CONSERVATION STATUS AND PRELIMINAR RESULTS ON THE PHYLOGENETICS OF *ISOPLEXIS* (LINDL.) BENTH. (SCROPHULARIACEAE) **AN ENDEMIC MACARONESIAN GENUS**

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With 4 figures and 4 tables

ABSTRACT. A survey of the populations of *Isoplexis* in 1995 shows *I. canariensis* occupying a broad habitat spectrum with a distribution that seems naturally wide in Tenerife but suggests its extinction on La Gomera and La Palma; *I. isabelliana* has few large populations with very little sign of regeneration; *Isoplexis chalcantha* is highly threatened and is already subject to protection; *I. sceptrum* has been found to be more widespread than previously believed. Data on the ecology, distribution and IUCN classification status of the four species is presented.

The phylogenetic relationship of *Isoplexis* to *Digitalis* is examined using cladistic analysis of molecular data from the *ITS* region of nuclear ribosomal DNA. This analysis presents the first cladistic view of relationships among taxa of the tribe Digitaleae. *Isoplexis* is shown to be a recent derivative taxon from *Digitalis.*

INTRODUCTION

The archipelagos of Madeira and Canaries have suffered loss of most of their natural vegetation since human colonization. What remains occurs largely in very fragmented patches (BRAMWELL, 1990; SANTOS, 1983) distributed over the islands. This continuous loss of natural habitats and the occurrence in these islands of several genera which are considered relicts from an ancient flora (SUNDING, 1979) make the evaluation of the present status and conservation needs of each one of the endemic taxa a priority.

The genus *Isoplexis* (LINDL.) BENTH. is considered to be a relict from the Tertiary and is endemic to Macaronesia. It comprises four species, three of which occur in the Canary Islands, *I.isabelliana* (WEBB & BERTH) MASF. and *I.chalcantha* SVENT. & O'SHANAHAN endemic to Gran Canaria, *I.canariensis* (L.) G.DON, reported to occur on Tenerife, La Palma

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and La Gomera (BRAMWELL, 1990; SANTOS, 1983; CEBALLOS & ORTUÑO, 1951; SANTOS & FERNÁNDEZ, 1981). The fourth one occurs only in Madeira, *I.sceptrum* (L.*fil.*) LOUD.

In contrast, the genus *Digitalis* L., commonly believed to be closely related to *Isoplexis*, shows an African-Eurasian distribution of the 18 species (WERNER, 1966). Of these species only *D. purpurea* L. and *D. grandiflora* Mill. are widespread, the remaining species each are restricted endemics around the Mediterranean basin. *D. purpurea* is unique in *Digitalis* in spreading into Macaronesia, though it is probably introduced.

BRAMWELL and RODRIGO (1982), established some priorities and guidelines for the conservation of the macaronesian endemics occuring in the Canary Islands. They prioritised the genera with one to four species. These were classified using the first IUCN categories of threat (IUCN, 1980). In contrast with the recent categories (IUCN, 1994), the 1980 categories do not utilise detailed information on demography. Canary Island *Isoplexis* species were categorised as: *I. canariensi*s, **R**; *I. isabelliana*, **V** and *I. chalcantha*, **E**.

The two genera under study are an example of the Macaronesian / West Mediterranean disjunctions (BRAMWELL, 1976), which is represented in this case by the iberian-moroccan endemics, *Digitalis obscura* L. and *Digitalis laciniata* Lindl., and the species of *Isoplexis* (BRAMWELL, 1972). The former two are considered by MEUSEL (1952) to be relict survivors of the Tertiary flora, whereas the modern European species of *Digitalis* are considered to be derived from the frutescent Macaronesian genus *Isoplexis*.

The woodiness of the taxa in the genus *Isoplexis*, and their taxonomic isolation, caused BRAMWELL (1972) to consider the genus to be relictual and he refers to it as one of the possible examples of paleoendemism (paleopolyploid), 2n=56 (8x).

In contrast with BRAMWELL and MEUSEL, HUMPHRIES (1979) sugested a possible monophyletic origin of *Digitalis* and *Isoplexis* from a single island ancestor on the basis of shared synapomorphies, which also would indicate that *Isoplexis* could be of equivalent age to *Digitalis*. In disagreement, PEREZ DE PAZ & ROCA (1982) reinforce the position that *Digitalis* has probably evolved from *Isoplexis*, considering *Digitalis laciniata* and *Isoplexis chalcantha* as 'bridge species' between the two genera.

The above statements on the origin and phylogenetic relationships between the two genera are intuitive and are based on limited morphological data. These conflicting opinions indicate the need for a better understanding of patterns of evolution in the Macaronesian isles and the Mediterranean region.

In this paper we present preliminary results on the:

A. Evaluation of *Isoplexis* status, ecology and conservation implications.

B. Phylogeny of *Digitalis* and *Isoplexis*.

A. The status of *Isoplexis* species on Madeira Island and The Canary Islands

A study of micro-scale phytogeography in Madeira and the Canary Islands was made

during May and June 1995. Ecological data on every sampled population was gathered and the location of each one of the populations recorded using UTM (Universal Transverse Mercator) coordinates (Fig.1, 2 and 3; Table 4). Complementary information, mainly chorological data, was added from the bibliography and herbarium specimen collections held at the Jardin de Aclimatacion de La Orotava (ORT) and at the Jardin Canario "Viera y Clavijo" (JVC). The *Isoplexis* associated species are listed in table 1 with the respective authorities.

