



CONSERVATION BIOLOGY OF *SERAPIAS PEREZ-CHISCANOI* ACEDO IN THE GUADIANA RIVER BASIN IN EXTREMADURA (SPAIN)

C. VENHUIS, J.G.B. OOSTERMEIJER and J.Th. P. TONK

Samenvatting

De zeldzame orchideeënsoort *Serapias perez-chiscanoi* ACEDO komt alleen voor in het stroomgebied van de rivier Guadiana in de Extremadura (Spanje) en in aangrenzend Portugal. Hoewel sommige auteurs de taxonomische waarde betwijfelen, beschouwen wij *S. perez-chiscanoi* als aparte soort vanwege zijn stabiele en uniforme verschijningsvorm, zijn afwijkende habitat en voortplantingswijze. Alle planten hebben groene bloemen en kunnen worden onderverdeeld in twee kleurvarianten: de 'groene' en de 'rode', hoofdzakelijk betrekking hebbend op de nervatuur van alle plantendelen. Er vindt zelfbestuiving plaats, omdat de pollinia snel op het stigma uiteenvallen, soms nog voor de bloemen zijn opengegaan (cleistogamie). Sommige bloemen gaan in het geheel niet open, maar dragen wel vrucht. De bladeren van vegetatieve bladrozetten zijn altijd veel langer dan die van bloeiende planten, waarschijnlijk om hun overleving zeker te stellen door het doen toenemen van hun wortelstelsel en biomassa voordat de eerste bloeiwijze wordt aangemaakt. We hebben de tien bekende vindplaatsen bezocht, maar de soort was van vijf daarvan in de laatste twaalf jaar verdwenen. Gelukkig vonden we tijdens latere zoektochten zes nieuwe populaties, waarvan we de leeftijdsopbouw hebben onderzocht, uitgaande van drie leeftijdsklassen. Slechts in drie populaties kwamen jonge (vegetatieve) planten voor, hetgeen betekent dat de populaties in meerderheid weinig of geen aanwas hebben, wat kan wijzen op een suboptimale levensvatbaarheid. De habitat kan worden beschreven als: vochtige weilanden, gekenmerkt door soorten uit de geslachten *Asphodelus* en *Scirpus*. De bodem is licht zuur en zeer voedselarm. Er is een groot verschil in de gemiddelde hoogte van planten in verschillende

Foto omslag:

Serapias perez-chiscanoi. 'Green' variation. Embalse de Cornalvo, 21-04-2004.

Foto's pagina 48: 1 2
3 4

1. *Serapias perez-chiscanoi*. Obando, 29-04-2003.
2. *Serapias perez-chiscanoi*. 'Red' variation. Aljucén, 17-04-2004
3. *Serapias perez-chiscanoi*. Trujillanos, 19-04-2003
4. *Serapias perez-chiscanoi*. 'Pink' variation. Aljucén, 05-05-2003

Foto's 1, 2 en 4: Cas Venhuis. Foto 3: Rob Poot.

Overige foto's bij dit artikel: pag. 72.

populaties, waarschijnlijk in verband met verschillen in de habitat. *S. perez-chiscanoi* heeft een zeer hoge mate van vruchtzetting (92%), kenmerkend voor een zelfbestuivende soort. Op dezelfde vindplaatsen heeft *Serapias lingua* een mate van vruchtzetting van slechts 10%, kenmerkend voor een kruisbestuivende orchidee zonder beloning voor de bestuiver. Het Allee-effect doet zich niet voor bij *S. perez-chiscanoi*. Kleine populaties van *S. lingua* hadden evenwel een geringe vruchtzetting, middelgrote hadden een hoge vruchtzetting en grote weer een geringe vruchtzetting. Kleine maar lijnvormige populaties (langs wegranden) en planten aan de rand van grote populaties hadden een hoog vruchtzettingspercentage. Dit wordt waarschijnlijk veroorzaakt door het feit dat naïeve insecten een populatie aan de buitenkant binnengaan en dan leren deze planten, die geen beloning bieden, over te slaan naarmate zij vorderen. Vanwege de alarmerend hoge snelheid waarmee *S. perez-chiscanoi* verdwijnt, moet er dringend een soortbeschermingsplan worden opgesteld om hem voor uitsterven in de nabije toekomst te behoeden.

