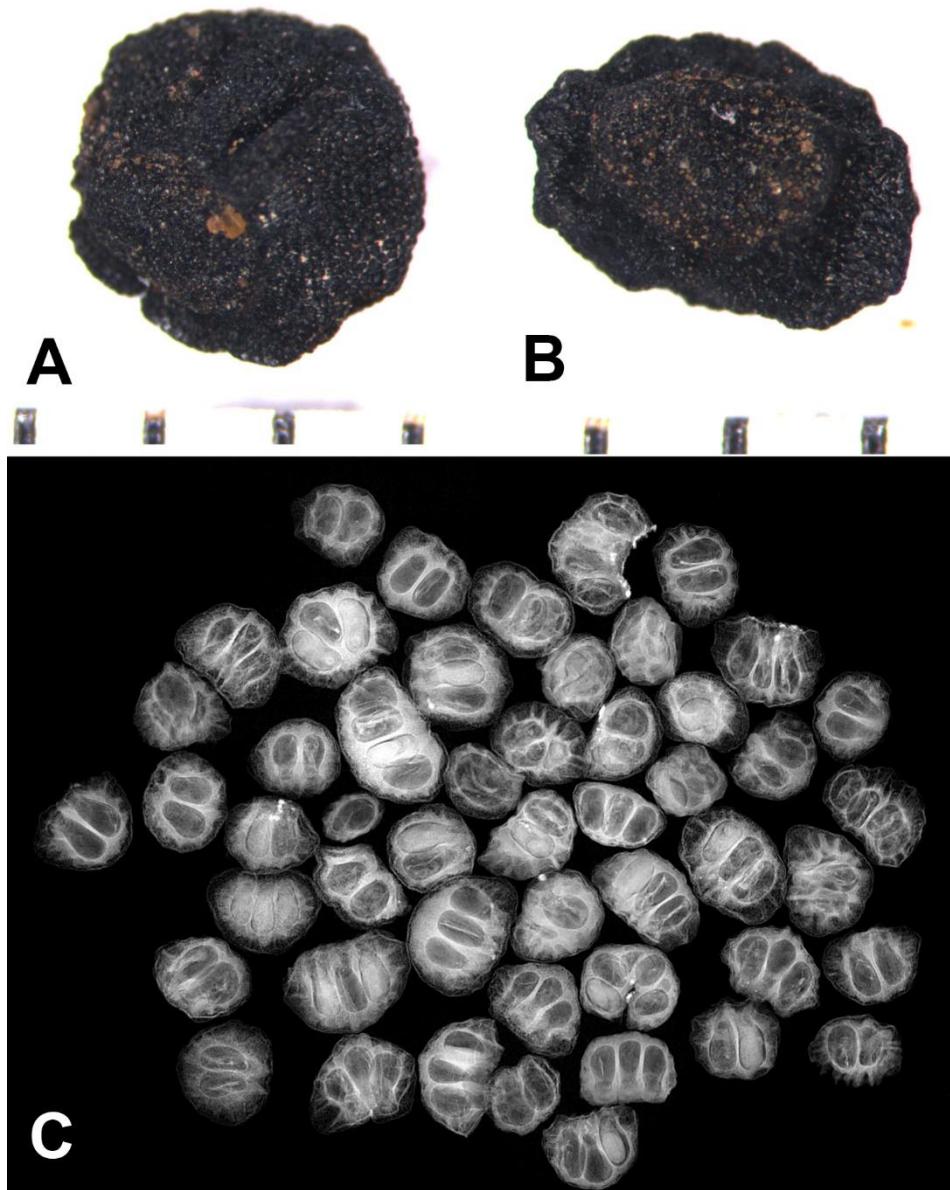


Fig. 4. *Nolana patachensis* mericarps. A. Abaxial view with stereoscopic magnifying glass images, scale marks = 1 mm. B. Adaxial view with stereoscopic magnifying glass images, scale marks = 1 mm. C. X-ray photograph showing internal seed chambers within each mericarps.