1. CANARY ISLANDS - Population, geographical and ecological data

In this archipelago, the three native species of *Isoplexis* were studied in most of their natural populations. All the wild localities visited have previously been reported (BRAMWELL, 1990; SANTOS, 1983; SANTOS & FERNÁNDEZ, 1981; CEBALLOS & ORTUÑO, 1951; RODRIGUEZ, 1994).

TENERIFE - *I.canariensis*

On this island all the populations of *I.canariensis* found in the wild occur within the so called "Zonas de Interés Botanico", i.e. areas of botanic interest (BRAMWELL, 1990). In most of the populations found (Fig. 1) there were mixed size classes of plants, indicating regular regeneration.

The north-east of Tenerife - Sierra de Anaga is the visited locality with the highest number of *Isoplexis canariensis* populations per locality. Here, the number of individuals per population was found to vary from eleven to more than 50 individuals. Sites [3] **1** and **2** (Fig. 1) have a scattered population of about 40 and 11 individuals, respectively. These two populations are growing in dappled shade amongst dense stands of *Erica arborea, Prunus lusitanica* and *Picconia excelsa* (Laurel forest). There was no evidence of regeneration. At site **3,** a small roadside population of 10 individuals amongst low heath, mixed size classes were found and some specimens were flowering. Sites **4** and **5** hold the populations with the highest number of individuals, approximately 45-50 mature and young individuals in each site. These two extensive colonies are growing on roadsides on the edge of low "Laurisilva" vegetation. With exception of site 1, no excursions from the road into the vegetation were made and therefore the number of individuals and populations are minimum estimates.

The two largest populations of all the *I.canariensis* sampled were found on Montaña de La Venta , in the north-west of Tenerife. At site **6,** on a minor road near La Musita, about 80 individuals were found on old abandoned terraces dominated by *Erica arborea* scrub with *Cistus symphitifolius*, *Teline canariensis*, some *Ilex canariensis*, *Rhamnus glandulosa*

[3] Each site no. corresponds to a different population

and *Picconia excelsa*. Site **7,** a NW facing slope between Las Portelas and Erjos, amongst open scrub of *Chamaecytisus proliferus* with *Erica arborea* and *Ulex* and pockets of secondary mesic woodland of *Myrica faya*, *Laurus azorica* and *Viburnum rigidum*. Extensive colonies of more than one hundred plants of *Isoplexis* were found growing in open areas or on roadside embankments.

Above Santa Ursula, east of Aguamansa, on a forestry track north west from Mirador Ortuño two populations were found. The vegetation in site **8** is mainly *Pinus canariensis* mixed with residual *Laurus* and *Viburnum*. Twenty individuals were found scattered along the roadside. Site **9**, is a scattered population of *circa* 20-30 plants, in natural *Pinus canariensis* woodland with an understorey of *Erica arborea* and *Cistus symphitifolius*.

The last location visited in Tenerife was near Barranco de Guimar. Site **10** is above Los Nitos in a south facing barranco with abandoned old agricultural terraces. The vegetation was a secondary succession of young *Pinus canariensis* woodland. In open areas dense scrub dominated by *Cistus symphitifolius*, *Rubus inermis*, *Erica arborea*, *Arbutus canariensis* and the introduced *Castanea sativa* was seen. This area was severely affected by drought during 1995. About 17 adults and no young plants were found, all showing evidence of drought stress. Site **11**, in Barranco del Herque, is a south facing valley with mixed scrub and elements of laurel forest. It is a site at the lowest margin of the cloud base. The vegetation consists of a broken low forest canopy (*circa* 2.5m) of *Ilex canariensis*, *Arbutus canariensis*, *Apollonias barbujana*, *Erica arborea*, *Laurus azorica* alternating with patches of *Phyllis nobla*, *Urtica morifolia*, *Sideritis soluta*, *Sonchus radicatus*, *Aeonium holochrysum*, *Davallia canariensis*, *Echium virescens* and other herbs. On shaded rock faces large colonies of *Adiantum reniforme* were seen. About 20 individuals of *Isoplexis* were observed.

La Palma - *I.canariensis*

Historical sites for *I.canariensis* (CEBALLOS & ORTUÑO, 1951; SANTOS, 1983) were visited, but no plants were located. Extensive exploration was made of a valley south of Roque del Faro, Barranco de Los Hombres in the north of the island where the plant had last been seen in 1984 (SANTOS-GUERRA, *pers. comm.*). The area is of dense 'Laurisilva'.

La Gomera - *I.canariensis*

The Cañada de los Loros, where *I.canariensis* has been recorded for the last time on the island (SANTOS-GUERRA, *pers. comm.*) was explored extensively. This species appears to be Extinct or Critically Endangered (sensu IUCN, 1994) in the wild on La Gomera.

Gran Canária

The two species found on this island are thought to have originally occured in two different types of vegetation. *I.chalcantha* was limited to "Laurisilva" and "Fayal-brezal*"* and *I.isabelliana* to *Pinus canariensis* woodland (SUAREZ, 1994).

I.isabelliana

This species is restricted to *Pinus* woodland and degraded areas. There are six main populations reported (SUAREZ, 1994) but only three were visited and sampled (Fig. 2; Table 4).