Summary

The rare orchid species *Serapias perez-chiscanoi* ACEDO is found only in the Guadiana river basin of Extremadura (Spain) and adjacent Portugal. Though some authors have doubts about the taxonomic validity, we consider *Serapias perez-chiscanoi* as a distinct species because it has a very stable and uniform morphological appearance, a distinct habitat and reproductive behaviour. All plants have pale green flowers and can be divided into two colour variations; the 'green' and the 'red' variation, which mainly involves the venation of all plant parts. Self-pollination takes place because the pollinia disintegrate rapidly onto the stigma, sometimes even before the flowers have opened (cleistogamy). Some flowers don't open at all, but do produce a fruit. The leaves of vegetative rosettes are always much longer than the leaves of flowering individuals, probably to ensure their survival by increasing their root system and vegetative biomass before producing the first inflorescence. We visited the ten known locations, but the species had disappeared from five of them during the past 12 years. Fortunately, during subsequent searches of the region, we found six new populations, of which we examined the life stage structure, using three different age states. Only in three populations, juvenile (vegetative) plants occurred, meaning that the majority of the populations show little or no recruitment, which might indicate sub-optimal viability. The habitat can be described as humid pastures, characterized by species of the genera *Asphodelus* and *Scirpus*. The soil is slightly acidic and very poor in nutrients. A big difference existed between the average height of plants in different populations, probably related to habitat differences. *Serapias perez-chiscanoi* has a very high average fruit-setting (92%), typical for a self-pollinating species. At the same locations, *Serapias lingua* had a low average fruit-setting of only 10%, typical for a cross-pollinating orchid lacking pollinator reward. The Allee-effect does not apply to *Serapias perez-chiscanoi*. Small populations of *Serapias lingua*, however, showed a very low fruit-setting, intermediately sized populations a high fruit-setting and large populations again a low fruit-setting. Small but linear populations (along road verges) and plants along the borders of large populations showed high fruit-setting percentages. This is probably caused by the fact that naïve insects enter a population at its border and then learn to avoid the plants lacking pollinator reward as they move inwards. Due to the alarming rate at which *Serapias perez-chiscanoi* disappears, a species conservation plan urgently needs to be set up to prevent its extinction in the near future.

