

COMPARATIVE VEGETATIVE ANATOMY OF *NEOSPARTON DARWINII* AND *N. EPHEDROIDES* (VERBENACEAE)

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Summary: *Neosparton darwinii* is endemic to the coastal dunes of Pehuen-có and one of the four species of the genus endemic to Argentina. It is considered an "endangered species" because of the extreme restriction of its area of occurrence. *Neosparton darwinii* is a woody shrub with globose spikes of yellowish-cream colored flowers. Its seeds are heavily parasitized by a bruchid, so its reproduction is basically vegetative. Primary roots are triarch to heptarch. The cylindrical, ridged, stem is covered by a thick cuticle; cyclocitic stomata and hairs occur in the furrows, fibers in the ridges, and scattered sclereids in the chlorenchymatous cortex. Collateral vascular bundles surround a parenchymatic pith. The ephemeral leaves are decussately arranged and nodes are unilacunar with two traces. The primary vascular system is of the closed type and consists of four sympodia, each mirroring the one next to it. *Neosparton ephedroides* occurs in disjunct areas and is very similar to *N. darwinii*. The presence of cortical sclereids in the stem of the latter suggests that they are only one species since this character-absence vs. presence of sclereids-seemed the most evident to separate them. Thus, we conclude that the population of Pehuen-có is another population of *N. ephedroides* and, on the basis of priority, *N. darwinii* is a synonym of the latter.

Key words: *Neosparton darwinii*, *Neosparton ephedroides*, vegetative anatomy, root anatomy, stem anatomy, leaf anatomy, synonymy.

Resumen: Anatomía vegetativa comparada de *Neosparton darwinii* y *N. ephedroides* (Verbenaceae). *Neosparton darwinii* es endémica de las dunas costeras de Pehuen-có y una de las cuatro especies del género, endémico de Argentina. Está considerada como una "especie amenazada" por la extrema restricción de su hábitat. Es un arbusto leñoso con espigas breves de flores color crema amarillento. Las semillas son intensamente parasitadas por un brúquido y la reproducción es básicamente vegetativa. Las raíces primarias son triarcas a heptarcas. El tallo cilíndrico, estriado, está cubierto por una cutícula gruesa; en los surcos hay estomas ciclocíticos y pelos, fibras en las costillas y esclereidas en la corteza. Hacedillos colaterales rodean una médula parenquimática. Las hojas son efímeras, decusadas, y los nudos unilacunares con dos trazas. El sistema vascular primario es del tipo cerrado y consiste en cuatro simpodios; cada uno es una imagen especular del contiguo. *Neosparton ephedroides* habita en áreas disjuntas y es muy parecido a *N. darwinii*. La presencia de esclereidas corticales en esta última especie sugiere que son sólo una especie ya que este carácter-ausencia vs. presencia de esclereidas-parecía ser el más evidente para separarlas. Concluimos que la de Pehuén-có es una más de las poblaciones de *N. ephedroides* y, en base a las prioridades, *N. darwinii* es un sinónimo de aquella especie.

Palabras clave: *Neosparton darwinii*, *Neosparton ephedroides*, anatomía vegetativa, anatomía radical, anatomía caulinar, anatomía foliar, sinonimia.

INTRODUCTION

The genus *Neosparton* Griseb. is endemic to Argentina and consists of four species: *N. ephedroides* Griseb., *N. darwinii* Benth. & Hook. f., *N. aphyllum* (Gillies & Hook.) Kuntze and *N. patagonicum* Tronc. (Troncoso, 1974). *Neosparton darwinii* is endemic to the coastal dunes of Pehuen-có. It occurs in a very restricted area—a 2,300 ha band parallel to the shore, 25 km

long by 1.5 km wide (Zalba & Villamil, 1989; Zalba & Nebbia, 1999)—extending East and West of Pehuen-có, a small village on the South of the Province of Buenos Aires, Argentina (Fig. 1). It has been considered a "vulnerable species" (Villamil *et al.*, 1996) or an "endangered species" (Zalba & Nebbia, 1999) because of the extreme restriction of its area of occurrence and the tourist expansion of the locality; thus, a thorough biological study is necessary to provide a better understanding of its morphology, reproductive biology, etc. to try to prevent its extinction due to habitat modifications as the urban and tourist areas expand.