Above Cueva Grande approached from Teror via Sán Mateo (site **12**) a population was visited with 26 adult plants in flower. These were scattered in open scrub and *Erica* heath.

In an agricultural track from Cueva Grande to Hoya del Gamonal (site **13**) on a steep slope of loose rubble, shaded by *Eucalyptus* sp. with clumps of nitrophilous plants such as *Urtica* sp. a scattered colony of 25 -35 plants were seen. The adults are on the top of the cliff and seedlings were seen on the slope caused by road cutting.

The largest population of *Isoplexis* was found on a 25% slope within an even aged *Pinus canariensis* plantation by side of the road, near the Presa de los Hornos (site **14**). Very extensive colonies of *I. isabelliana* were seen growing in the dappled shade of high canopy cover and formed the main component of the understorey with scattered thickets of *Cistus symphytifolius*. At least 400-500 adult plants plus seedlings were seen to be spread over 4 hectares.

On the roadside from Pozo de Las Nieves south to Telde (site **15)**, above Cazadores, on the roadside one plant was located at the base of a newly constructed boundary wall. Inspection of the area behind the wall revealed 23 plants of flowering age plus seedlings. The long term security of this site is not certain due to the plantation of ornamental trees.

The Pinar de Tamadaba was visited. This is an historical location for *I. isabelliana* (SUAREZ, 1994), however, no plants were located.

I.chalcantha

Los Tiles Reserve (site **16,** Fig. 2; Table 4) was visited but no wild plants were located above Los Tiles nursery due to logistic difficulties. Four reintroduced plants were seen growing along the path. Several more healthy plants were in cultivation in the nursery but five adults in the fenced reintroduction area all seemed to be in poor health and a further one was dead. This species appears to be Critically Endangered and restricted to a few remnant populations in Los Tiles vicinity (GONZALEZ-MARTIN, *pers. comm.)*.

2. MADEIRA ARCHIPELAGO - Population, geographical and ecological data.

Madeira Island - *I. sceptrum*

There is surprisingly little information on the location of populations and status in the wild. Though an incomplete survey, this is the first detailed account of the distribution and ecology of *I.sceptrum* populations in the wild.

Four different localities separated by very deep valleys in the northern mountains of Madeira were visited. All the populations were at altitudes of 900-1100m, within the cloud

zone community, "Clethro-Laurion*"* (SJOGREN, 1972). The *I.sceptrum* populations were always associated with a high percentage of Macaronesian endemics. The habitat is mainly near streams, on very steep slopes, and because the valleys where they grow are very deep and narrow the number of hours of direct sunlight is small.

This species was supposed to be very rare (PRESS & SHORT, 1994). However, the field data indicates a high frequency of individuals in particular localities and a very wide distribution within Madeira. It was found to grow from the extreme east to the extreme west of the island, but only in the northern mountains. On the other hand, it is true that this is a species with a localised occurrence, i.e., it is confined to a narrow band of altitudes where the relative air humidity is very high (>90 %) and with values of precipitation above 1700 mm per year (SJOGREN, 1974).

The central and western part of the island were visited using the paths along the "levadas" (canals of water) as an easy way to access the inland valleys and mountains. In total, 21 populations of different sizes were surveyed and its locations recorded (Fig. 3; Table 4).

In the São Vicente valley, from Encumeada to Ribeira do Inferno, nine populations were seen (sites **1-9**). Almost isolated individuals were found in sites **1** (three individuals) and **2** (one individual). They present an oblique or almost horizontal growth on very steep slopes, 2 to 2.5 meters above the base of the slope. The specimens are about 2 meters high. In both cases, surrounding the *Isoplexis* specimens, were several species typical of "Laurisilva": *Laurus azorica*, *Heberdenia excelsa*, *Euphorbia mellifera, Clethra arborea, Phyllis nobla* (abundant) *Bystropogon* sp., *Pericallis aurita, Sibthorpia peregrina, Sonchus fruticosus, Aichryson divaricatum, Erica scoparia* (abundant), *Ocotea foetens, Picconia excelsa, Woodwardia radicans* (abundant), *Ageratina adenophora* and a large number and variety of ferns and bryophytes. At site **3**, a small river very rich in madeiran and macaronesian endemic species with *Erica scoparia* ssp. *maderinicola Geranium maderensis, Sambucus lanceolata, Aichryson divaricatum, Oenanthe divaricata, Sonchus fruticosus, Dactylorhiza foliosa, Erysimum bicolor, Clethra arborea, Deschampsia argentea, Euphorbia mellifera, Phyllis nobla*, *Ranunculus cortusifolius* and other non-endemic species such as *Woodwardia radicans.* There were observed about 15 adult *Isoplexis* specimens with a good number of seedlings. Sites **4** and **5** are in narrow valleys, the first with a waterfall. A population of 13 adult specimens of *Isoplexis* was found and a small population of the rare madeiran endemic, *Musschia wollastonii.* Many plantlets of *Isoplexis* were observed giving evidence of good regeneration. The surrounding vegetation in sites 4 and 5 is basically the same as that in site 3. Site **6** is impossible to reach from the path. As far as can be seen from the path it is a population of 10 adult individuals. With this population were: *Cirsium latifolium, Euphorbia mellifera, Clethra arborea, Teline maderensis, Geranium maderense* (very abundant with big specimens), *Melanoselinum decipiens, Laurus azorica, Sonchus fruticosus, Hypericum grandifolium, Aichryson divaricatum, Ranunculus cortusifolius, Phyllis nobla, Argyranthemum pinnatifidum* ssp. *pinnatifidum* and *Ruscus streptophyllus*. This site is much

more exposed to the sunlight than previous ones as confirmed by the occurrence of *T. maderensis* and *A. pinnatifidum*. Site **7** has a population of about 20 specimens. A lot of seedlings were found indicating good regeneration. Sites **8** and **9** each consist of about 8 adult specimens of *Isoplexis*. The surrounding vegetation is composed of the same species as site 3 but with a small population of *Musschia wollastonii.* However, at site 9, *Sinapidendron* sp. is recorded for the first time.