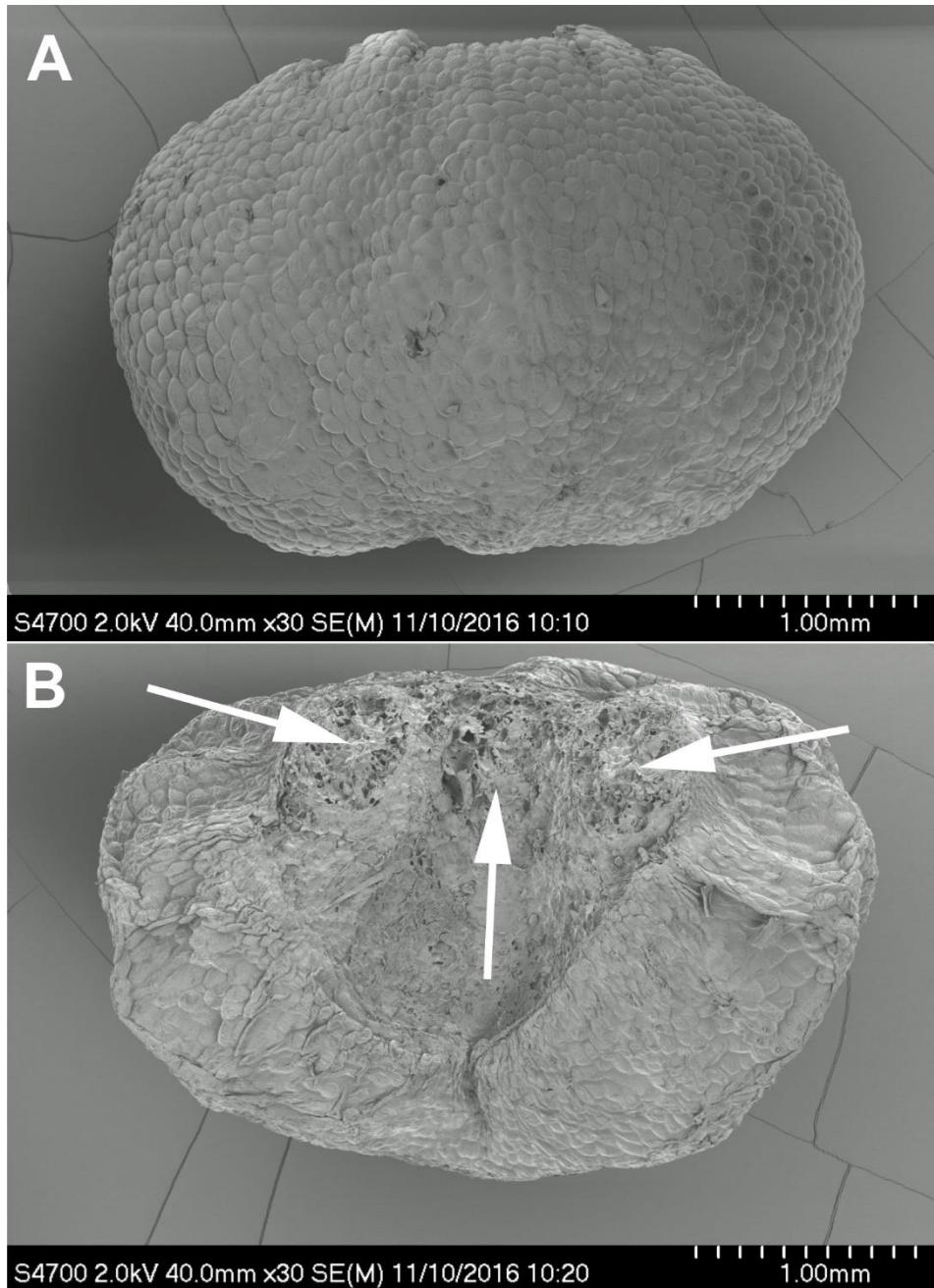


Fig. 5. *Nolana patachensis* mericarp. A. SEM of adaxial mericarp surface. B. SEM of abaxial mericarp surface, the arrows indicates the position of the funicular scars where the radicle appears during germination.

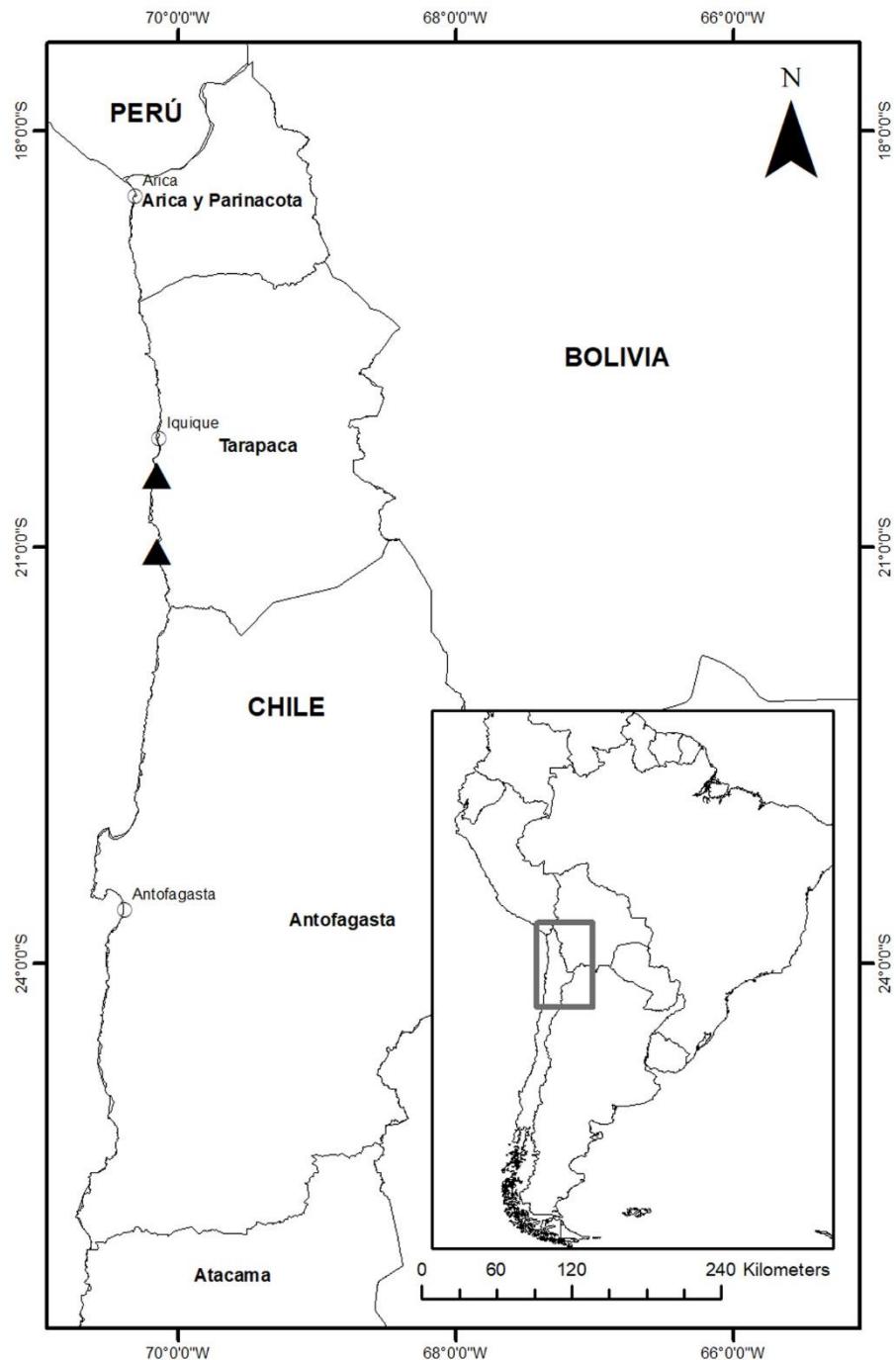


Fig. 6. Distribution of *Nolana patachensis*.

