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Phylogenetic Relationships among Chilean *Sophora* Species

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Abstract—Phylogenetic affinities among Chilean *Sophora* species are not clear. We suggest a new hypothesis for the origin of the section *Edwardsia* on the basis of parsimony analysis, which allows a South American origin to be established for the species of this section. The seed alkaloid composition did not provide useful information for the filiation of *Edwardsia* species, and the shortest tree was obtained using morphological characters only. Two branches are clearly distinguishable by the pubescence of the leaflets and the flag/wings length ratio: one of them includes *S. chrysophylla*, *S. tetraptera*, *S. toromiro*, *S. howinsula* and *S. denudata*; the other one includes *S. macnabiana*, *S. microphylla*, *S. masafuerana*, *S. prostrata* and *S. fernandeziana*. In contrast, *S. macrocarpa*, an ancient element of the South American flora, is closely related to species belonging to the section *Sophora* represented in the region by *S. tomentosa*, *S. linearifolia* and *S. rhynchocarpa*. Sections *Calia* and *Styphnolobium* are clearly related to each other, both morphologically and chemically. Copyright © 1996 Elsevier Science Ltd

Introduction

The genus *Sophora* comprises about 45–50 species, 10 of which are included in the section *Edwardsia*. A segmentation of *Sophora* was suggested originally by Yakovlev (1967), who revived the genera *Calia* and *Styphnolobium* primarily on the basis of morphological arguments. More than a decade later Tsoong and Ma (1981) merged *Calia* into *Styphnolobium*. Sousa and Rudd (1993), in their revision of *Styphnolobium*, have acknowledged that this taxon is close to *Calia* although its chromosome number $2n=28$ contrasts with $2n=18$ shared by *Sophora* and *Calia* (cf. Palomino *et al.*, 1993).

Bailey (1974) found support for this segregation based on the polysaccharide composition of the seeds, which appear to be a reliable material for chemotaxonomic studies, as their composition is presumably quite stable over time and does not depend on the vegetative state of the plant. Bailey recognized *Calia*, *Sophora* (most of them representing the section *Edwardsia*) and *Styphnolobium* as separate genera. The former two genera contain no galactomannans, which again distinguish them from *Styphnolobium* (*S. affinis* Torrey *et* A. Gray). *Calia* (*S. secundiflora* Lag. *ex* DC) seeds contain starch or amyloid and polymers of galactose and arabinose, and *Sophora tomentosa* seeds (section *Sophora*) contain galactose–arabinose polymers. Recently, Professor A. S. Cerezo (personal communication) confirmed that the Chilean *Edwardsias S. macnabiana* and *S. macrocarpa* also contain arabinogalactans as the major seed polysaccharides.

Murray (1986) studied the electrophoretic patterns of seed proteins from *S. microphylla* and the Chilean *S. macrocarpa*, finding them undistinguishable. Nevertheless, this author had noted earlier that the protein pattern of *Pisum* was very similar to that of *S. macrocarpa* (Murray and Porter, 1980), thus showing, in our opinion, that seed protein electrophoretic patterns are of little use to distinguish anything much below the family level. Therefore, although Murray (1986)

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used these data to derive both Chilean species, *S. macnabiana* and *S. macrocarpa*, from taxa growing in New Zealand, it seems more reasonable to refrain from drawing any conclusion other than that seed protein electrophoresis shows strong similarities between rather distantly related Papilionaceous genera. In line with these conclusions, Markham and Godley (1972) had found no support for the segregation of *S. macnabiana* from Chile and *S. microphylla* from New Zealand considering the similarity of their leaf flavonoid composition.