On the western part of the island, in the Ribeira do Seixal valley, above Chão da Ribeira (900-1100 m) other samples were collected from four small populations. This valley is one of the least disturbed places of madeiran "Laurisilva". Four sites (Fig. 3) were found to shelter *I.sceptrum* populations. At site **10,** ten individuals were observed, but most of them are unreachable. This site is near a waterfall. *Musschia wollastonii* was also found here. Site **11**, is forest dominated by *Ocotea foetens*, *Clethra arborea* and *Laurus azorica*. Three adult *Isoplexis* specimens were found. Site **11** is within the same river channel and forms a continuum with site 12. Site **12** is a very humid site near a water fall, with low luminosity and a large population of *I. sceptrum*, about 20 adult individuals. Most of them were unreachable. The surounding vegetation is formed mainly by: *Euphorbia mellifera, Ranunculus cortusifolius, Pericallis aurita, Rubus grandifolius, Festuca donax, Deschampsia maderensis, Sonchus fruticosus, Clethra arborea, Woodwardia radicans, Sibthorpia peregrina* and *Huperzia selago*. The last site visited in this valley (site **13**), is very humid with a luxuriant vegetation. Above 20 adult individuals and many seedlings were seen. This site, of all sites visited, presents the highest species diversity of macaronesian endemics with 27 taxa: *Cirsium latifolium, Laurus azorica, Vaccinium padifolium, Festuca donax, Ocotea foetens, Clethra arborea, Erica scoparia, Sibthorpia peregrina, Saxifraga maderensis, Ilex perado, Geranium maderense, Viola odorata, Sonchus fruticosus, Euphorbia mellifera, Teline maderensis, Bystropogon sp., Erica scoparia, Erica maderensis, Argyranthemum pinnatifidum, Bupleurum salicifolium, Aichryson divaricatum, Phyllis nobla, Pericallis aurita, Erisymum bicolor, Rubia sp., Ranunculus cortusifolius, Hypericum grandifolium*.

The eastern central part of the island, Levada do Caldeirão Verde, Santana, is much more disturbed than the sites previously visited (Fig. 3). The populations of *Isoplexis* have a very small number of plants and some isolated individuals were also found. The first site is against a slope with permanent draining water (site **14**) with only one adult specimen observed. On the site **15** three specimens were recorded in a very small population, surrounded by a vegetation dominated by *Laurus azorica, Clethra arborea, Erica scoparia, Sonchus fruticosus, Tolpis machrorrhiza, Sibthorpia peregrina, Phyllis nobla, Hypericum sp., Festuca donax, Dactylorhyza foliosa, Blechnum spicant* and *Argyranthemum pinnatifidum.* The site **16** with the same type of vegetation of the above site, two specimens of *Isoplexis* were found. At sites **17** and **18** with a high degree of exposure two and three individuals were observed. In a very narrow valley (site **19**), very humid and with a low degree of luminosity, it was found a high diversity of species. The surrounding vegetation is basically the same as in site 15,

plus *Musschia wollastonii* and *Geranium maderense*. Four *Isoplexis* specimens were found.

The eastern populations sampled are within Ribeira do Faial valley, from Levada do Ribeiro Frio to Fajã da Nogueira. This locality is only very rich in its botanic composition in some undisturbed sites It is in general very much impoverished in its natural vegetation due to human activities through the last centuries. Here, only two populations were found and some other isolated individuals were impossible to reach. Near a river (site **20**), there were two specimens of *Isoplexis*. The tree cover surrounding this site is very poor and the vegetation is mainly composed by: *Erica scoparia* ssp. *maderinicola* (abundant), *Ocotea foetens, Clethra arborea, Bystropogon* sp., *Sibthorpia peregrina, Erica maderensis*. This site has a very low species diversity and this is the site of all that is more exposed to direct sunlight. On site **21**, in a very narrow valley near a water stream with low luminosity and high humidity, a population of 10 adult individuals, above the levada from which only one is reachable.

B. DNA PHYLOGENY

The nrDNA *ITS* (internal transcribed spacer) region is very useful for assessing phylogenetic relationships at lower levels such as among genera or species (SUH & al, 1993). In this preliminary analysis, each one of the five subgeneric groups within *Digitalis* (HEYWOOD, 1972) is represented at least by one species (Table 2). *Isoplexis* is represented by all three species occuring in the Canary Islands. The outgroup, *Erinus alpinus* is from the tribe Veroniceae, believed to be closely linked to Digitaleae.