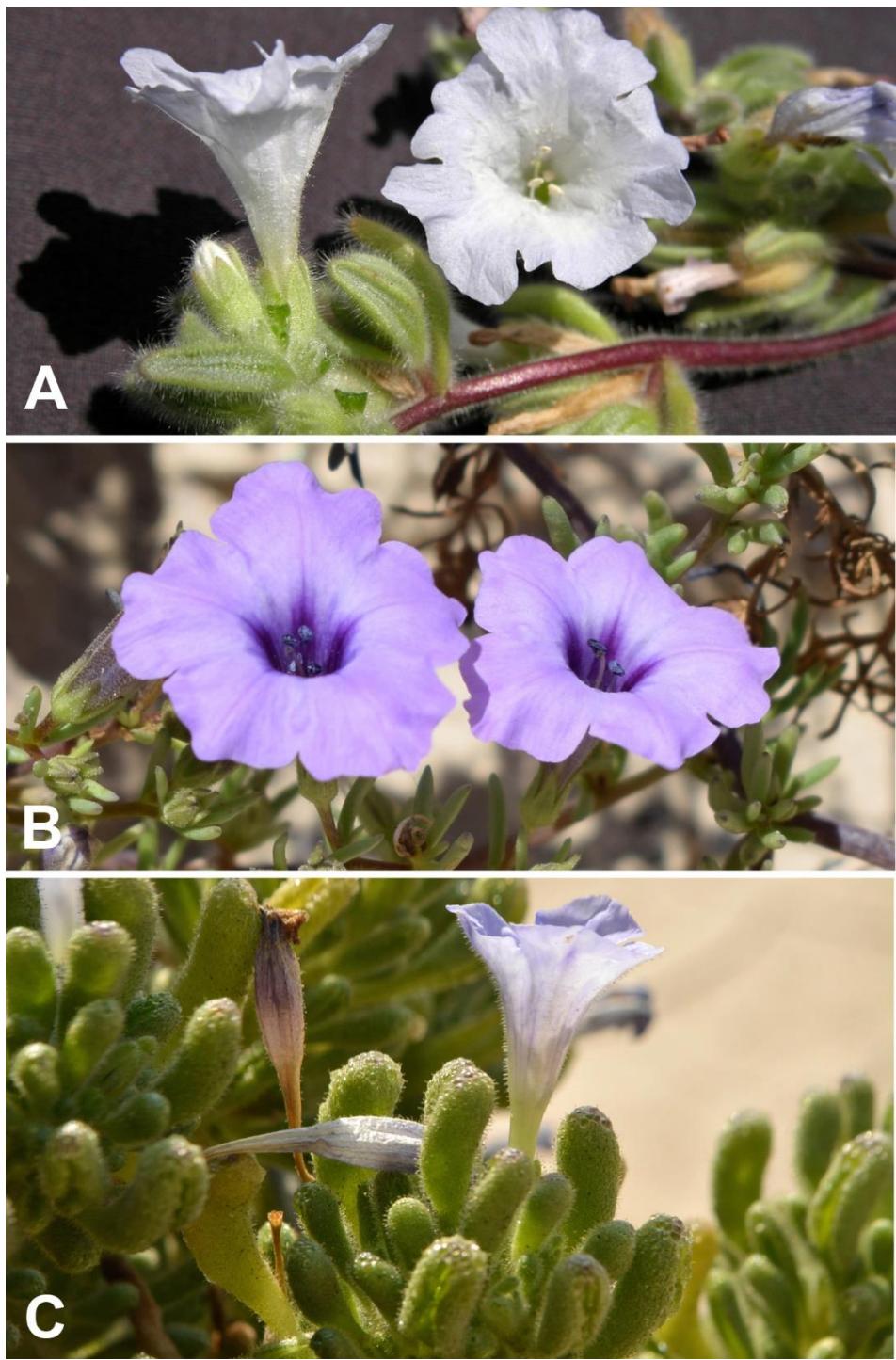


Fig. 7. *Nolana* species confused with *N. patachensis*. A. *N. aplocaryoides*, close-up of leaves and flowers. Voucher: Dillon 9055 (F). B. *N. gracillima*, close-up of flowering branch. Voucher: Quipuscoa et al. 6728 (F). C. *N. onoana*, close-up of leaves and flowers. Voucher: Dillon 9050 (F).

Table 1.

Alphabetical list of *Nolana* species recorded from Región Tarapaca, Chile, distribution and phylogenetic position as suggested by membership in clades (adapted from Dillon *et al.* 2009).

	<b>Species</b>	<b>Distribution</b>	<b>Clade</b>
1	<i>N. adansonii</i> (Roem. & Schult.) I.M. Johnst.	Chile-Peru	F
2	<i>N. foliosa</i> (Phil.) I.M. Johnst.	Chile	E
3	<i>N. gracillima</i> (I.M. Johnst.) I.M. Johnst.	Chile-Peru	E
4	<i>N. intonsa</i> I.M. Johnst.	Chile	F
5	<i>N. jaffuelii</i> I.M. Johnst.	Chile	B
6	<i>N. leptophylla</i> (Miers) I.M. Johnst.	Chile	G
7	<i>N. lycioides</i> I.M. Johnst.	Chile-Peru	D
8	<i>N. patachensis</i> J. Hepp & M.O. Dillon	Chile	G
9	<i>N. peruviana</i> (Gaudich.) I.M. Johnst.	Chile	G
10	<i>N. sedifolia</i> Poepp.	Chile	G
11	<i>N. tarapacana</i> (Phil.) I.M. Johnst.	Chile	E
12	<i>N. tocopillensis</i> (I.M. Johnst.) I.M. Johnst.	Chile	G

## **Chapter VI. A study of fruit development in *Nolana paradoxa* (Solanaceae) from the coast of Chile**

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### **Abstract**

*Nolana paradoxa* (Solanaceae) is the species with the greatest latitudinal distribution of the genus, and one of the more extensively studied, in terms of flower morphology and fruit development; however, aspects of its germination requirements and dormancy mechanisms, and the role and formation of the funicular scar or germination plug, are still unclear. The complete fruits (schizocarps) of *N. paradoxa* (Solanaceae) were analysed structurally using light microscopy. The development of fruits, which were collected at 2, 5, 9, 12, 17, 25 and 35 days after flowering (DAF), was monitored, with particular focus on the development of the funiculus and the formation of the germination plug. Ripe mericarps (dispersal units) were also analysed using a Scanning Electron Microscope, a stereoscopic magnifier and X-rays. In the anatomical analysis, it was possible to visualize the monolayer of the endocarp and the origin of two zones within the mesocarp of the fruits. In addition, the formation of the germination plug was observed, which sclerifies as mericarps mature; this structure, however, is not impermeable to water.

Keywords: schizocarp, mericarp, funicular scar, germination plug

### **Introduction**

The genus *Nolana* L.f. (Solanaceae), originally described by Linnaeus in 1762, derives its name from the Latin word *nola* or small bell (Freyre *et al.*, 2005). It currently comprises 90 species from Perú (40 endemic species) and Chile (46 endemic species), with both countries sharing only three species; *N. galapagensis* is the only one endemic to the Galápagos Islands (Dillon *et al.*, 2009). The genus is considered monophyletic and is distinguished by the fundamentally 5-carpellate ovary that develops sclerified fruits, a unique character in the Solanaceae family (Knapp, 2002; Freyre *et al.*, 2005). The complete fruit is a schizocarp consisting of several uni or pluriseminate mericarps of different sizes (Bruno, 1994); when the schizocarp is mature, it often splits up into several mericarps, the dispersal units.