Zusammenfassung

Die seltene Orchideenart *Serapias perez-chiscanoi* kommt nur im Guadiana Flussbecken in Extremadura (Spanien) und im benachbarten Portugal vor. Trotz Zweifel einiger Autoren bezüglich der Taxonomie, behandeln wir *Serapias perez-chiscanoi* auf Grund seiner stabilen und uniformen morphologischen Erscheinung sowie seines abweichenden Habitats und Reproduktionsverhaltens als eigenständige Art. Alle Pflanzen besitzen blassgrüne Blüten und können zwei Farbvarianten zugeordnet werden, der 'roten' und der 'grünen' Variante, bezüglich der Nervatur aller Pflanzenteile. Selbstbestäubung findet statt, wenn Pollen schnell auf der Narbe zerfallen, manchmal schon vor Öffnung der Blüte. Einige Blüten öffnen sich überhaupt nicht, produzieren jedoch Früchte (Kleistogamie). Blätter vegetativer Rosetten sind immer deutlich länger als die der blühenden Individuen, wahrscheinlich um durch Vergrößerung von Wurzelsystem und vegetativer Biomasse ihr Überleben zu sichern bevor die ersten Blüten produziert werden. An fünf der zehn bekannten, von uns besuchten Standorten ist die Art innerhalb der letzten zwölf Jahre verschwunden. Glücklicherweise fanden wir bei späterer Suche in der Region sechs neue Populationen, deren Altersstruktur wir untersuchten und wobei wir drei Altersklassen unterschieden. Nur in drei Populationen kamen juvenile (vegetative) Pflanzen vor; in der Mehrheit der Populationen findet somit geringe oder keine Verjüngung statt, was möglicherweise auf suboptimale Viabilität hinweist. Die Habitate sind feuchte Weiden, charakterisiert durch Arten der Gattungen *Asphodelus* und *Scirpus*, mit leicht saurem und sehr nutrientenarmem Boden. Große Unterschiede gab es zwischen den mittleren Höhen der Pflanzen der verschiedenen Populationen, wahrscheinlich bedingt durch Habitatunterschiede. *Serapias perez-chiscanoi* weist im Durchschnitt einen hohen Fruchtansatz (92%) auf, was typisch für autogame Arten ist. An den gleichen Standorten hatte *Serapias lingua* einen geringen Fruchtansatz von nur 10%, typisch für nicht belohnende, allogame Orchideenarten. Bei *Serapias perez-chiscanoi* ist der Allee-Effekt nicht von Bedeutung. Dagegen findet man in kleinen Populationen von *Serapias lingua* sehr geringen Fruchtansatz, in Populationen mittlerer Größe hohen Fruchtansatz und in großen Populationen wiederum geringen Fruchtansatz. Kleine, lineare Populationen (an Straßenrändern entlang) sowie Pflanzen am Rand großer Populationen weisen hohe Fruchtansatzraten auf. Dies wird wahrscheinlich durch das Verhalten naiver Insekten bedingt, die zunächst Pflanzen am Rand der Population besuchen und während sie sich weiter in die Population hineinbewegen, lernen, die nicht belohnenden Pflanzen zu meiden. Auf Grund der alarmierenden Rate, mit der *Serapias perez-chiscanoi* verschwindet, ist es erforderlich einen Plan zum Artenschutz zu entwickeln, um ihr Aussterben in naher Zukunft zu verhindern.

Resumen

La rara orquídea de la especie *Serapias perez-chiscanoi* es encontrada solamente en la cuenca del río Guadiana de Extremadura (España) y el Portugal limítrofe. Aunque algunos autores tienen dudas acerca del valor taxonómico, nosotros consideramos *Serapias perez-chiscanoi* como una especie distinta porque tiene una apariencia morfológica muy estable y uniforme, un hábitat irregular y una conducta reproductiva. Todas las plantas tienen flores verde pálido y pueden ser divididas en dos tipos de colores; la variante 'verde' y la variante

‘roja’, la cual incluye mayormente las venas de todas las partes de la planta. La autopolinización ocurre cuando las polinias se desintegran rápidamente en el estigma, a veces mucho antes de que las flores se hayan abierto (cleistogamia). Algunas flores no llegan ni abrirse, pero producen fruto.

Las hojas de las rosetas vegetativas son siempre mucho más largas que las hojas de los individuos que están en flor, probablemente para asegurar su supervivencia al incrementar su sistema radicular y biomasa vegetativa antes de producir su primera inflorescencia. Nosotros vistamos los diez lugares conocidos, pero la especie ha desaparecido en cinco de estos lugares a lo largo de los últimos 12 años. Afortunadamente, durante búsquedas posteriores de la región, nosotros encontramos seis nuevas poblaciones, de las cuales examinamos la estructura del estadio de vida, utilizando tres estados de edad diferentes. Solamente en tres poblaciones, existían plantas juveniles, lo que significa que la mayoría de las poblaciones muestran muy poco o nada de probabilidades de extenderse, lo cual puede indicar una viabilidad sub-optimal. El hábitat puede ser descrito como pastizales húmedos, caracterizados por las especies del género *Asphodelus* y *Scirpus*. El suelo es ligeramente ácido con un componente nutritivo muy pobre. Observamos una gran diferencia en la media de altura de las plantas en diferentes poblaciones, probablemente relacionado a las diferencias de hábitat. *Serapias perez-chiscanoi* tiene un alto porcentaje de fructificación (92%) típico de una especie que se autofecunda. En los mismos lugares, *Serapias lingua* tenía un porcentaje bajo de fructificación solamente 10%, típico de una orquídea con polinización cruzada (alogama) sin néctar y sin polen. El efecto-Allee no es aplicable en *Serapias perez-chiscanoi*. Pequeñas poblaciones de *Serapias lingua*, sin embargo, mostraban una fructificación muy baja, poblaciones medianas una fructificación alta y poblaciones grandes una fructificación baja otra vez. Poblaciones pequeñas pero alineadas (a lo largo de arcones de carreteras) y plantas a lo largo de los bordes de poblaciones grandes mostraban un porcentaje de fructificación alto. Esto es probablemente causado por el hecho de que insectos ingenuos rodean una población se posan en las plantas de los bordes y aprenden a evitar las plantas sin néctar si penetran la población. Debido al alarmante grado de desaparición de *Serapias perez-chiscanoi*, un plan de conservación de la especie necesita ser puesto en marcha de manera urgente para prevenir su extinción en un futuro cercano.