On the other hand, pioneering chemotaxonomic studies carried out by Briggs and Ricketts (1937) and Briggs and Mangan (1948) recognized *S. microphylla* var. *fulvida, S. chathamica* and material from Anawhata, New Zealand as separate species on the basis of their seed alkaloid composition. Urzúa and Cassels (1970), using the same criterion and methodology, found the Chilean *S. tetraptera sensu* Reiche (=*S. macnabiana non S. microphylla*) to differ from the previously studied material from New Zealand. These conclusions, however, although they are the direct precedent of the present chemical study, can no longer be considered reliable due to the coarseness of the analysis (by vacuum distillation of the alkaloid mixture) and the fact that they were not based on individual samples.

The present study has attempted to integrate morphological characters and seed alkaloid composition, analyzed by GLC at the individual level, into a scheme providing a more secure foundation for our hypothesis regarding the South American origin and affinities of the Chilean *Sophora* species (Peña *et al.*, 1993).

Materials and Methods

See Table 1. The following taxa were studied, all of which have specific status according to Allan (1961), Yakovlev (1967), Green (1970) and Isely (1981): *S. chrysophylla* (Salisb.) Seem, (including *S. unifoliata* (Rock) Deg. et Scherff.), *S. denudata* Bory, *S. fernandeziana* (Phil.) Skottsb., *S. howinsula* (Oliv.) Green, *S. macnabiana* (Grah.) Skottsb., *S. macrocarpa* J.E. Sm., *S. masafuerana* (Phil.) Skottsb., *S. microphylla* Ait. (including *S. chathamica*), *S. prostrata* Buchan., *S. tetraptera* J. Mill., *S. toromiro* (Phil.) Skottsb. (all included in sect. *Edwardsia*), *S. tomentosa* L., *S. linearifolia* Griseb., and *S. rhynchocarpa* Griseb. (sect. *Sophora*), *S. secundiflora* Lag. ex DC. (sect. *Calia*) and *S. japonica* (sect. *Styphnolobium*). Voucher specimens were deposited in the Herbarium of the Escuela de Química y Farmacia, Universidad de Chile (SQF). Ripe seeds of all species from the localities indicated in Table 2 were used in extraction and alkaloid determination procedures.

Extraction and analysis. Air-dried seeds (one to five, depending on their size) from each individual plant were coarsely ground and extracted with methanol until a fresh extract gave no Dragendorff reaction. The concentrated residue was dissolved in 1% sulfuric acid, saturated with NaCl, and extracted with

TABLE 1. DATA MATRIX FOR *SOPHORA* SPECIES STUDIED: 9 INDICATES UNDETERMINED OR INAPPLICABLE CHARACTERS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
MACRO	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	2	2	0
CHRYS	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0	0	2	2	1
DENUD	1	0	1	0	0	1	1	1	1	1	0	0	0	1	1	1	0	1	2	1	1
MACNA	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	2	2	0	0
FERN	1	0	1	0	1	1	0	1	0	1	0	0	0	0	1	1	1	0	2	2	1
MASAF	1	0	1	1	1	1	0	1	0	1	0	1	1	0	1	1	9	9	9	9	9
TORO	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	0	1
HOW	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	9	9	9	9	9	9
TETRA	1	1	0	0	0	1	2	1	0	1	1	1	1	1	1	1	0	2	2	0	0
MICRO	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	0	1
PROST	0	0	1	1	1	1	0	1	1	1	0	0	1	1	0	1	0	0	2	2	0
LINEA	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0
RHYNC	0	1	1	0	0	0	0	0	0	0	0	0	1	0	9	1	9	9	9	9	9
TOMEN	0	0	0	0	0	0	0	0	0	1	0	1	0	0	9	1	1	2	1	0	1
SECUN	1	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	2	0
JAPON	1	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Character states 1–21 are given at the end of the paper.