Most *Nolana* species are found in the fog-dependant *lomas formations* in the coast of the Atacama Desert, but some species occupy habitats at higher altitudes (> 1,000 masl). *N.*

*paradoxa* Lindl. is the sole member of the family south of the Valparaíso area (Johnston, 1936), its distribution extending from Huasco ( $28^{\circ}13'S$ ) to the south of Chile, in Chiloé Island ( $42^{\circ}43'S$ ). It is usually found in sandy sea-shore environments, from 0 to 100 masl. It is an annual to perennial succulent herb with a fleshy taproot (Freyre *et al.*, 2005), and the elongate stems are prostrate and loosely branched (Johnston, 1936).

*Nolana paradoxa* was the second *Nolana* (after *N. humifusa*) to be introduced to European gardens in the early 1820s, and today remains the most popular commercially available *Nolana*, sold under the name of Chilean bellflower, with existing cultivars “Blue Bird” and “Cliff Hanger Blue” (Freyre *et al.*, 2005). For this reason, this species has been studied extensively, with a focus on flower morphology and fruit development (Lindley, 1824; Payer, 1857; Saunders, 1936; di Fulvio, 1969; di Fulvio, 1971; Huber, 1980; Bondeson, 1986), although it is not yet fully understood how the seeds break dormancy and germinate in their natural habitat.

Many seeds, across several families, contain specialized structures that regulate the uptake of water by closing the natural openings in the seed or fruit coat (Bewley *et al.*, 2013) and thus relate to physical dormancy by preventing water to reach the embryo. For example, a chalazal cap or plug is found in members of the Malvaceae (Bewley *et al.*, 2013; Baskin & Baskin, 2015); an imbibition lid (gap adjacent to hilum) in Cannaceae (Baskin & Baskin, 2015); and a lens gap in Fabaceae (Gama-Arachchige *et al.*, 2013). Before seeds can germinate, these structures are ruptured, causing the seeds or fruits to become permeable to water; they are called water-gaps or water-plugs (Gama-Arachchige *et al.*, 2013; Baskin & Baskin, 2015). A germination plug, constituted by remains of the funiculus (Saunders, 1936), has also been described for species of *Nolana* in the Solanaceae family (Bondeson, 1981 and 1986; Douglas and Freyre, 2006), and an impermeability to water, i.e. physical dormancy, has been reported for the genus (Cabrera *et al.*, 2015). However, the role this plug plays in germination is not yet clearly established.

The objective of this study is to characterize the development of fruits of *Nolana paradoxa*, particularly in relation to the germination plug of the mericarps, to better understand its role in the germination and dormancy mechanisms of *Nolana* species.

## Materials and methods

### Plant material

Mericarps of *Nolana paradoxa* (Fig. 1) were collected in Guanaqueros ( $30^{\circ}11.734'S$  /  $71^{\circ}25.377'W$ ) and germinated according to Hepp *et al.* (2019, in preparation), removing the germination plug and imbibing the fruits in gibberellic acid (500 ppm). The obtained seedlings were transplanted to the greenhouse at the Facultad de Agronomía e Ingeniería Forestal in Santiago, Chile, where they were watered twice a week and kept at ambient temperature. The transplant was carried out in pots (14 cm diameter), with previous disinfection with sodium hypochlorite (Clorox ©) at 5%. The substrate used was peat, sand and perlite in 8:3:1.5 ratio, respectively. When flowers were open, they were hand-pollinated

and marked, and then fruits in development (complete schizocarps) were harvested at 2, 5, 9, 12, 17, 25 and 35 days after flowering (DAF).



Fig. 1. Flowering plant (A) and developing fruits with persistent calyx (B) of *Nolana paradoxa* growing at the sandy sea-shore in Guanaqueros, Coquimbo region, Chile.

### Morphology of mericarps

The colour, size and external appearance of the mericarps (dispersal unit) were recorded. A sample of fruits of *N. paradoxa* was photographed using a stereoscopic magnifier Olympus SZ2-ILST (Olympus Corporation, Tokyo, Japan).

Fruits were also x-rayed using the Faxitron MX20 Digital X-ray (Faxitron Bioptics, LLC, Arizona, USA). A sample of fruits were x-rayed and digital images of each sample were recorded. Identification of full seeds and of cavities appearing empty in each fruit were made based on differences in contrast within recorded images.

Scanning electron microscopy (SEM) was used to examine the structure of the fruit, the surface of the funicular scar and the longitudinally sectioned mericarp. Samples of *N.*

*paradoxa* were mounted on aluminium SEM stubs and sputtercoated with a platinum-gold alloy, using a Quorum Sputter Coater Q150T (Quorum Technologies Ltd, East Sussex, United Kingdom). Mericarps were examined and photographed using a HITACHI S-4700 SEM (Hitachi High-Technologies Corporation, Tokyo, Japan) at an accelerating voltage of 2.0 kV and working distance of 12.0 mm. Images were saved as Tagged Image File Format (TIFF).

To evaluate if water was able to penetrate and reach the embryo in intact mericarps of *N. paradoxa*, methylene blue (1gr/100 mL) was used. A sample of mericarps were left in metal containers with enough dye to cover them and were evaluated after 48 hours (room temperature). They were washed with distilled water and dried with paper towel, then allowed to air dry for 20 minutes at room temperature. They were observed under magnifier and sectioned using a scalpel (longitudinal cut).

### **Histological analysis**

Two or three complete fruits (schizocarps) corresponding to each collection day (at 2, 5, 9, 12, 17, 25 and 35 DAF) were selected and fixed in formol acetic alcohol (FAA), then dehydrated and preserved in paraffin to be sectioned off. Cross and longitudinal histological sections were made through the schizocarp, using a microtome (to a thickness of 16 µm) and safranin-fast green staining. Sections were observed under light microscope and photographed.

## **Results and discussion**

### **3.1 General structure of the mericarp**

The dispersal unit in the case of *N. paradoxa* is a polyhedral fruit, violet to black in colour (Johnston, 1936) called mericarp (Fig. 2A), usually 3-6 mm long (Johnston, 1936). Each mericarp of *N. paradoxa* has 1 or 2 (even 3) seeds (Reiche, 1910) which are enclosed in a separate chamber (Lindley, 1824). These embryonic chambers normally contain one embryo but are sometimes empty (Fig. 2B).