Introduction

In the spring of 2002 we did our research training for the Larenstein University of Velp (The Netherlands) in Extremadura (Spain). The aim of that project was to investigate the flora and fauna along the Ruta de la Plata (the Silver road) that runs from North to South through the autonomous province of Extremadura. We performed this project for ADENEX (Asociación para la DEfensa de la Naturaleza y sus recursos de EXtremadura), which is the biggest nature conservation organisation in Spain. Along the river basin of the Guadiana, we found the Green tongue-orchid (*Serapias perez-chiscanoi*) for the first time. We learned that *S. perez-chiscanoi* is a very rare species and that in the Guadiana river basin the number of populations is declining very rapidly, which immediately sparked our interest. We therefore decided to focus our research project on the conservation of this orchid.

Jose Luis Pérez Chiscano first discovered the species on the 30th of April 1976 near the small town of Santa Amalia (Badajoz, Extremadura). He named it *Serapias viridis* PEREZ

CHISCANO in 1988 due to its green appearance (*viridis* means green). After a subsequent study at the University of León by Acedo, it appeared that Da Conceição Vellozo had already in 1825 allocated the name *S. viridis* VELLOZO to a Brazilian orchid species, which is peculiar, because the genus *Serapias* only occurs in the Mediterranean zone. To prevent confusion, Acedo decided to rename the species *S. perez-chiscanoi* ACEDO, after the discoverer of the species (Acedo, 1990).

In this article, we report on plant characteristics, its reproduction, colour variances, distribution, taxonomic status of the species, habitat characteristics, differences to other *Serapias*-species, conservation and population structure.

Characteristics of *S. perez-chiscanoi*

The plants of *S. perez-chiscanoi* are (9-) 15-40 (-46) cm tall and usually contain two ovoid stolons. The 2-10 lanceolate leaves are 4 to 25 cm long and have a width of 0.4 to 2.0 cm. Young, vegetative plants have 2-5 (-6) leaves and older plants 4-10. The inflorescence is short, dense and 'twisted' and contains (1-) 3-11 (-13) rather large flowers that are spontaneously self-pollinating and often cleistogamous. The bracts are oval-lanceolate shaped and 32-52 mm long and 8-17 mm wide. The sepals have the same shape and are 20-26 mm long and 5-7 mm wide. The petals are 18-24 mm long, orbicular at the base and finely pointed at the tip. The labellum is 22-31 mm long and, due to a constriction, divided into the hypochile and the epichile. The hypochile is 6-10 mm long and 14-19 mm wide, its lateral lobes hidden inside the hood and with two separate, parallel lamellae at the base. The epichile (tongue) is oval-lanceolate with divergent edges and measures 16-22 mm long and 9-15 mm wide. The erect ovary is sessile and 10-22 mm long. The flowers of *S. perez-chiscanoi* bloom 2-3 weeks from the beginning of April until mid-May, depending on the weather.

Colour variations

The plants we found in the eleven populations can be divided into two groups, which can be described as the "green" and the "red" variation.