TABLE 2. QUINOLIZIDINE ALKALOID LEVELS IN THE SEEDS OF *SOPHORA* SPECIES

Species	Locality	Voucher (principal component code)	1	2	3	4	5	6	7
S. macrocarpa	Espinalillo 33°00′S 70°55′W	284-b (a2)	+++	+	+++	+	—	0	0
		284 (a)	+	—	++++	+	—	0	0
		296 (a7)	++	+	++++	0	0	0	0
		296-3 (a9)	++	+	+++	—	0	—	—
	Lag. Amargo 36°18′S 71°24′W	318-b1 (b3)	+	—	+++	0	—	—	0
		318-b2 (b4)	+	+	+++	+	0	0	—
	Salto del Laja 37°20′S 72°40′W	326-b* (b8)	++	++	+++	+	—	—	—
		326-b (b9)	++	+	++	+	0	0	0
	Chicauma 33°12′S 70°58′W	459 (e)	+++	+	+++	+	0	0	0
	Cta. Elefantes 35°03′S 72°03′W	BKC-1 (d8)	++	+	+++	+	0	0	—
		BKC-2b (d9)	++	++	+++	0	0	0	0
S. macnabiana	Lag. Amargo 36°21′S 71°20′W	311-b (b)	++	+	+++	—	0	—	—
		321-c (b5)	+++	+	+++	+	—	—	—
		322 (b6)	++	+	++	—	—	—	
		322-1 (b5)	++	+	+++	+	—	—	0
		322-2 (b7)	++	++	+++	+	—	—	—
	Salto del Laja 37°20′S 72°40′W	331 (c1)	++	+	+++	—	—	0	—
		331* (c2)	++	+	+++	—	—	0	0
	Caunahue 40°08′S 72°15′W	341 (c4)	++	++	+++	0	0	0	0
		341-c (c5)	++	+	+++	—	0	0	—
		342 (c6)	++	+	+++	0	0	—	—
		342-3 (c8)	++	+	+++	+	0	—	—
		344-5 (c9)	+	++	+++	0	—	—	—
		344-5 (d)	+	++	+++	0	—	—	0
	Trumao 40°21′S 73°10′W	353-3 (d1)	++	++	+++	0	0	0	0
		355 (d2)	++	+	+++	—	—	—	—
		355-2 (d3)	++	+	++	—	—	—	0
	Pucatrihue 40°32′S 73°41′W	363 (d4)	++	+	++	—	0	—	+
	Frutillar 40°09′S 70°30′W	383-1 (d5)	++	++	+++	—	0	—	—
		389-2 (d7)	++	+	+++	+	0	0	0
	Valdivia 39°49′S 73°14′W	AR-d (e1)	+	+	+++	0	0	0	0
	Los Ruiles 35°50′S 72°30′W	475 (e2)	+	—	+	—	0	—	—
		475* (e3)	++	+	++	0	0	0	0
S. secundiflora	Camargo 26°15′N 98°50′W	Calia-E (e4)	+	++++	+	0	0	—	—
		Calia-E (e5)	+	++++	+	0	—	—	—

TABLE 2—*CONTINUED*

Species	Locality	Voucher (principal component code)	1	2	3	4	5	6	7
S. denudata	Maïdo 21°10′S 55°30′E	Maïdo-c (e7)	++	+++	0	+	0	0	0
		Maïdo-c (e7)	++	+++	0	0	0	0	0
	Nez de Boeuf 21°10′S 55°30′E	JF-b (e9)	+++	+	+++	0	0	0	0
		JF-d (f)	++	+	++	0	—	—	0
S. toromiro ex hortus		PT-b (f2)	++	+	++	+	0	—	0
		MM-a (f4)	+++	+	+++	—	—	+	++
		MM-a (f5)	+++	0	+++	—	—	+	+
		MM-c (f7)	++	0	+++	0	—	+	—
		MM-c (f8)	++	0	+++	0	—	+	—
S. fernandeziana	J. Fernández 33°40′S 79°00′W	SF-1 (g1)	++++	+	++	+	+	—	—
		SF-2 (g2)	+++	+	++	0	0	—	0
S. chrysophylla	Hawaii 20°14′N 160°13′W	DL-6337-a (g3)	+	—	0	0	++	+	—
		DL-6337-c (g4)	+	—	0	0	+++	+	0
S. tomentosa	Pr. do Inglês 9°45′S 35°50′W	Cassels s.n. (g5)	+++	+	+++	+	0	+	+
S. microphylla	New Zealand	Knowles s.n. (g6)	+++	+	++	0	0	—	++
S. prostrata	New Zealand	Knowles s.n. (g7)	+	++	++	0	0	0	0
S. tetraptera	New Zealand	Knowles s.n. (g8)	+	+	+++	+	—	—	—