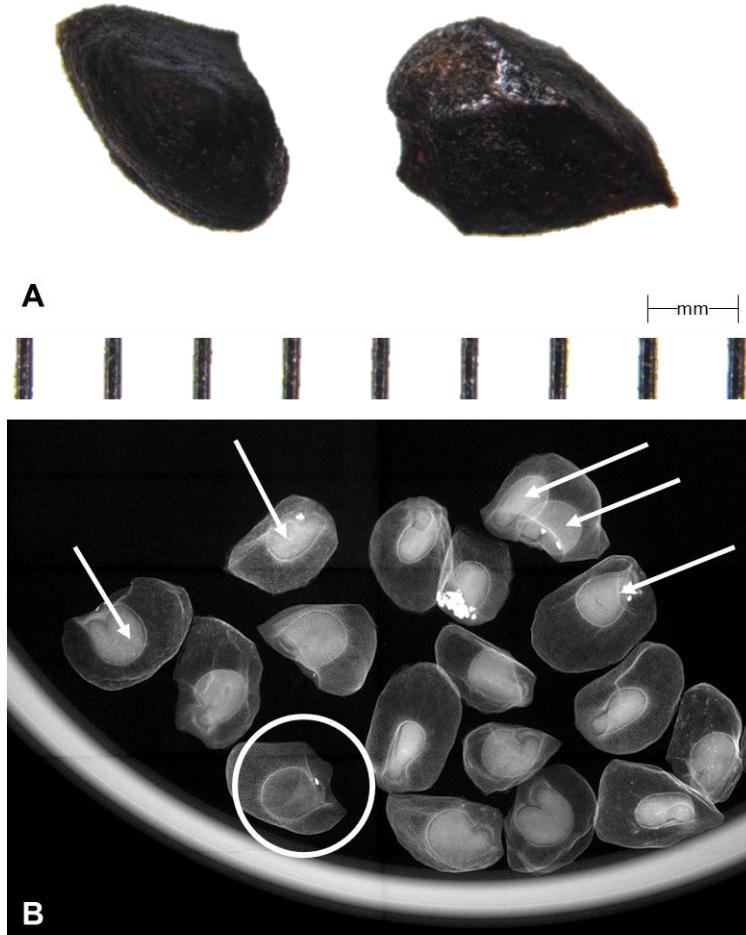


Fig. 2. Dispersal units (mericarps) of *N. paradoxa*, stereoscopic magnifier (A) and x-ray images (B). Arrows indicate full embryonic chambers; circle encloses an empty embryonic chamber.

In the morphological analysis with SEM images, it is possible to distinguish a funicular scar in the basal region of the mericarp (Fig. 3A and 3B), which corresponds to the area of union with the floral receptacle (Saunders, 1936). This slightly depressed, elliptical area indicates the location of a structure which has been termed a “germination plug” (Fig. 3C and 3D), since the protruding radicle push the plug out during germination (Bondeson, 1981). Figure 4 shows in detail the union of the receptacle with individual mericarps, which altogether form the complete fruit or schizocarp.

For the longitudinally cut mericarps, the intermediate layer corresponding to the mesocarp stands out (Fig. 3C); it is composed of medium to large, almost empty cells with undulated but lignified walls, forming an airy or porous tissue in appearance, as described in Hepp *et al.* (2019, *in press*); not corky, as reported by Johnston (1936), since cell walls are not suberized. For the majority of *Nolana* species, mericarps are passively distributed (Knapp, 2002); in the case of *N. paradoxa*, the porous pericarp might be an adaptation to floating. Johnston (1936) even suggests that the wide distribution of this species may have been

aided in part to detached leafy stems (that readily root) washed by the waves and currents, and also to the floating mericarps of the species.

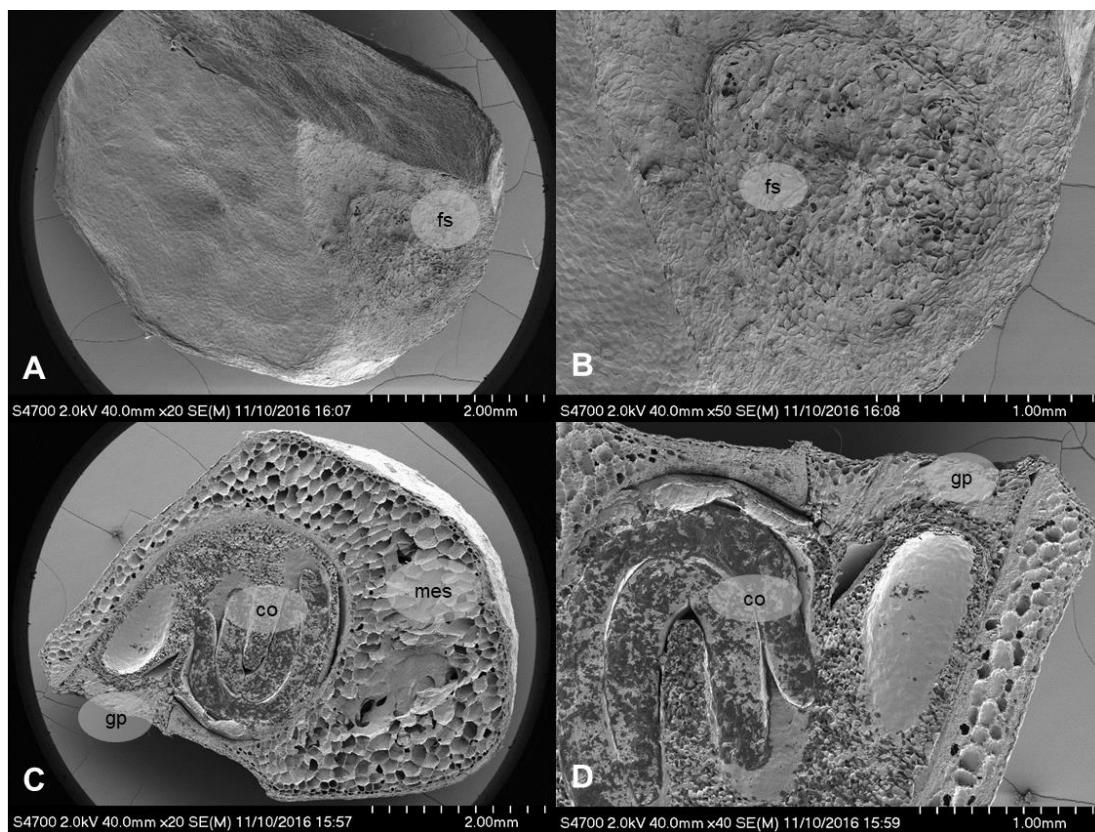


Fig. 3. SEM images of mericarps of *Nolana paradoxa*. A: small funicular scar in the area of attachment to the floral receptacle. B: Detail of funicular scar. C: Longitudinal section of mericarp. D: Detail of embryo and germination plug. Abbreviations: co: cotyledons; fs: funicular scar; gp: germination plug; mes: mesocarp.