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Being endemic to such a restricted zone, very few authors have studied this species. Bentham & Hooker (1876) described the first specimen collected by Charles Darwin on October 2, 1832 during the voyage of the Beagle as a new species: *N. darwinii*. Later on, Troncoso (1957, 1974) considered it in taxonomic works where exomorphology in general and anatomy of the stem are described. More recently, Zalba (1989) characterized the dispersion of the species, considered its way of propagation, phenology, and proposed a possible conservation strategy. This study has led the Municipality of Coronel Rosales to declare *N. darwinii* a protected species. Michetti *et al.* (1994) and Zalba & Nebbia (1996, 1999) concluded that the reduced distribution and the lack of its range expansion might be related to reproductive restrictions rather than to limitations on availability of adequate or favourable habitats. In addition, fruits and seeds are heavily parasitized preventing seed germination (Nebbia & Zalba, 2001), and seriously compromising sexual reproduction. This is another reason to consider its protection against anthropogenic encroachment.

Neosparton darwinii has been described as the only species in the genus lacking sclereids (Troncoso, 1957), the most consistent feature to separate it from *N. ephedroides*. Sclereids do occur, however, in the stem cortex of *N. darwinii*, which makes it even more similar to *N. ephedroides*. Thus, a comparative study of the vegetative structures of both species has been undertaken in an attempt to find out whether they actually should be maintained as two different taxa and to describe the anatomy of a species considered "vulnerable" or "endangered".

MATERIALS AND METHODS

Material of *Neosparton darwinii* and *N. ephedroides* was collected by several collectors including the authors. The vast majority of the observations were done on fixed material collected at Pehuén-có, Province of Buenos Aires, Argentina (*N. darwinii*) and by Route 20 near 25 de Mayo, Province of La Pampa (*N. ephedroides*) (Fig. 1).

Despite fungicide treatments germination essays for *Neosparton darwinii* were unsuccessful; only one seed germinated and as soon as a very small portion of the radicle emerged it was immediately

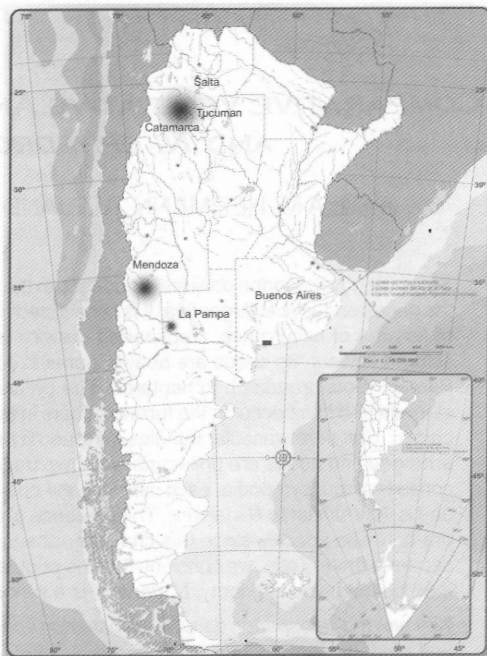


Fig. 1. Population distribution of *Neosparton darwinii* (rectangle) and *N. ephedroides* (circles).

covered by fungi and growth stopped. No other treatments were tried since the aim was to obtain primary roots for anatomical study; we used adventitious roots to describe root morphology.

Herbarium specimens examined: *Neosparton darwinii*. Type: Argentina, Prov. Buenos Aires. Monte Hermoso, outside of Bahía Blanca, coast of Patagonia, Darwin 528, 2-X-1832 (K). Prov. Buenos Aires, Pdo. Coronel Dorrego, 1 al 5-XI-1924, Doello Jurado (BA 24-1711). Pdo. Coronel Rosales, Dos leguas al Norte de Monte Hermoso, 1894, Ameghino (LPS 21934); Pehuén-Có, 19-XI-1962, Cabrera y Fabris 14910 (SI); 31-X-1985, Villamil y Cazzaniga 3444 (BBB); 11-XI-1984, Villamil 9621 (BBB).