1 *N*-methylcytisine, 2 cytisine, 3 matrine, 4 sophoranol, 5 anagyrine, 6 rhombifoline, 7 baptifoline.

++++ over 60%, +++ 30–60%, ++ 10–30%, + 1–10%, — less than 1%, 0 not detected.

chloroform. The aqueous phase was made basic with sodium bicarbonate and partitioned into chloroform. The chloroform extract of the alkaline aqueous phase was dried with sodium sulfate and concentrated to dryness.

GLC was performed on a 20 m SE-30 capillary column at a constant temperature of 120°C for 3 min, then with a constant rate of increase (6°C/min) up to 210°C, followed by a plateau lasting 20 min. The N/P selective detector temperature was 250°C. Caffein was injected with each sample as internal standard. Authentic samples of the following alkaloids were available: baptifoline, cytisine, *N*-methylcytisine, matrine and 5-hydroxymatrine (sophoranol). Anagyrine and rhombifoline were identified tentatively by comparing relative retention times with published data.

The characters were polarized by the out-group technique. Character states are given at the end of the paper.

Results

The cladogram

The data matrix shown in Table 1 was analyzed using Swofford's (Swofford, 1985) PAUP 2.4 program package. *Styphnolobium* was chosen as the ancestor and the Lundberg option was applied. This technique minimizes the homoplasies due to the out-group. The shortest tree is 68 steps long, consistency index 0.412, for 21 characters and 16 taxa (Fig. 1).

A heuristic analysis requiring only 52 steps, 16 fewer than in the original tree, was obtained using 16 characters, neglecting 5, 7, 9 and 13. This strategy supports a New Zealand origin for the section *Edwardsia*.

Another heuristic development using only the morphological characters (1–16) further reduced the length of the tree to 46 steps, 22 less than the original for 21 characters (Figs 1 and 2), consistency index 0.435 with a topology showing two clades in the *Edwardsia* group (2,9),(7,8)3) and (4,10)6)11)5).

The percentages of *N*-methylcytisine, cytisine, matrine, sophoranol, anagyrine, rhombifoline and baptifoline in the diferent specimens were subjected to principal component analysis. Baptifoline was not recognized as a principal component in this treatment. The distribution of specimens into the planes F1 × F2 (accounting for 52.11% of the total variation) revealed three groups corresponding to *Calia*, *Edwardsia* and *Sophora* (Fig. 3). No additional groups could be distinguished considering factor 3 (F3 = 10.72% of the variation).