Total cellular DNA was extracted from fresh or herbarium material using a CTAB protocol modified from SAMBROOK *et al.*(1989) and DOYLE & DOYLE (1987). Double stranded PCR (polymerase chain reaction) products were generated using ITS5 and ITS4 primers (WHITE *et al.* 1990) encompassing the ITS1, 5.8S and ITS2 regions in entirety. The double stranded products were sequenced either manually using AmpliTaq® cycle sequencing with P-33 or automatically on an ABI373 using the DyeDeoxy® termination method. The sequencing primers used for the *ITS* region were *ITS1* (internal) and *ITS4* (terminal).

Sequences were aligned using the ClustalV algorithm and, in the set used for cladistic analysis, gaps in one species relative to any other were scored as missing data. Comparisons included 684 bp of aligned sequence of which 530 positions were used in the analysis. Phylogenetic analysis was performed using PAUP version 3.1 (SWOFFORD, 1993) using the exhaustive search option (with collapse of zero length branches) with all changes weighted equally. To assess the support for the clades found in the analysis a bootstrap analysis (FELSENTEIN, 1985) was conducted in PAUP with 100 replicates using the Branch & Bound option.

Three equally parsimonious trees were found, differing only in the relative positions of the three *Isoplexis* species to each other. One of the most parsimonius trees (Fig. 4) is topologically identical with the strict consensus tree. The other two show either *I. isabelliana* or *I. canariensis* sister to the remaining pair of *Isoplexis* species. In these cases the terminal

branch lengths are short (1 change) and not supported in the bootstrap analysis. In contrast the terminal branch lengths in *Digitalis* are long (9-21 changes). This may be due to low sampling at the subgeneric level in *Digitalis*.

The bootstrap analysis (Fig. 4) strongly supports the basal branching patterns in *Digitalis* and the inclusion of *Isoplexis* as a derived group within *Digitalis*. The derived clade to which *D.grandiflora* is sister shows limited resolution which may be due to limited taxon sampling.

DISCUSSION AND CONCLUSIONS

Isoplexis does not show wide adaptive radiation in contrast with other endemic genera (BRAMWELL, 1976) and it is restricted to a narrow range of habitats, which in turn makes conservation of the genus a particular challenge.

I.sceptrum and *I.chalcantha* are the only two *Isoplexis* species to be restricted to "Laurisilva". The second species was seen only in its natural habitat conditions at Los Tiles de Moya reserve but not in the wild and therefore, no considerations on its ecology could be made by us at the present time. SUAREZ (1994), refers to only four populations and GONZALEZ-MARTIN (*pers. comm.*) working on a monitoring program of this species in the wild refers to a worrying decrease in the number of individuals during the last years. Despite this, and assuring the continuity of this species, there is an increase in numbers of cultivated specimens within the Los Tiles de Moya reserve from wild collected seeds.

I.sceptrum is restricted mainly to the vicinity of permanent streams where the tree cover is less dense so enabling the sunlight to get through the canopy and there is high air humidity. Its occurence on steep slopes seems to show a preference to places where there is good drainage and the tree cover is low. Of course, only more specific studies on the ecology of this species will give us information on the factors which most strongly influence its habitat preferences and determines the generally small population size. This species' widspread occurrence, possibly in a continuum almost from one extreme of the island to the other, make us believe that higher number of populations and individuals occurs in the wild than in recorded here. Further field work is being carried out by the authors in order to get a better knowledge of this species' distribution, population numbers and size.

I.isabelliana is restricted to another type of habitat, mainly *Pinus canariensis* woodland. This species also occurs in open disturbed places localised within the so called potential distribution of "Monte Verde" (SUAREZ, 1994) and areas of transition between the *Pinus* woodland and the "fayal-brezal". The small number of populations, some with very small numbers of individuals, and the existence of threats to some of the populations makes the need for localized conservation actions urgent.

In contrast with the above species, *I.canariensis* has been recorded for three islands. Tenerife was the only island where this taxon was seen and sampled during this field trip. It

occupies a broad habitat spectrum. It is common in the humid "Laurisilva", frequent in the also humid *Pinus canariensis* woodland in the north but rare in a much more dry *Pinus canariensis* woodland in the south, its extreme range, indicating a preference for moister habitats. Despite the total numbers of individuals and populations found and refered here, this species distribution seems naturally wide in Tenerife and therefore it does not present any immediate conservation needs.

On La Palma and La Gomera this species was not found in its few reported localities bringing in to question the continued natural existence of this species on these two islands. It has been suggested (Santos-Guerra, *pers. comm.*) that these reports could be the result of a recent introduction event that did not result in the long term establishment of this species on these two islands.

In summary, the habitat requirements and conservation status for each species are different (Table 3) and while the genus as a whole is as secure as any island endemic taxon some individual species are in need of, or already receiving, conservation action.

The preliminar explicit phylogenetic hypothesis for *Isoplexis* and *Digitalis* presented here contradicts previous implicit theories (MEUSEL, 1952; BRAMWELL, 1972; HUMPHRIES 1979, PERÉZ DE PAZ & ROCA, 1982) by suggesting derived origin of the woody *Isoplexis* and *Digitalis* from herbaceous *Digitalis*. This result makes questionable the justification of conservation of these endemics on the basis that they are some kind of ancient relict taxon. Their value is more in the study of rapid change associated with a change in pollinator, bee to bird, and change in herbivore pressure, mammals to insects and gastropods.