Neosparton ephedroides. Prov. La Pampa. Dep. 25 de Mayo, Ruta a Colonia Chica, a 1300 m del Vivero Provincial, 20-X-1989, Villamil 6578 (BBB); Entre Colonia Chica y 25 de Mayo, 26-XI-1980, Steibel y Troiani 6466 (BBB). Prov. Mendoza. Dep. Malargüe, Bardas Blancas, 14-XI-1969, Ancibor, Cano, Crespo (SI 90129); entre Agua Botada y Arroyo Chequenco, I-1942, Ruiz Leal 7700 (BA 46381). Prov. Salta, Dep. Cafayate, Médanos de Cafayate, I-1943, Castellanos (BA 46953); X-1948, Cárdenas 4222 (LIL 532043); cauce seco del Río Santa María, 22-X-1948,

Burkart 17634 (SI). *Dep. Guachipas*, Quebrada de Guachipas, XII-1896, Spegazzini (LPS 21979). *Prov. Catamarca. Dep. Andalgalá*, Campos del Arenal, 10-III-1916, Joergensen 1731 (BA 23968); *Dep. Antofagasta de la Sierra*, Antofagasta de la Sierra, 26-II-1981, Cabrera, Botta, Deginani 32564 (SI); 17-II-1974, Ulibarri 641 (SI); 6-IV-1950, Vervoorst 730 (LIL 362694); I-1885, Philippi (SGO 42500); *Dep. Tinogasta*, Agua Negra, 7-II-1930, Castellanos (BA 30/603); Vallecito a Agua Negra, II-1930, Schreiter 6314 (LIL); *Dep. Santa María*, Camino Santa María al Cajón, I-1915, Castellón (LIL 32413); Fuerte Quemado, 19-X-1948, Cozzo (BA 52800); *Dep. Belén*, Río Hualfín, X-1875, Schickendantz 105 (CORD). *Prov. Tucumán. Dep. Tafí*, Río Santa María, 3-II-1935, Castellanos (BA 14807).

Herbarium material was restored following Venning's technique (1953). Both fresh and restored herbarium material were fixed in formalin-acetic acid-alcohol, dehydrated in an ethyl alcohol-tertiary butyl alcohol series and embedded in Paramat. Cross and longitudinal sections were cut at 10 μ m, stained with safranin-fast green and observed under a compound microscope. Leaves were cleared following Dizeo de Strittmatter's technique (1973). Observations were recorded through drawings and photomicrographs.

RESULTS

Neosparton darwinii is a woody shrub 2-2.20 m high that grows on coastal dunes in Pehuen-có contributing to fixing them. Its ridged branches seem aphyllous due to the early falling of the small leaves. Inflorescences are globose spikes of 15-25 yellowish-cream colored flowers of a peculiar, rather unpleasant, odor. They bloom from September to November and fruits mature from November to January. Reproduction through seeds seems almost nonexistent since they are heavily parasitized by a bruchid, *Lithraeus ferreroi* (Belenguer & Ferrero, 1994). Reproduction is thus essentially vegetative, via stolons with adventitious roots at the nodes, which are responsible for dune fixation.

Root

The epidermis of the primary root has rather thick outer tangential cell walls and long root hairs

(Fig. 2B). The hypodermal layer has larger cells with thinner walls (Fig. 2B). The rest of the cortical cells—approximately 5-14 layers—have very thin walls, are isodiametric in cross section and rather elongated in longitudinal section, increasing in size toward the center of the cortex and decreasing toward the endodermis (Fig. 2B). Large intercellular spaces occur among the cortical cells (Fig. 2B). The endodermal cells are small, squarish and present the characteristic casparian thickenings. The vascular cylinder is surrounded by a one-, sometimes two-, layered pericycle; its cells are similar to those of the endodermis except for the absence of casparian thickenings. Primary xylem forms 3-7 arms of protoxylem alternating with 3-7 groups of phloem cells. The center of the vascular cylinder is occupied by an area, variable in size, of sclerified parenchyma cells (Fig. 2C).