Of the green variation (page 48, photo 1 & cover photo), the leaves, stem, bracts, ovary, gynostegium, lamellae, flowers and veins are all pale green, with whitish hairs on the labellum. The lateral lobes of the flower are yellowish, greenish to light pinkish.

The red variation (page 48, photos 2 & 3) is also a greenish plant but with red venation on the leaves, stem, bracts and ovary. The gynostegium is green, like the green variation. The green flowers also have red veins and reddish hairs on the labellum and pink to reddish lateral lobes and lamellae. Also intermediate colour variations frequently occurred.

A couple of very strange plants were found near the hamlet of Aljucén. The three plants contained pink bracts, sepals, petals, gynostegium and pinkish flowers with whitish hairs (page 48, photo 4). The inflorescences were slightly lax, more resembling *S. cordigera*. Also, one plant contained flowers with mixed colours, the majority were pink but one of the top flowers was intensely red. The edges of the lamellae were slightly divergent, which is intermediate between *S. perez-chiscanoi* and *S. cordigera*. The ovaries were all swollen (seeds were forming) before the flowers had withered, suggesting self-pollination, similar to *S. perez-chiscanoi*. The leaves were completely green and rather broad, like *S. cordigera*. It

is very difficult to determine the taxonomic status of these plants. A possibility could be hybridisation between *S. perez-chiscanoi* and *S. cordigera*, because a lot of intermediate features were present.

Distribution

S. perez-chiscanoi is a very rare tongue-orchid, which, as far as we know, only occurs in the river basin of the Guadiana in Extremadura (Spain) (Perez Chiscano, 1988, Perez Chiscano *et al.*, 1991, Delforge, 1995, 2002) and adjacent Portugal (Jansen, 1993, Delforge, 2002, Caraça & Pereira, pers. comm., 2003). Perez Chiscano (1988) describes twelve populations in Extremadura scattered over the Guadiana river basin, but two years later Perez Chiscano *et al.* (1991) describe only ten populations, Keitel - personal communication with Pérez Chiscano in 1991 – presents only four populations (Keitel, 1991). The last fifteen years, several new populations were discovered but in the same period as many populations disappeared (Kreutz, 1995). Helped by information of Dr. Pérez Chiscano, we attempted to relocate all known populations but only found five of them. Because we could recognize the habitat of *S. perez-chiscanoi*, we drove by car along the river basin to search for new populations. At the end of our fieldwork period, we had located six new populations, bringing the total to eleven.

Reproduction

During our fieldwork we noted that some flowers of *S. perez-chiscanoi* didn't open at all, which makes cross-pollination impossible. All these flowers had a 100% fruit-setting however, suggesting cleistogamy (page 72, photo 1). It was also clear that insects very rarely visited the flowers of *S. perez-chiscanoi*, in contrast to other *Serapias*-species where we regularly saw small bumblebees. Other *Serapias*-species are pollinated by small bumblebees that use the hoods for shelter during bad weather, for passing the night and also by inexperienced bumblebees searching for nectar. The pollination of *S. perez-chiscanoi* takes place when the pollinia, which are placed at the back of the gynostegium, disintegrate on the stigma. This frequently occurs very rapidly, sometimes even before the flower has opened. After several weeks the seeds are formed and when the fruit opens the seeds are blown away by the wind. During the first year after germination they normally produce two small, narrow leaves and in following years they produce more and taller leaves and the plants can form vegetative shoots. The years before the first inflorescence is formed, several long leaves are produced. These leaves are always much longer than the leaves that are produced in the reproductive life stage. Probably, this occurs because the plant needs to ensure its survival by increasing its root system and vegetative biomass before flowering for the first time. After a couple of years, the first inflorescence appears which normally contains (1-2-) 3 flowers. More flowers are produced in subsequent years. When the seeds are ripe, the plant itself has completely withered. The plant survives the summer, autumn and part of the winter underground. *S. perez-chiscanoi* can also reproduce vegetatively by producing shoots. The maximum number of shoots found is six and these normally have a distance from the mother plant of approximately one to five centimetre.