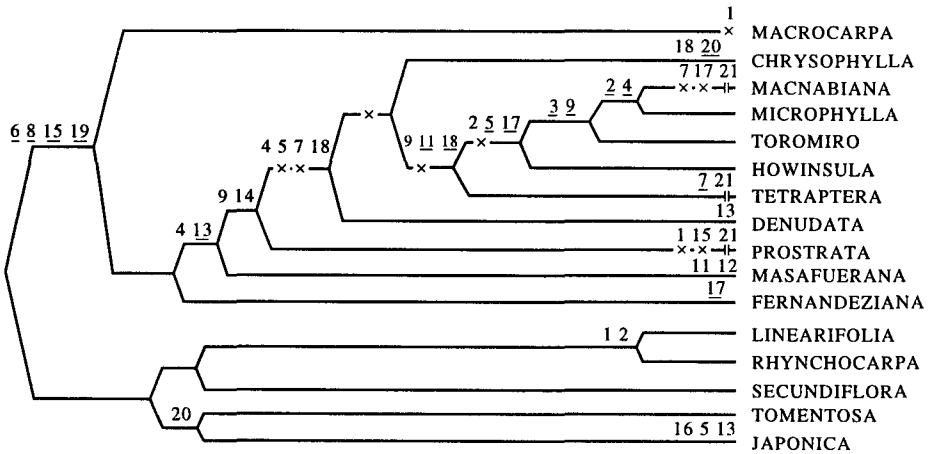

FIG. 1. A WORKING CLADOGRAM FOR *SOPHORA* SECTION *EDWARDSIA*, SHOWING REVERSION AS X, SYNAPOMORPHIES AS UNDERLINED CHARACTERS, PARALLELISMS AS ||. Chemical characters: 17–21.

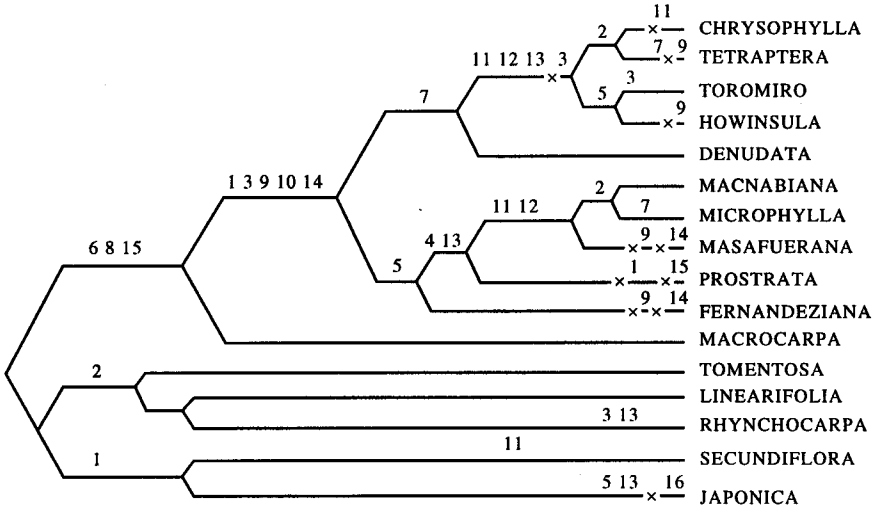

FIG. 2. A REDUCED CLADOGRAM FOR *SOPHORA* SHOWING RELATIONSHIPS GROUNDED ON MORPHOLOGY BETWEEN SECTIONS *EDWARDSIA, SOPHORA* (*S. TOMENTOSA, S. LINEARIFOLIA, S. RHYNCHOCARPA*) AND *STYPHNOLOBIUM SENSU* TSOONG (*SOPHORA SECUNDIFLORA* AND *S. JAPONICA*).