Stem

A: Anatomy. A cross section of the stem internode shows it is circular and ridged (Figs. 2D, G), the ridges being 24-37. The epidermis is commonly biseriate (Fig. 2G), occasionally uniseriate at the furrows and three-, seldom four-seriate at the ridges. It is covered by a thick cuticle which thins markedly over the cyclocytic stomata that occur in the furrows (Fig. 2E). This area is protected from water loss through the occurrence of different sorts of hairs in and very close to the furrows (Figs. 2D, G). There are long, unicellular, cylindrical, hairs, depleted of cytoplasm at maturity but with cuticular teeth; 4-cell-headed hairs; and, less frequently, palisade-cell-headed hairs. At the ridges, directly beneath the epidermis, there is a group of fibers arranged in a triangle, the base of which is in contact with the epidermis (Figs. 2D, G). It is flanked by elongated chlorenchyma cells resembling the palisade parenchyma cells of leaves (Figs. 2D, G). This tissue flanking one side of a fiber triangle continues in a band 3-4 cell layers thick beneath the epidermis of the furrows to the flank of the fiber triangle of the contiguous ridge. Thus, each furrow is surrounded by a semicircle (or horseshoe-shaped band) of palisade chlorenchyma (Figs. 2D, G). Closer to the center of the stem, chlorenchyma cells are larger and more isodiametric, and scattered among them there appear sclereids, solitary or in groups of two to seven, most commonly closer to the furrows (Figs. 2D, G). They are very thick

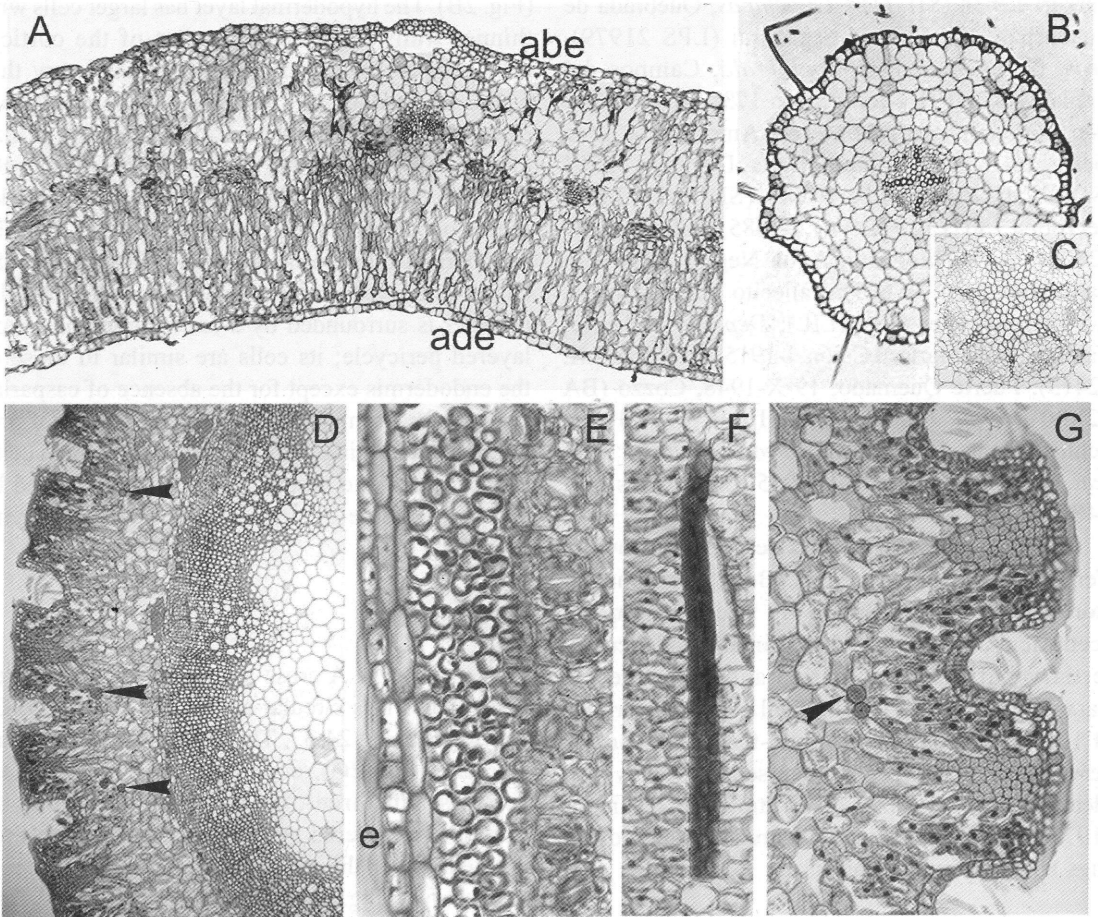


Fig. 2. A - G: *Neospartan darwinii*: leaf, root and stem. **A:** leaf, central portion. x 45. **B:** tetrach primary root. x 80. **C:** vascular cylinder of heptarch primary root. x 58. **D:** part of a stem showing four ridges and corresponding furrows. Notice sclereids at arrowheads. x 54. **E:** part of a furrow with cyclocytic stomata in surface view. x 270. **F:** sclereid in stem cortex. x 135. **G:** detail of ridges, furrows, and sclereids (arrowhead). x 157. **A - D, G:** cross sections. **E, F:** longitudinal sections. References: abe, abaxial epidermis; ade, adaxial epidermis; e: epidermis.