Habitat

The habitat in which *S. perez-chiscanoi* occurs can be described as humid pastures. The soils of these pastures are very poor in nutrients. Our investigation shows an average Electric Conductivity of 48 $\mu\text{S}/\text{cm}^2$. The soils are rich in silica and slightly acidic, our investigation showing an average pH of 5.3.

The plant communities in which it occurs can be assigned to three alliances. *S. perez-chiscanoi* can be found frequently in vegetations of the *Agrostidion castellanae*-alliance. This group of plant communities is characterised by an open, slightly humid pasture, which almost always contains *Asphodelus ramosa* and *Briza maxima*. The second vegetation type, in which the species occurs less frequently, belongs to the *Molinio-Holoschoenion*-alliance. Vegetations of this alliance can be characterised by an open, humid pasture, which always contains species of the genera *Scirpus* (*Scirpus holoschoenus*) and *Juncus*. The third plant community, in which *S. perez-chiscanoi* can be found, is the *Tuberarion guttatae*-alliance. This community represents a more advanced successional stage than the first two. It comprises slightly humid, rather open pastures mixed with dwarf shrubs of the genera *Cistus* and *Lavendula*. The fourth plant community, in which the species occurs sporadically, belongs to the *Poetalia*-alliance. This can be characterised by an open, very humid pasture, which contains species of the genera *Isoetes* and *Vulpia*. *S. perez-chiscanoi* can be found at altitudes of up to 600 meters.

Taxonomic status of the species

Most authors describe *S. perez-chiscanoi* ACEDO as a close relative of *S. vomeracea* (BURMAN) BRIQUET (Perez Chiscano, 1988, Baumann & Kunkele, 1989, Perez Chiscano *et al.*, 1991, Delforge, 1995, 2002). It seems however, that *S. vomeracea* does not occur in southern-, mid-Spain and Portugal. In these regions, we are probably dealing with *S. strictiflora* WELWITSCH ex VEIGA (Tyteca, 1986, 1997), which is distributed from East-Algeria to Morocco, South-Portugal and Andalusia (Baumann & Kunkele, 1989, Delforge, 1995, 2002). The species, formerly identified in Portugal as *S. vomeracea*, was later recognised as a representative of another species, namely *S. mauretanica* SCHLECHTER, which name in its turn was a little later proved to be a synonym of *S. strictiflora* (Tyteca, 1997). We rarely found this species in Extremadura. *S. strictiflora* is intermediate between *S. lingua* L. and *S. parviflora* PARLATORE (Delforge 1995, 2002, Tyteca, 1997). It is characterised by a small and rather stretched, sometimes undulated epichile, large sepals and petals and with one deeply grooved swelling on the hypochile. Another recently described species is *S. stenopetala* MAIRE & STEPHENSON (Delforge, 1995, 2002), which is rather similar to *S. strictiflora* but has larger bracts, which are sometimes much taller than the hood. This species is intermediate between *S. lingua* and *S. strictiflora* (Delforge, 1995, 2002) and is also characterised by a deeply grooved swelling on the hypochile. Landwehr (1977) presents similar plants from Portugal, but describes them as striking, ecological forms of *S. vomeracea* subsp. *vomeracea*. *S. vomeracea*, however, always has two small, separate lamellae so probably we are dealing here with *S. strictiflora* and/or *S. stenopetala*. Delforge (1995, 2002) describes the distribution of *S. stenopetala* from the east coast of Algeria, to central Portugal and southern Spain.

The photographs used by Perez Chiscano *et al.* (1991) to illustrate *S. vomeracea* are certainly not typical and the specimens depicted are difficult to distinguish from *S. cordigera* L. (Tyteca, 1997). The absence of *S. vomeracea* in southern and mid-Spain and Portugal does not necessarily mean that *S. perez-chiscanoi* could not be closely related to *S. vomeracea*. The species could have differentiated in a separate area (i.e. through allopatric speciation), but morphologically it much more resembles *S. cordigera*. Because of this, we hypothesize that *S. perez-chiscanoi* is more closely related to *S. cordigera* than to *S. vomeracea*.