HUMPHRIES (1979) highlighted the scarcity of detailed explicit phylogenies for macaronesian endemics. Since then, and only recently, some studies have been done using molecular data to access the pylogenetic relationships involving different groups of mcaronesian taxa (BOEHLE *et al.*, 1994; MES & HART, 1994; RAY, 1995; FRANCISCO-ORTEGA *et al.*, 1995; FRANCISCO-ORTEGA *et al.*, 1996; MES & HART, 1996; KIM *et al.*, 1996). In contrast with these studies, all focusing on groups of taxa which have undergone a strong adaptive radiation on the Macaronesian islands, this work investigates a taxon which show a week adaptive radiation response.

With this study and future work we hope to contribute with complementary data to better understand the complex biogeography of the Macaronesian islands.

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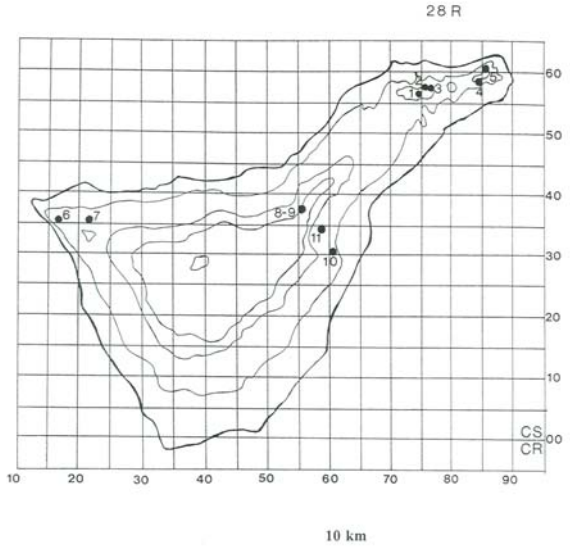

Fig. 1 - Location of the *I.canariensis* populations visited in Tenerife. Numbered populations are cited in the text.

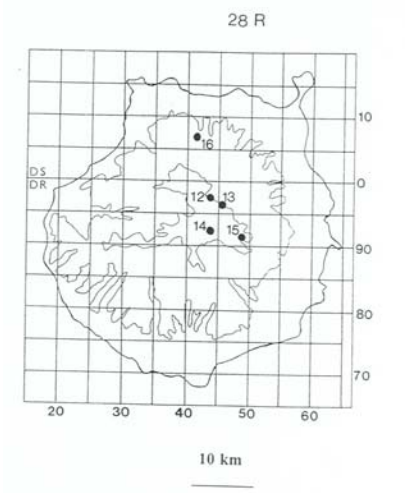

Fig. 2 - Location of the *I.isabelliana* populations visited in Gran Canaria. Numbered populations are cited in the text.

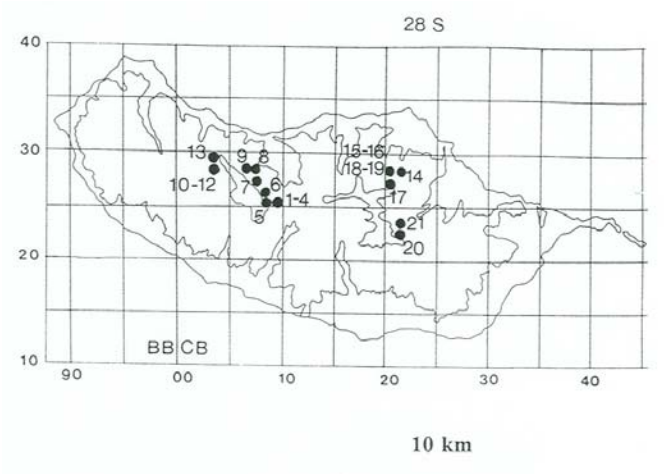

Fig. 3 - Location of the *I.sceptrum* populations visited in Madeira. Numbered populations are cited in the text.

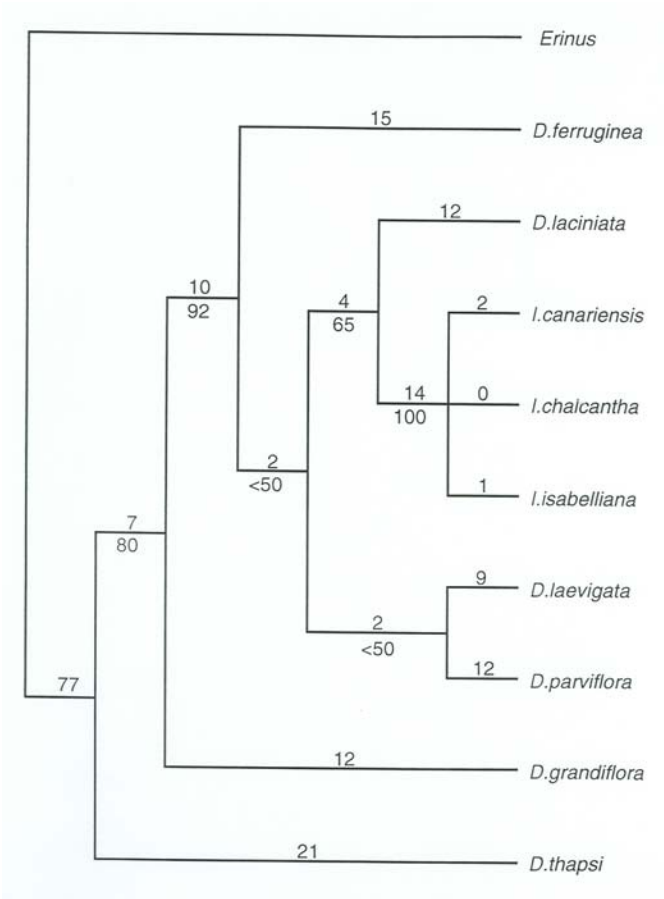

Fig. 4 - The most parsimonius tree topologically identical with the strict consensus tree. Upper values indicate branch lengths and lower ones are bootstrap values

TABLE 1 - List of taxa associated with *Isoplexis* populations which are cited in the text.