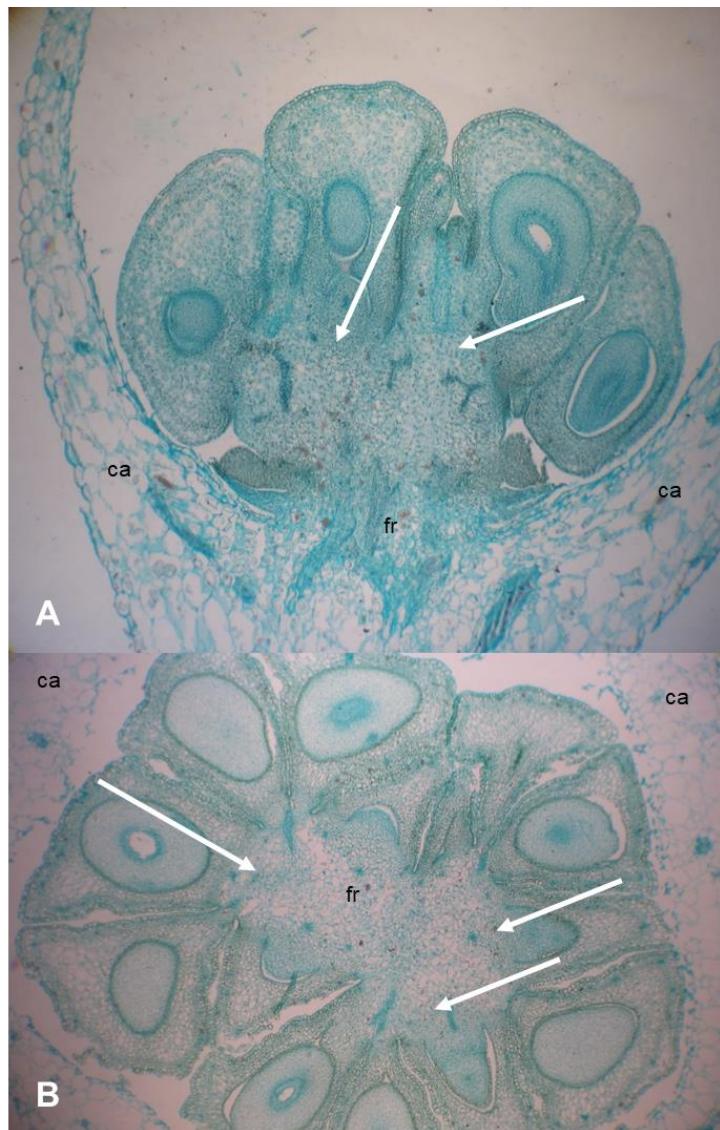


Fig. 4. Histological sections of the complete fruit of *N. paradoxa*. Longitudinal cut (A: 2 DDF, 40x) and transversal cut (B: 2 DDF, 40x) of schizocarps showing the calyx (ca) and the floral receptacle (fr). Arrows indicate the area of union of mericarps with floral receptacle.

### 3.2 Anatomy of the pericarp

By following the development of the fruits, it was possible to appreciate the formation of the different layers of the pericarp. By the definition of Richard (1819, quoted in Pabón-Mora & Litt, 2011), we consider a single-layered endocarp which is easily distinguishable in early stages (Fig. 5A-5C). The outer layer or exocarp also corresponds to a mono layered epidermis with cells that contain vacuoles with tannins and a thin protective cuticle, which appear collapsed when mericarps are fully mature (Fig. 5D).

The tissue in between would correspond to the mesocarp, for which two histologically differentiated zones can be visualized (Fig. 5A-5C): an internal one, which in the case of *N.*

*paradoxa* is constituted by sclereids, empty cells with undulated but lignified walls, and which has been previously described as endocarp (Bondeson, 1986; Cabrera *et al.*, 2015, working with *N. jaffuelii*). The external one, formed by parenchymatic cells (Fig. 5B and 5C), is reduced in size as mericarps mature (Fig. 5D).

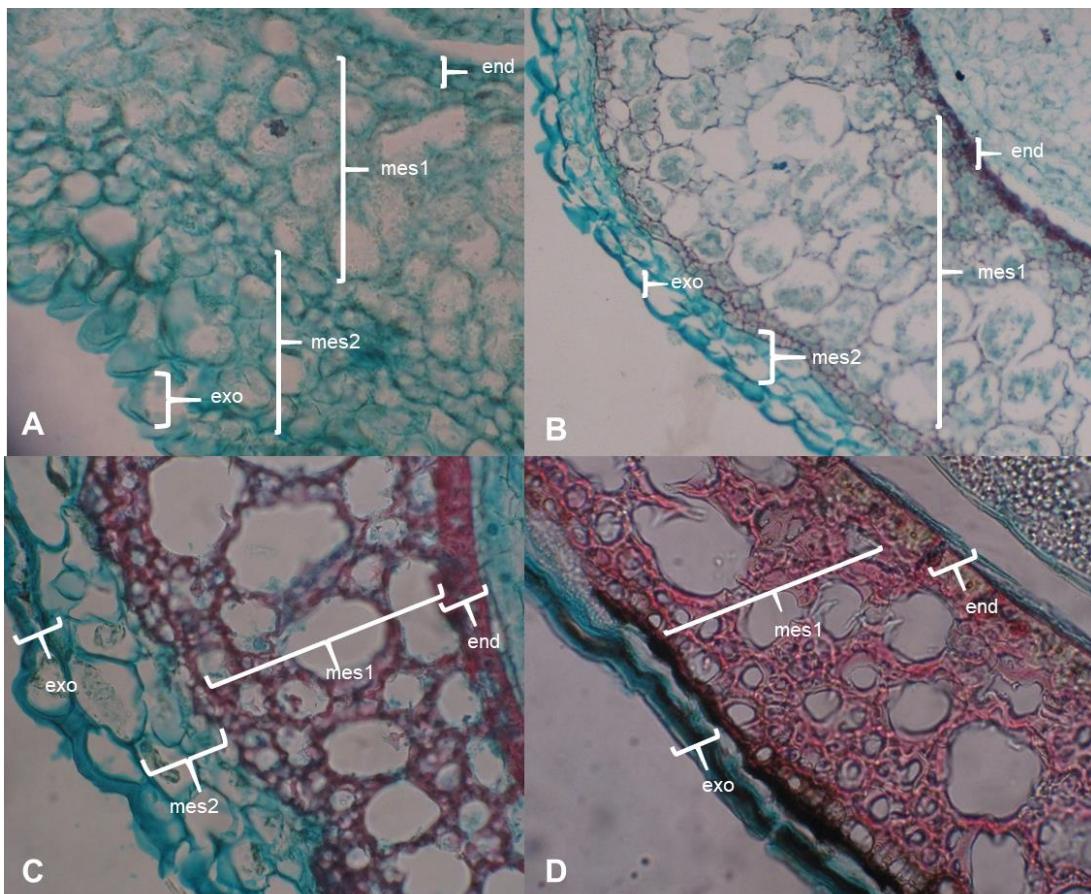


Fig. 5. Layers of the pericarp of *N. paradoxa*. A: 2 DAF (40x). B: 12 DAF (10x). C: 17 DAF (400x). D: Dispersed mature mericarp (400x). Abbreviations: end: endocarp; mes1: internal zone of the mesocarp; mes2: external zone of the mesocarp; exo: exocarp.