Kreutz (1995) describes yellow-flowering *Serapias*-plants from Barranco do Velho (Algarve, Portugal) and comes to the conclusion, like Jansen (1993), that they are not identical to *S. perez-chiscanoi* in Extremadura. Reasons are the yellow colour of the flowers, the intensely red epichile, the pinkish bracts, sepals and petals and the completely different habitat. Although Tyteca (1997) also presents the same *Serapias*-plants from Barranco do Velho (Algarve), he however, came to the conclusion that "all greenish, yellowish or pinkish, large-flowered *Serapias* plants from Extremadura as well as the Algarve represent no more than degenerate forms of *S. cordigera*, that at most deserve the status of form or variety".

Based on our observations in Extremadura we do not support Tyteca's conclusion. We do agree that *S. perez-chiscanoi* is closely related with *S. cordigera*, but we consider it a distinct species for several reasons. Firstly, *S. perez-chiscanoi* is 100% self-pollinating and *S. cordigera* is a typical cross-pollinator. Secondly, the habitat of *S. perez-chiscanoi* is completely different from that of *S. cordigera*. In all eleven populations we never saw *S. perez-chiscanoi* co-occurring with *S. cordigera*. *S. perez-chiscanoi* often grows together with *S. lingua* and sometimes with *S. parviflora*. *S. cordigera* is a very large, robust plant (up to 55 cm) with wider leaves, and bigger flowers with a very wide, intensely red-coloured, hairy labellum. The average height (329 plants) of *S. perez-chiscanoi* is only 24 cm. A characteristic of *S. perez-chiscanoi* is that it is often cleistogamous. This has never been observed in other *Serapias*-species in Extremadura, with the exception of *S. parviflora*. The inflorescence also differs; *S. perez-chiscanoi* has a short, dense, and 'twisted' inflorescence, whereas *S. cordigera* has a more stretched, more lax and 'straight' inflorescence. The gynostegium of *S. perez-chiscanoi* is always greenish, that of *S. cordigera* always red. Another difference is the angle of the flowers to the stem. *S. perez-chiscanoi* has flowers of which the labellum is normally pointing forwards (45 - 100 degrees with the stem) which gives the inflorescence a wide, pointed appearance. The inflorescence of *S. cordigera* is much more "slender", because the labellum is reflexed to a position parallel to the axis of the inflorescence. Finally, the colour differs a lot, although this is not necessary relevant because hypochromatic individuals (without colour) do occur in many *Serapias*-species in Spain and Portugal. The yellowish, greenish and pinkish flowering plants near Barranco do Velho probably consist of (semi) hypochromatic (incorrectly called degenerate by Tyteca) individuals of *S. cordigera*. *S. cordigera* (page 72, photo 2) normally has (at least in Extremadura) deep red – sporadically semi-hypochromatic pink – flowers, whereas those of *S. perez-chiscanoi* are always greenish.

It is, however, difficult to differentiate between most *Serapias*-species because some species have a similar appearance, some species are highly variable, like *S. lingua* (Kapteyn den Boumeester, 2001), and hybrids occur frequently. A thorough investigation, including a description of morphological characteristics, habitat, soil and genetic variation is necessary to get a better understanding of the taxonomic status of every species. For now, we consider *S. perez-chiscanoi* as a distinct species, because it has a very uniform and stable appearance.

We hypothesize that it has probably evolved from *S. cordigera* or maybe originates from a hybrid between *S. cordigera* and another *Serapias*-species.