Taxa	**Family**
Adiantum reniforme L.	Adiantaceae
Bupleurum salicifolium R.Br. in Buch	Apiaceae
Melanoselinum decipiens (Schard et Wendel.) Hoffm.	Apiaceae
Oenanthe divaricata (R.Br.) Mabb.	Apiaceae
Ilex canariensis Poir.	Aquifoliaceae
Ilex perado Ait.	Aquifoliaceae
Ageratina adenophora (Spreng.) King et Robins.	Asteraceae
Argyranthemum pinnatifidum (L. *fil*.) Lowe ssp. *pinnatifidum*	Asteraceae
Cirsium latifolium Lowe	Asteraceae
Pericallis aurita (L'Hér.) B.Nord.	asteraceae
Sonchus fruticosus L.*fil*.	Asteraceae
Sonchus radicatus Ait.	Asteraceae
Tolpis macrorhiza (Lowe) Lowe	Asteraceae
Blechnum spicant (L.) Roth.	Blechnaceae
Woodwardia radicans (L.) J.E.Sm.	Blechnaceae
Echium virescens DC.	Boraginaceae
Erysimum bicolor (Hornem.) DC.	Brassicaceae
Sinapidendron sp.	Brassicaceae
Musschia wollastonii Lowe	Campanulaceae
Sambucus lanceolata R.Br. in Buch	Caprifoliaceae
Viburnum tinus L. ssp. *rigidum* (Vent.) P. Silva	Caprioliaceae
Cistus symphytifolius Lam.	Cistaceae
Clethra arborea Ait.	Clethraceae
Aeonium holochrysum Webb et Berth.	Crassulaceae
Aichryson divaricatum (Ait.) Praeger	Crassulaceae
Davallia canariensis (L.) J.E.Sm.	Davalliaceae
Arbutus canariensis Veill.	Ericaceae
Erica arborea L.	Ericaceae
Erica maderensis (DC.) Bornm.	ericaceae
Erica scoparia L. ssp. *maderinicola* McClintock	Ericaceae
Vaccinium padifolium J.E. Sm. ex Rees	Ericaceae
Euphorbia mellifera Ait.	Euhorbiceae
Chamaecytisus proliferus (L.*fil*.) Link	Fabaceae
Teline canariensis (L.) Webb et Berth.	Fabaceae
Teline maderensis Webb et Berth.	Fabaceae
Ulex europaeus L.	Fabaceae
Castanea sativa Mill.	Fagaceae
Geranium maderense P.F.Yeo	Geraniaceae
Hypericum grandifolium Choisy	Hypericaceae
Hypericum sp.	Hypericaceae
Bystropogon sp.	Lamiaceae
Sideritis soluta Webb ex Clos	Lamiaceae
Apollonias barbujana (Cav.) Bornm.	Lauraceae
Laurus azorica (Seub.) Franco	Lauraceae

Ocotea foetens (Ait.) Baill.	Lauraceae
Ruscus streptophillus P.F. Yeo	Liliaceae
Huperzia selago (L.) Bernh. ex Schrank et Mart.	Lycopodiaceae
Heberdenia excelsa (Ait.) Banks ex DC.	Mirsinaceae
Myrica faya Ait.	Myricaceae
Eucalyptus sp.	Myrtaceae
Picconia excelsa (Ait.) DC.	Oleaceae
Dactylorhiza foliosa (Verm.) Soó	Orchidaceae
Pinus canariensis Chr. Sm.ex DC. in Buch	Pinaceae
Deschampsia argentea (Lowe) Lowe	Poaceae
Festuca donax Lowe	Poaceae
Ranunculus cortusifolius Willd.	Ranunculaceae
Rhamnus glandulosa Ait.	Rhamnaceae
Prunus lusitanica L.	Rosaceae
Rubus inermis Pourr.	Rosaceae
Phyllis nobla L.	Rubiaceae
Rubia sp.	Rubiaceae
Saxifraga maderensis D. Don	Saxifragaceae
Sibthorpia peregrina L.	Scrophulariaceae
Urtica morifolia Poir.	Urticaceae
Viola odorata L.	Violaceae

Taxonomy follows Press & Short (1994) and Hansen & Sunding (1993).

TABLE 2 - Taxa used for DNA extraction and ITS sequencing.