### 3.3 Formation of the funicular plug

It can be observed that the seed is generally protected by the pericarp (Fig. 6), except for the aperture or opening left by the invagination of the ovule and occupied by the funiculus (Bondeson, 1986). The epidermis (or endocarp) appears as a layer of cells that cover the inner wall of the embryonic chamber, the funiculus and the embryo (Bondeson, 1986).

As the mericarp matures, cells of a portion of the funiculus begin to lignify and sclereids are formed (Fig. 6A-F). This sclerified funicular plug (Fig. 7), or germination plug (Saunders, 1936) protects the opening after dispersal of the mericarps; then, when germination begins, the pressing radicle will break the testa and push the funicular plug out (Bondeson, 1986).

Additionally, the presence of starch stored in the abscission zone (of the funiculus) was observed (Fig. 7B), where the mericarp is attached to the floral receptacle. According to Bondeson (1986), all cells that are to become endocarp cells accumulate starch; the cell walls thicken and lignify.

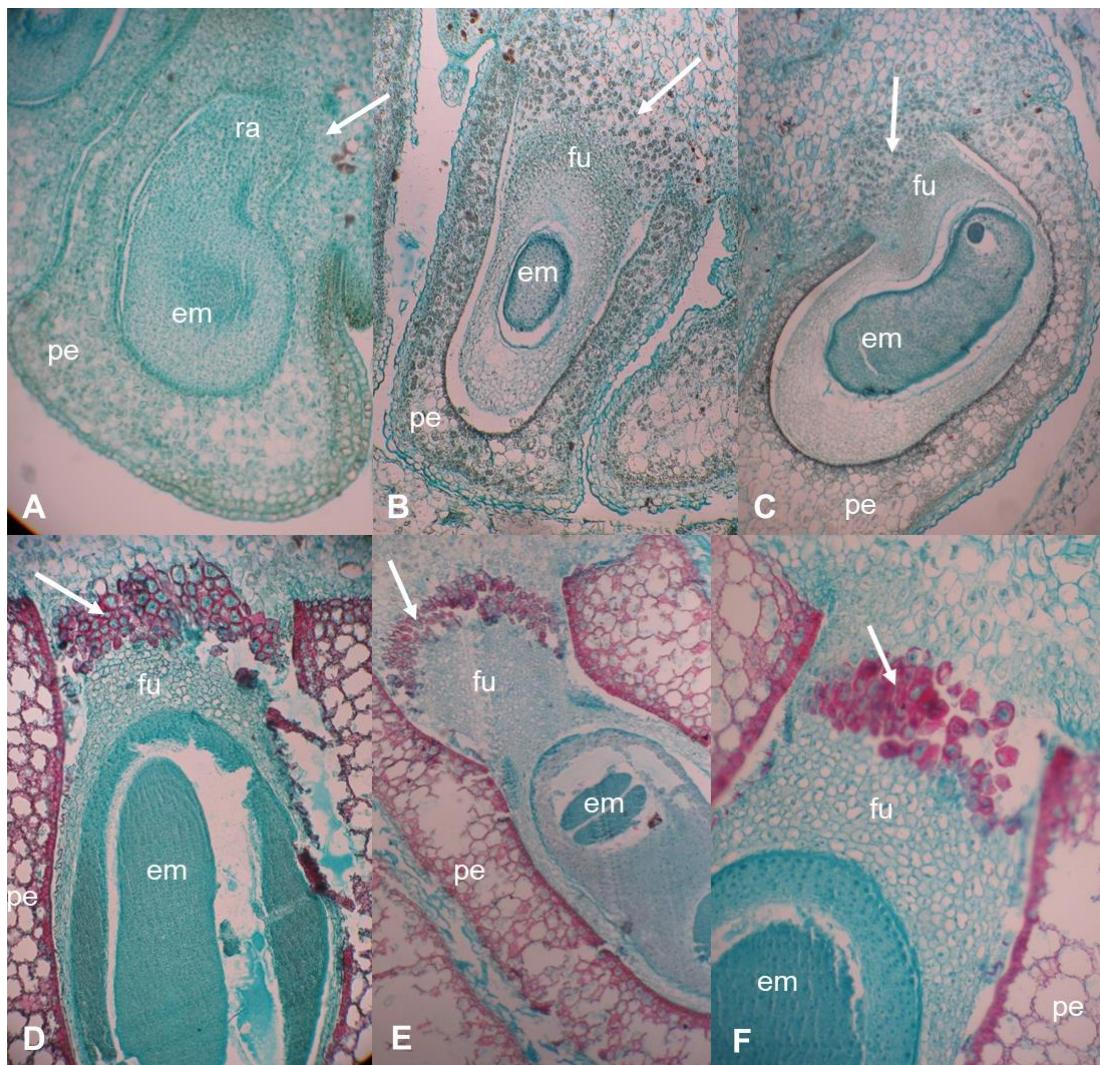


Fig. 6. Detail of mericarps and developing embryo. The germination plug in formation is indicated with an arrow. A: 2 DAF (100x). B: 9 DAF (40x). C: 12 DAF (40x). D: 17 DAF (40x). E: 25 DAF (40x). F: 35 DAF (100x). Abbreviations: em: embryo; fu: funiculus; pe: pericarp.