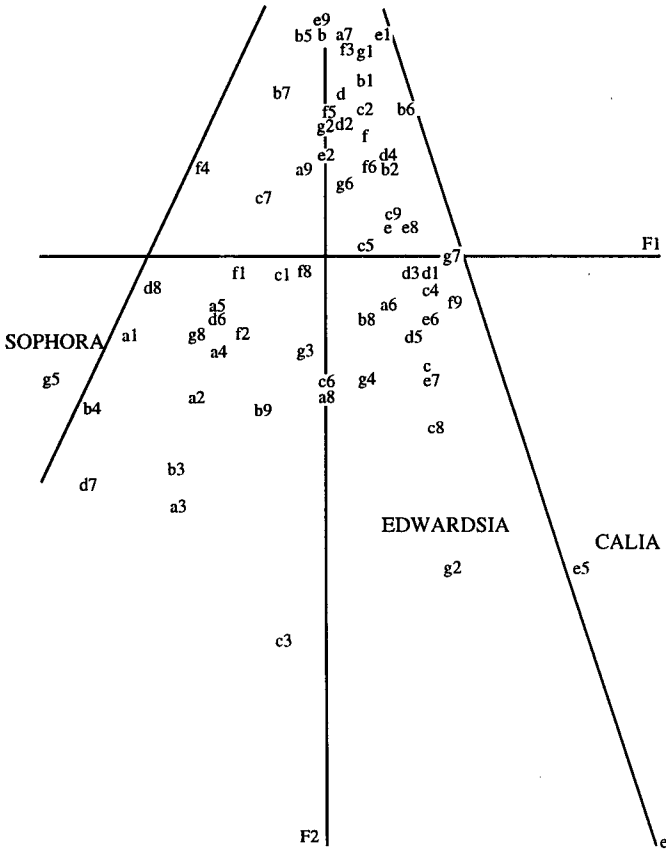

FIG. 3. TWO PLANE F1 × F2 PRINCIPAL COMPONENT ANALYSIS FOR QUINOLIZIDINE ALKALOID CHARACTERS CORRESPONDING TO SEEDS OF SECTIONS *CALIA, EDWARDSIA* AND *SOPHORA*.

Discussion

A cladogram including seed alkaloid composition shows the greatest number of homoplasies. Section *Edwardsia* is represented as a group with the following characteristics: 6, 8, and 15 (extended flag, exerted stamens, and petals without auricules). Character 15 is reversed in *S. prostrata*. Two branches are defined by characters 5 and 7 (pubescence of the leaflets and flag/wings ratio): the *tetraptera* clade, with leaflets hairy below, and the *microphylla* clade with the petals of equal length (with the only exception of *S. microphylla* itself whose petals are unequal). Characters 4 and 13 (short leaflets and fruits less pubescent) join *S. prostrata* to this branch. Characters 11, 12 (color and number of seeds), and 13 join the *tetraptera* group, excluding *S. denudata*, to the *microphylla* group, through *S. toromiro*. Character 3 (length of leaflets) is reversed in the *tetraptera* branch, when *S. denudata*, with longer leaflets, is excluded. Characters 1, 3, 9, 10, and 14 (trees, with little leaflets, winged and constricted fruits, and small seeds) delimit *Edwardsia*, excluding *S. macrocarpa*.

In a biogeographical discussion, one could recognize as the South American group of *Sophora* sect. *Edwardsia*, the continental *S. macrocarpa* and the species from Juan Fernández Islands, *S. fernandeziana* and *S. masafuerana*. Another branch associates *S. macnabiana* and *S. toromiro* to *S. microphylla*. Finally, *S. chrysophylla* and *S. denudata*, with some ancestral characters (orange or reddish seeds, low relative cytisine content) are left out. Characters 11 and 18 (yellow–ocher seeds and a greater content of cytisine), are common to the the New Zealand species, including *S. howinsula*, *S. toromiro* and *S. macnabiana*, which appear to be more advanced, with *S. prostrata* being a sister group for the latter clade.

Although principal component analysis does not show any difference between the *Edwardsias* from the South American continent, *S. macrocarpa* and *S. macnabiana*, morphologically *S. macrocarpa* represents a transition between sections *Sophora* and *Edwardsia*, with strong affinities to Argentinian species belonging to the former section. Considering the flora of the Juan Fernández Islands, *S. fernandeziana* seems closely related to *S. macrocarpa* (2, 4, 6–8, 10–19), but parallel evolution appears to be the simplest explanation for this. Similarly, *S. masafuerana* is closer to the *microphylla* group. The palynological characteristics of the first pair, *S. fernandeziana* and *S. macrocarpa* (exine heterobrocate), contrast with the homobrocate exine of *S. macnabiana, S. masafuerana*, and most of the insular *Edwardsia* species (Peña *et al.*, 1993). Therefore, derivation of the insular species, particularly *S. fernandeziana*, from South American species cannot be dismissed. Similar suggestions have been previously raised for the flora of Juan Fernández (see Hoffmann and Marticorena, 1987). The other species from these islands, *S. masafuerana*, is here considered as derived from New Zealand stocks related to the *microphylla* group. Chemical characteristics could clarify this point.