walled, roundish in cross section (Figs. 2D, G), and elongated longitudinally (Fig. 2F). Continuing toward the center of the stem there are scattered packages of fibers that correspond to the fiber cap of the collateral primary vascular bundles (Fig. 2D) and beneath these, primary phloem is squashed between the fiber caps and secondary phloem. Between contiguous fiber caps, cortical cells are smaller and abut directly into secondary phloem (Fig. 2D). The two cortical layers closest to the secondary phloem have small crystals. Continuing on to the center of the stem, cambium leads on to secondary xylem and this to remnants of primary xylem (part of primary vascular bundles). The center of the stem is occupied by a thin-walled parenchymatic pith (Fig. 2D).

B: Primary vascular system. This description is based on the three nodes that occur between the apical meristem and the beginning of secondary growth. Leaves are decussately arranged and nodes are unilacunar with two traces. The primary vascular system, illustrated in Fig. 3, is of the closed type and consists of four sympodia. Close to each node the axial bundle of each sympodium branches producing one lateral leaf trace to one side and half median leaf trace plus a small bud trace to the other. This pattern is repeated alternately along the stem. Each sympodium is the mirror image of the one next to it. The lateral traces traverse almost a complete internode before entering the corresponding leaf. The halves of the median trace fuse with each other at the level of the next node and continue upwards

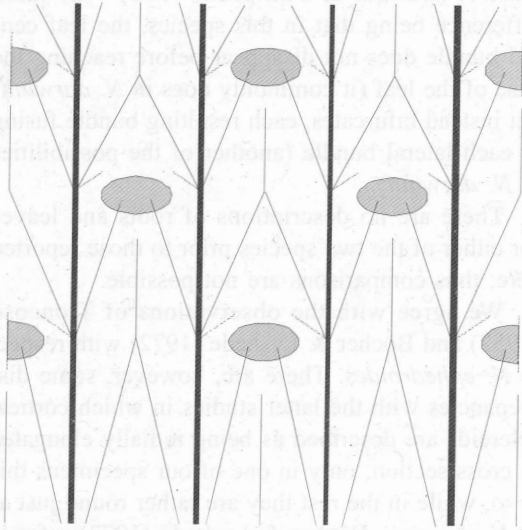


Fig. 3. Primary vascular system along the three nodes between the apical meristem and the beginning of secondary growth. References: leaf; main bundle of sympodium; leaf trace; bud trace.

(fused) for most part of another internode, thus traversing almost two internodes; it never reaches the base of the leaf, however, commonly fading out close to it, sometimes fusing to either one of the lateral traces, or bifurcating, with each resulting branch fusing to a lateral trace. Both lateral traces and continue a parallel course upward or they may fuse somewhere between the middle and the distal part of the leaf.

Leaves

The ephemeral leaves are decussate, small, simple, sessile, somewhat fleshy, slightly connate at the base, oblong-lanceolate to ovate-spathulate with smooth margins, glabrous on the abaxial side and hairy on the adaxial one. Variable in size, the smaller ones are 4 mm long by 2 mm wide whereas the larger ones are 10 mm long by 4 mm wide; occasionally they reach a length of 15 mm and a width of 7 mm. Both epidermis consist of cells that are squarish to rectangular both in surface view and in cross section (Fig. 2A); the outer walls are relatively thick. A thin cuticle covers the epidermis. Stomata occur on both adaxial and abaxial sides of the leaves (Fig. 2A) and are essentially anomocytic, showing in some cases a slight tendency to cyclocytic. Quite abundant simple unicellular and