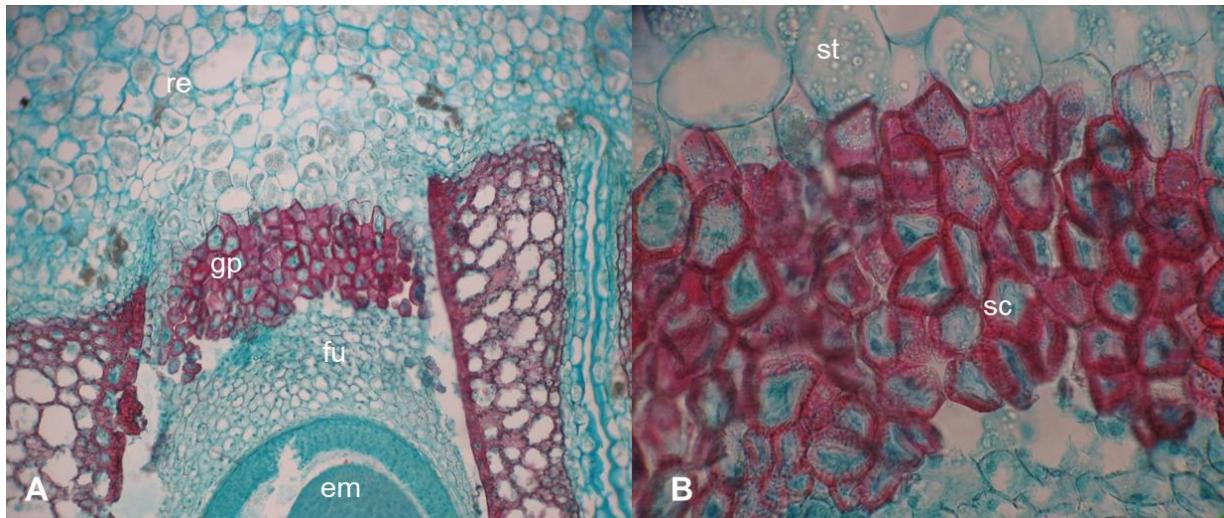
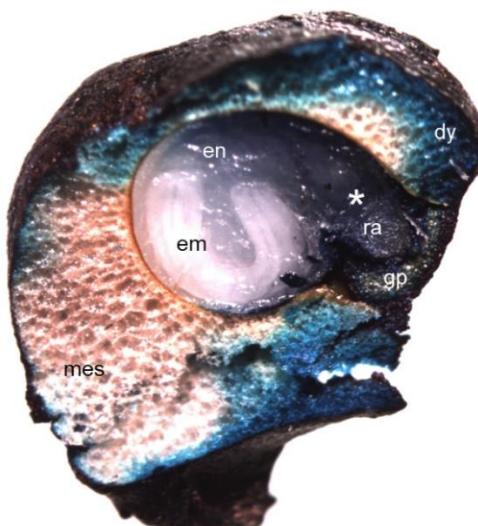


Fig. 7. A. Funicular plug at 17 DAF (100x). B: Detail of sclereids of the funicular plug, 17 DAF (400x). Abbreviations: em: embryo; fu: funiculus; gp: germination plug; re: receptacle; sc: sclereids; st: starch.

Baskin & Baskin (2014) and Gama-Arachchige *et al.* (2013) describing the water gaps or water plugs in different species, identify three types of complexes based on the morphology of openings and the anatomy of occluding structures; type-III corresponds to a narrow-linear or circular water-gap opening that is occluded by a plug-like structure formed by water-impermeable sclerenchyma cells (Baskin & Baskin, 2014), which is similar to the structure described in this study. However, the germination plug in *Nolana* is not impermeable to water, as can be observed in Figure 8, since dye-tracking of intact mericarps of *N. paradoxa* showed the imbibition route and water reaching the embryo within 48 hours. Therefore, *N. paradoxa* does not present physical dormancy, according to the definition of impermeability to water of the seed or fruit coat (Bewley *et al.*, 2013; Baskin & Baskin, 2014).



— mm —

Fig. 8. Stereoscopic image of longitudinal section of mericarp of *N. paradoxa*, imbibed in methylene blue for 48 hrs. Abbreviations: dy: dye; em: embryo; en, endosperm; gp: germination plug; mes: mesocarp; ra, radicle; \* indicates regions which have imbibed.

## Conclusions

The observation of the development of complete fruits (schizocarps) of *N. paradoxa*, allowed the determination of three histologically different tissues: the monolayer of the endocarp, an internal and an external tissue of the mesocarp, and an exocarp monolayer that appears crushed at maturity. The tissues of the mesocarp develop differently, and one of them, the external, is reduced in size as the mericarp matures. Therefore, at least for this species, it is difficult to observe the external mesocarp when making cuts in fully mature, already dispersed mericarps.

Within each mericarp, it was also possible to observe the development of a germination plug that is constituted by a portion of the funiculus and which closes the opening of the embryonic chamber; although it is sclerified, it is not impermeable to water, therefore it does not constitute physical dormancy.

## Acknowledgments

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## VII. General conclusions

- It was found that *Nolana* species do not have physical dormancy, as previously reported, but physiological dormancy, with different levels of depth between the twelve species studied. In this context, the importance of the endosperm as a germination regulator in the studied species could be determined.
- It was possible to germinate, and in fact seedlings were obtained for most of the twelve species studied; the only one that did not show germination was *N. intonsa*, for which a very deep dormancy was defined. The best treatment for all species included the removal of the germination plug (scarification) and partial removal of the endosperm or alternatively, removal of the germination plug and addition of gibberellic acid.
- It was possible to find a dormancy pattern for the species studied; the level of dormancy was related to phylogeny, that is, to the clade to which the species belong. Neither latitudinal distribution nor niche diversity was related to the level of dormancy; it is therefore likely that the dormancy trait has a greater genetic influence than the environment under which the seed develops (maternal environment).
- Under a climate change scenario in the future, the distribution pattern of the genus *Nolana* will contract towards the coast, but some species will keep their distribution area stable, and some will even expand. However, some species whose distribution will contract, have high values according to the dormancy index (i.e. lower percentages of germination). Special efforts should be directed to include these species in an integrated conservation programme, including both *in situ* and *ex situ* initiatives.
- As for the anatomy, according to the histological sections of mericarps of all the species studied, three different tissues were identified: an external one (exocarp), corresponding to a mono-stratified epidermis; a middle one (mesocarp), of variable thickness, occupying the bulk of the pericarp and the one that presents more variation between species; and an inner one (endocarp), constituted by one layer of very dense sclereids. It was determined that the thick layer composed of dense sclereids, which had previously been described as endocarp, corresponds to the inner layer of the mesocarp. This hard layer provides protection to the embryo, as it would allow the mericarps to remain relatively intact in the soil seed bank for many years.
- It was not possible to determine a clear relation between the anatomy and morphology of mericarps and the position of the species within clades of the genus. However, trends were observed, and the new information generated could be of use for the identification of species. In fact, it was possible to describe a new species for the genus, *Nolana patachensis* from the fog oasis of Alto Patache, in Tarapacá, based mainly on the morphological differences of mericarps between

otherwise very similar species. This means that by the end of this thesis, the genus *Nolana* is composed of 90 species.

- For a selected species, *N. paradoxa*, it was possible to observe the development of the germination plug, constituted by a portion of the funiculus. As the mericarp matures, this portion begins to lignify and sclereids are formed. The germination plug protects the opening of the mericarp after dispersal of the mericarps; then, when germination begins, the pressing radicle will break the seed cover and push the funicular plug out.