In 1979, Godley had speculated " ... that *S. microphylla* arose in New Zealand, derived some of its present variation from *S. prostrata*, and that the large-leafleted southern species, *S. chrysophylla* of Hawaii, *S. macrocarpa* of central Chile, and *S. tetraptera–S. howinsula* of New Zealand and Lord Howe Island, each distinct, are older". This view is supported by our own initial analysis including for the first time morphological and chemical characters (Fig. 1). Nevertheless, when the seed alkaloid composition is neglected, a more parsimonious cladogram is obtained supporting the alternative hypothesis, i.e. that only *S. macrocarpa* is ancient and close to *S. tomentosa*, *S. linearifolia*, and *S. rhynchocarpa* belonging to section *Sophora*. *S. macnabiana* and all the insular species, including those from New Zealand, thus appear to be derived from an ancestor common to them and *S. macrocarpa*.

S. microphylla and *S. macnabiana*, in agreement with Godley's observations, are very closely related. *S. prostrata*, a taxon from New Zealand, retains some unspecialized characteristics, particularly auricules on the wing petals and an almost unwinged pod. It is thus possible that it represents a relictual species in the South Island. We refer to our 52 steps long heuristic cladistic treatment, in which this species appears at the bottom of the *Edwardsia* branch of *Sophora*. Cockayne (1912, *fide* Godley, 1979) regarded it as a juvenile form of *S. microphylla*. Quinolizidine alkaloid contents also separate *S. prostrata* from *S. microphylla*. Godley (1979) speculated that *S. microphylla* had parents in an old hybrid swarm of *S. tetraptera* and *S. prostrata*. Our data do not support this filiation: *S. prostrata* is closer to *S. tetraptera* than to *S. microphylla*. The seed alkaloid composition of these last two species is also closer to that of insular species, like *S. fernandeziana* or *S. toromiro*, with higher contents of tricyclic cytisine derivatives.

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Character states

Coding numbers, characters and states.

1. Life form: shrub: 0, tree: 1.
2. Number of leaflets: less than 15: 0, 15–25: 1.
3. Length of leaflets: over 1 cm: 0, less than 1 cm: 1.
4. Length/width of leaflets ratio: more than 2: 0, less than 2: 1.
5. Pubescence of the leaflets: face and back: 0, back only: 1.
6. Direction of flag: erect: 0, extended: 1.
7. Flag/wings ratio: 1 (same length): 0, longer flag: 1, much longer: 2.
8. Exertion of stamens: no: 0, yes: 1.
9. Strangulation of the lomentum: no: 0, yes: 1.
10. Length of seed: 1 cm or more: 0, less than 1 cm: 1.
11. Seed color: brown: 0, ocher: 1, orange: 2, reddish: 3.
12. Number of seeds per fruit: 2–5: 0, 6 or more: 1.
13. Pubescence of the lomentum: marked: 0, slight: 1.
14. Presence of wings on the fruit: no: 0, yes: 1.
15. Presence of auricules on the petals: yes: 0, no: 1.
16. Presence of stipulae: yes: 0, no: 1.
17. Methylcytisine percentage: below 30%: 0, over 30%: 1.
18. Cytisine percentage: below 30%: 0, over 30%: 1.
19. Sparteine percentage: over 1%: 0, below 1%: 1.
20. Matrine percentage: below 30%: 0, over 30%: 1.
21. Methylcytisine/Cytisine ratio: less than 0.5: 0, more than 0.5: 1.

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