glandular multicellular hairs occur on the adaxial surface of young leaves. A cross section of the leaf shows a small portion of collenchyma in the margins; this same tissue occurs hypodermally in the central zone—approximately the length occupied by four to seven central nerves (Fig. 2A) of the 27-36 observed in cross section. This tissue forms two to three layers under the abaxial epidermis and one to two under the adaxial one. Vascular bundles are collateral and occur in a row from margin to margin of the leaf, somewhat closer to the abaxial epidermis. Toward the adaxial surface, the mesophyll consists of rectangular, rather long, chlorenchyma cells vaguely reminiscent of palisade parenchyma (Fig. 2A); lack of stratification makes the tissue lose the characteristic appearance of palisade parenchyma. Quite compact toward the margins and adaxial surface, large intercellular spaces and more irregular cells occur toward the center giving it the aspect of a spongy parenchyma. Two to three central bundles are surrounded by a collenchymatous parenchyma that reaches the subepidermal collenchyma of the abaxial side of the leaf, whereas toward the adaxial side it consists of three to four cell layers which narrow towards the leaf surface until they contact the chlorenchyma or the subepidermal collenchyma. Scattered throughout the mesophyll some degrading cells (they stain rather reddish) may be found (Fig. 2A); similarly, numerous starch grains occur essentially toward the adaxial surface.

Neosparton ephedroides is also endemic to Argentina and occurs in three widely separated, disjunct, areas (Fig. 1): 1) NE of Catamarca, S of Salta and NW of Tucumán (Troncoso, 1957); 2) S of Mendoza (Troncoso, 1957); 3) W of La Pampa (Covas, 1965). It is a woody shrub 2-3 m high with similar characteristics to those described for *N. darwinii*. The globose spikes bear 11-21 flowers also similar in color and odor to that species.

Roots, stems and leaves look like those of *N. darwinii* although a slight variation occurs in the number of ridges of the stem, being 21-33 in *N. ephedroides*; several of the specimens observed lacked cortical sclereids, and the small number of leaves available of *N. ephedroides* show a larger amount of collenchyma on the abaxial side; this, however, may be a quite variable feature.

Although in a lower degree, flowers in this species are also parasitized apparently by Coleoptera

of the family Nitidulidae (Belenguer, pers. comm.); only adults have been seen in the flowers and so far have not been identified.

DISCUSSION AND CONCLUSIONS

Our observations on the stem of *N. darwinii* are in accordance with those of Troncoso (1957) except with respect to the occurrence of cortical sclereids; whereas this author did not detect sclereids—a character that she used to separate this species from *N. ephedroides*—our studies indicate that they almost always occur in the former species (with the exception of young stems with little or no secondary). Moreover, the specimen of *N. darwinii* collected by Doello Jurado on Nov. 1924 (BA 24-1711), cited by Troncoso (1957) as one of the two specimens she observed, possesses cortical sclereids; the other one, collected by Ameghino in 1894, however, does not. Nevertheless, among the material collected by the authors and other collectors in many different opportunities and from different plants, lack of sclereids has never been found.

Beck *et al.* (1982) point out that “closed vascular systems occur in both woody and herbaceous species, but are more common among herbaceous species”. *N. darwinii* is another woody species to have a closed primary vascular system. The same authors have found a strong correlation between closed systems and decussate phylotaxy, which is also the case with *N. darwinii*. Furthermore, they indicate that “closed systems are commonly characterized by an even number of axial bundles, often 4..., 6..., or 8...”. Four axial bundles occur in *N. darwinii*.

“Closed systems differ from open systems in being regularly reticulate” where “the axial bundles of sympodia are connected by the fusion of leaf traces..., or the axial bundles themselves anastomose...”. The first part of this statement by Beck *et al.* (1982) is in accordance with the characteristics of the primary vascular system of *N. darwinii* where axial bundles are connected by the fusion of leaf traces, those that make up what would be the median leaf trace.

In comparing *N. darwinii*'s primary vascular system with those of other species studied, we have found that it is very similar to that of a Lamiaceae—*Solenostemon scutellarioides* (L.) Codd (= *Coleus*

blumei) (Balfour & Philipson, 1962)—the main difference being that in this species, the leaf central bundle does not disappear before reaching the base of the leaf (it commonly does in *N. darwinii*) but instead bifurcates, each resulting bundle fusing to each lateral bundle (another of the possibilities in *N. darwinii*).

There are no descriptions of roots and leaves for either of the two species prior to those reported here, thus comparisons are not possible.