Taxa	**Tribe**	**Origin**
Erinus L.	Veroniceae	Alastair Culham
I.isabelliana (Webb.) Masf.	Digitaleae	CPG
I.chalcantha Svent. & O'Shanon	Digitaleae	CPG
I.canariensis (L.)Lindl.	Digitaleae	CPG
D.thapsi L.	Digitaleae	NJ
D.grandiflora Mill.	Digitaleae	Bot.Garden Justus Liebig
D.parviflora Jacq.	Digitaleae	NJ
D.laevigata Waldst.& Kit.	Digitaleae	Bot.Garden Justus Liebig
D.laciniata Lindl.	Digitaleae	PSL, herbarium (RNG)
D.ferruginea L.	Digitaleae	NJ

NJ - Noreen Jardine's Digitalis National Collection/UK
CPG - Chelsea Physic Garden living collection/ UK
PSL - Plant Science Laboratories/ The Universiy of Reading/ UK

All living plants were grown at School of Plant Sciences facilities. All specimens are kept as herborized material at the School of Plant Sciences, Univ. of Reading, UK

TABLE 3 - A summary of the results and conclusions obtained from the trips to Madeira and Canary Islands.

	Isoplexis sceptrum	*Isoplexis canariensis*	*Isoplexis isabelliana*	*Isoplexis chalcanta*
Islands	Madeira	Tenerife, La Palma and La Gomera	Gran Canaria	Gran Canaria
Localities visited	4	4	4	1
Populations sampled	21	11	4	1 (cultivated plants)
Estimated No. of individuals	• < 1000 mature individuals (< 200 observed)	• > 1000 individuals (< 500 observed)	• < 1000 mature individuals (< 600 observed)	• < 250 mature individuals
Community	• "Laurisilva"	• "Laurisilva" • *Pinus canariensis* woodland • disturbed habitats	• *Pinus canariensis* woodland • disturbed habitats	• "Laurisilva"
Habitat	• 900-1100 m • high humidity • near permanents streams	• 600-1300 m • dry to humid	• 800-1650 m (SUAREZ, 1994) • moderately dry	• 400-800 m (SUAREZ, 1994) • humid
Status	• widespread in "Laurisilva" • not as rare as supposed before • good population regeneration	• widspread in "Laurisilva" • restricted in the *Pinus canariensis* woodland • species not rare • good population regeneration • not found in La Palma and La Gomera	• biggest population within *Pinus canariensis* woodland • small number of populations • some population regeneration • continuing decline population	• few remnant and severely fragmented populations • little population regeneration • continuing decline
IUCN categories (1994)	• Vulnerable (V)	• Lower Risk (LR-nt)	• Endangered (EN)	• Critically Endangered (CR)

TABLE 4 - UTM coordinates on the location of the *Isoplexis* populations surveyed.

Site No.	Location	UTM Coordinates	Island
1	Sierra de Anaga	28R CS 70 55	Tenerife
2	Sierra de Anaga	28R CS 75 55	Tenerife
3	Sierra de Anaga	28R CS 75 55	Tenerife
4	Sierra de Anaga	28R CS 80 55	Tenerife
5	Sierra de Anaga	28R CS 85 55	Tenerife
6	Montana de La Venta	28R CS 10 35	Tenerife
7	Montana de La Venta	28R CS 25 35	Tenerife
8	Above Santa Ursula, east of Aguamansa	28R CS 55 35	Tenerife
9	Above Santa Ursula, east of Aguamansa	28R CS 55 35	Tenerife
10	Barranco de Guimar	28R CS 55 25	Tenerife
11	Barranco de Herque	28R CS 55 25	Tenerife
12	Above Cueva Grande	28R DR 97 43	Gran Canaria
13	Near Presa de La Lechucilin	28R DR 96 45	Gran Canaria
14	Near Presa de Los Hornos	28R DR 92 43	Gran Canaria
15	Above Cazadores	28R DR 91 48	Gran Canaria
16	Los Tiles de Moya	28R DS 07 41	Gran Canaria
1	Levada do Norte (São Vicente valley)	28S CB 09 25	Madeira
2	Levada do Norte (São Vicente valley)	28S CB 09 25	Madeira
3	Levada do Norte (São Vicente valley)	28S CB 09 25	Madeira
4	Levada do Norte (São Vicente valley)	28S CB 09 25	Madeira
5	Levada do Norte (São Vicente valley)	28S CB 08 25	Madeira
6	Levada do Norte (São Vicente valley)	28S CB 08 26	Madeira
7	Levada do Norte (São Vicente valley)	28S CB 07 27	Madeira
8	Levada do Norte (São Vicente valley)	28S CB 07 28	Madeira
9	Levada do Norte (São Vicente valley)	28S CB 06 28	Madeira
10	Ribeira do Seixal valley	28S CB 03 28	Madeira
11	Ribeira do Seixal valley	28S CB 03 28	Madeira
12	Ribeira do Seixal valley	28S CB 03 28	Madeira
13	Ribeira do Seixal valley	28S CB 03 29	Madeira
14	Levada do Caldeirão Verde, Santana	28S CB 21 28	Madeira
15	Levada do Caldeirão Verde, Santana	28S CB 20 28	Madeira
16	Levada do Caldeirão Verde, Santana	28S CB 20 28	Madeira
17	Levada do Caldeirão Verde, Santana	28S CB 20 27	Madeira
18	Levada do Caldeirão Verde, Santana	28S CB 20 28	Madeira
19	Levada do Caldeirão Verde, Santana	28S CB 20 28	Madeira
20	Levada do Ribeiro Frio-Fajã da Nogueira	28S CB 21 22	Madeira
21	Levada do Ribeiro Frio-Fajã da Nogueira	28S CB 21 23	Madeira

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