We agree with the observations of Troncoso (1957) and Böcher & Lyshede (1972) with respect to *N. ephedroides*. There are, however, some discrepancies with the latter studies in which cortical sclereids are described as being radially elongated in cross section; only in one of our specimens this is so, while in the rest they are rather round just as in *N. darwinii*. Böcher & Lyshede (1972) refer to two types of hairs in this species: the “cutinized covering hairs” and the glandular hairs (head two-celled with large nuclei); we have found, however, the same three types as in *N. darwinii*, including the glandular one with palisade-celled head that they describe only for *N. aphyllum*.

The presence of cortical sclereids in the stem of *N. darwinii* magnifies its similarity to *N. ephedroides*, since this character—absence vs. presence of sclereids—seemed the most evident to separate both species (Troncoso, 1957). The other characters used by Troncoso (1957)—height, number of ridges in the stem, bract/calyx length, inserted vs. exerted stamens—do not really contribute to a separation of the two species. There is much overlap in height (2-2.20 m in *N. darwinii*; 2-3 m in *N. ephedroides*), in the number of ridges in the stem (24-37 in *N. darwinii*; 21-33 in *N. ephedroides*) and in bract/calyx length (\geq in *N. darwinii*; \leq in *N. ephedroides*), and we have found didynamous stamens—two exerted and two inserted ones—in both species. Thus, the five differences pointed out by Troncoso (1957) to separate the two species are insufficient traits for identification.

Most of the specimens of *N. ephedroides* lacking cortical sclereids belong to a rather restricted zone around the borders between the Provinces of Catamarca, Salta and Tucumán, in the vicinity of the river Santa María. This could be driven by physical conditions—climatic, edaphic, etc.—but it also demonstrates that whereas in *N. ephedroides* cortical sclereids may be absent, this has rarely been observed so far in *N. darwinii*.

It is not uncommon to find species with a disjunct distribution such as occurs with *N. ephedroides*; however, as difficulties arise to detect clear differential characteristics between this species and *N. darwinii*, the tendency exists to confirm their identities on the basis of their place of origin. The distribution of *N. ephedroides* in three distant areas as stated above, makes possible some speculation: if the northernmost populations—those of Catamarca, Salta and Tucumán—occur little over 1000 km apart from those in the south of Mendoza, and these are 300 km away from those of 25 de Mayo (La Pampa), the distance between that locality and Pehuen-có—550 km—does not seem an important isolation factor that will lead to contemplate the population at Pehuen-có as a different species. Böcher & Lyshede (1972) consider that the absence of *N. ephedroides* from the provinces of La Rioja, San Juan and north of Mendoza could be attributed to climatic factors since this region constitutes the driest part of the most elevated areas of the Argentine western region. No observations have been made of the conditions occurring between the south of Mendoza and 25 de Mayo or between the latter and Pehuen-có, but here too, edaphic reasons or some others could be responsible for the distribution of the species in disjunct areas.

Synonymy

Troncoso (1957) indicates that *N. darwinii* is a "Especie indudablemente muy vecina de *N. ephedroides*. Aún requiere ser más estudiada a base de mejores ejemplares que los actualmente disponibles...". Moreover, one of the labels on the type specimen observed at the Royal Botanic Gardens (Kew) Herbarium reads: "*Neosparton ephedroides* Griseb. [TYPE of *N. Darwinii* Benth:]. Identified by H. N. MOLDENKE and cited by him in his Monograph of the Genus. November, 1938." The mentioned monograph, however, has apparently never been published since the same author cites *N. darwinii* as a valid species in a later work (Moldenke, 1941), and so did Troncoso (1957) and Múlgura (1999). Based on the above discussion, it is concluded that we are dealing with only one entity at the species level and the binomial *N. darwinii* should become a synonym of *N. ephedroides* for priority reasons. Therefore, the population at Pehuen-có is *Neosparton ephedroides* Griseb.

Even if this is one more population of *N. ephedroides*, it would still be important to prevent

its extinction since each population adds genetic diversity to the species gene pool (Ehrlich & Daily, 1993; Myers, 1994). Another important consideration is that it is the only one that occurs in a coastal habitat and that this environment could be a factor of selection towards certain phenotypes that may eventually become a new species; this could then certainly be called *N. darwinii*.

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BIBLIOGRAPHY

- BALFOUR, E. E. & W. R. PHILIPSON. 1962. The development of the primary vascular system of certain dicotyledons. *Phytomorphology* 12: 110-143.
- BECK, C. H. B., R. SCHMID & G. W. ROTHWELL. 1982. Stelar morphology and the primary vascular system of seed plants. *Bot. Rev.* 48: 691-815.
- BELENGUER, C. & A. FERRERO. 1994. Notas sobre la biología de *Lithraeus ferreroi* (Bruchidae), parásito de una verbenácea endémica del sudeste bonaerense. VI Congreso Latinoamericano de Botánica y XXIV Jornadas Argentinas de Botánica. Mar del Plata, Argentina. Abstract: 787.
- BENTHAM, G. & J. D. HOOKER. 1876. Verbenaceae. In: LOVELL REEVE/WILLIAMS (eds.), *Genera Plantarum*. U. K.: Northgate, London, 2 (2):1144.
- BÖCHER, T. M. & O. B. LYSHEDE. 1972. Anatomical studies in xerophytic apophyllous plants. Additional species from South American shrub steppes. *Biol. Skr.* 18: 1-137.
- COVAS, G. 1965. Especies interesantes de la Flora Pampeana. *Apuntes para la Flora de La Pampa* 14: 53-56.
- DIZEO DE STRITTMATTER, C. G. 1973. Nueva técnica de diafanización. *Bol. Soc. Argent. Bot.* 15: 126-129.
- EHRlich, P. R. & G. C. DAILY. 1993. Population extinction and saving biodiversity. *Ambio* 22: 64-68.
- MICHETTI, K., A. J. NEBBIA & S. ZALBA. 1994. Caracterización del habitat de *Neosparton darwinii*, especie vulnerable endémica del sudoeste de la provincia de Buenos Aires. I. Vegetación asociada. VI Congreso Latinoamericano de Botánica. Mar del Plata, Argentina. Abstract: 796.

- MOLDENKE, H. N. 1941. Contributions to the Flora of Extratropical South America. III. *Lilloa* 6: 419-440.
- MÚLGURA, M. E. 1999. Verbenaceae. In: ZULOAGA, F. & O. MORRONE (eds.), *Catálogo de las plantas vasculares de la República Argentina II. Monogr. Syst. Bot. Missouri Bot. Gard.* 74: 1136-1170.
- MYERS, N. 1994. Global Biodiversity II: Losses. In: MEFFE, G. K. & C. RONALD CARROLL (eds.), *Principles of Conservation Biology*, pp. 110-140. Sinauer Associates, Inc., Sunderland, Massachusetts.
- NEBBIA, A. J. & S. M. ZALBA. 2001. Fallas en la germinación de las semillas de una especie amenazada. XXVIII Jornadas Argentinas de Botánica. Santa Rosa, Argentina. *Bol. Soc. Argent. Bot.* 36 (Suplemento): 41-42.
- TRONCOSO, N. S. 1957. El género *Neosparton* (Verbenaceae). *Darwiniana* 11: 163-192.
- TRONCOSO, N. S. 1974. Los géneros de Verbenáceas de Sudamérica Extratropical. *Darwiniana* 18: 295-412.
- VENNING, F. D. 1953. *Manual of advanced plant microtechnique*. Dubuque, Wm. C. Brown Co. Publishers.
- VILLAMIL, C. B., G. DELUCCHI & M. A. LONG: 1996. Cincuenta especies prioritarias para su conservación en la provincia de Buenos Aires. XXV Jornadas Argentinas de Botánica. Mendoza, Argentina. Abstract: 517.
- ZALBA, S. M. 1989. Biología de *Neosparton darwinii* Benth. & Hook. Informe Final, Beca de Estímulo al Estudio, Dep. de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. Bahía Blanca, Argentina.
- ZALBA, S. M. & A. J. NEBBIA. 1996. ¿Por qué *Neosparton darwinii* sólo crece en una playa del sudoeste de Buenos Aires? XXV Jornadas Argentinas de Botánica. Mendoza, Argentina. Abstract: 435.
- ZALBA, S. M. & A. J. NEBBIA. 1999. *Neosparton darwinii* (Verbenaceae), a restricted endemic species. Is it also endangered? *Biodivers. conserv.* 8: 1585-1593.
- ZALBA, S. M. & C. B. VILLAMIL. 1989. *Neosparton darwinii*, especie endémica del partido de Coronel Rosales (Prov. de Buenos Aires). Evaluación de su status como especie amenazada. XXII Jornadas Argentinas de Botánica. Córdoba, Argentina. Abstract: 168.

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