Population structure

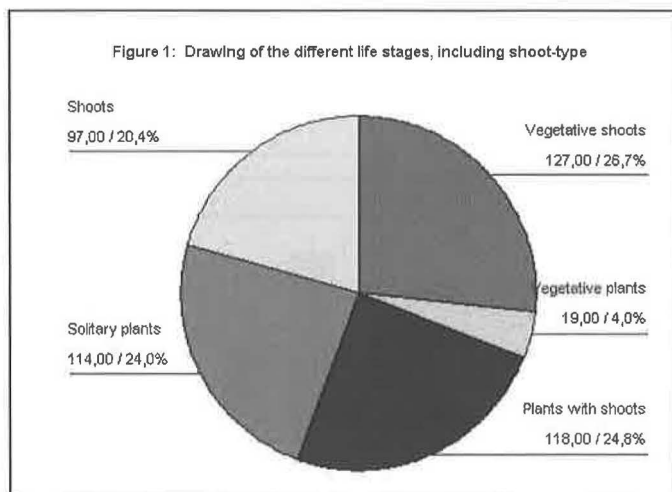
To get a better insight into the structure of the populations, we measured all plants of *S. perez-chiscanoi* in all populations. On the basis of these measurements, we distinguish three separate life stages:

1. Plants with vegetatively produced, flowering or non-flowering shoots (hereafter also referred to as motherplants)
2. Solitary plants (solitary flowering plants without vegetative shoot(s))
3. Vegetative plants (solitary non-flowering rosettes, without flowering stalk)

The shoots can be divided into two types:

- Flowering shoots (shoots with an inflorescence, found within 5 cm of a – generally larger – motherplant)
- Vegetative shoots (shoots without an inflorescence, found within 5 cm of a – generally larger – motherplant)

The number of motherplants (118 individuals) equalled the number of solitary flowering plants (114) (Figure 1). Solitary vegetative plants were very scarce, either because they were very difficult to locate or because there simply were few of them. The majority of the 224 shoots was vegetative (57%) and the rest was flowering (43%).



The size and the structure of the populations varies strongly with location (Figure 2). Only in the largest populations (Trujillanos, Obando and Valdecaballeros) solitary vegetative plants occurred, which suggests that there is recruitment of new individuals to these populations, a necessary requirement for longer-term viability. It is also obvious that the population near Valdebotoa does not have any solitary vegetative plants or shoots at all. The cattle density is very high at this site, introducing risks of direct damage by trampling and increased competition due to higher soil nutrient levels. Hence, without change in management, this population will probably soon disappear.

Variation among populations

The populations differed considerably in average plant height. Especially the plants in the population Badajoz were much taller than the rest (Figure 3), probably because of the surrounding agriculture. The adjacent areas were fertilised, which apparently also affects the habitat of this population. Probably due to higher soil nutrient levels, the vegetation was much higher than other vegetation types in which *S. perez-chiscanoi* occurred.

A lower number of flowers per plant can be a result of inbreeding, which occurs especially in small populations. We therefore expected that the largest populations would have more flowers per inflorescence. The number of flowers could also be correlated with habitat characteristics, such as vegetation structure, because higher vegetation

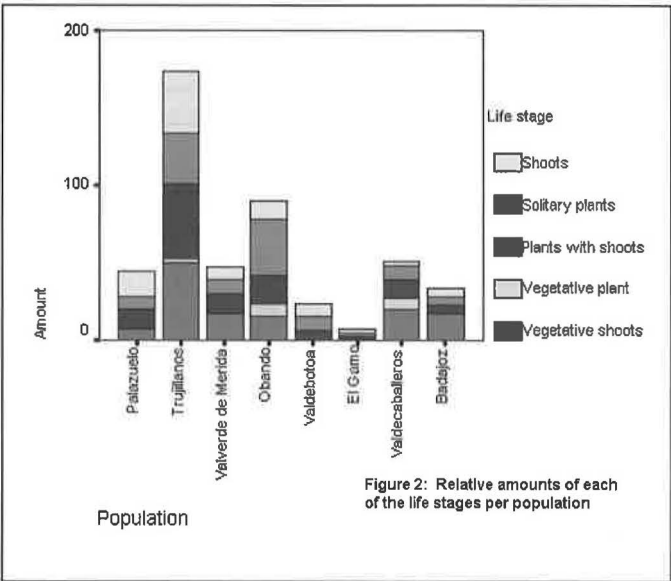


Figure 2: Relative amounts of each of the life stages per population

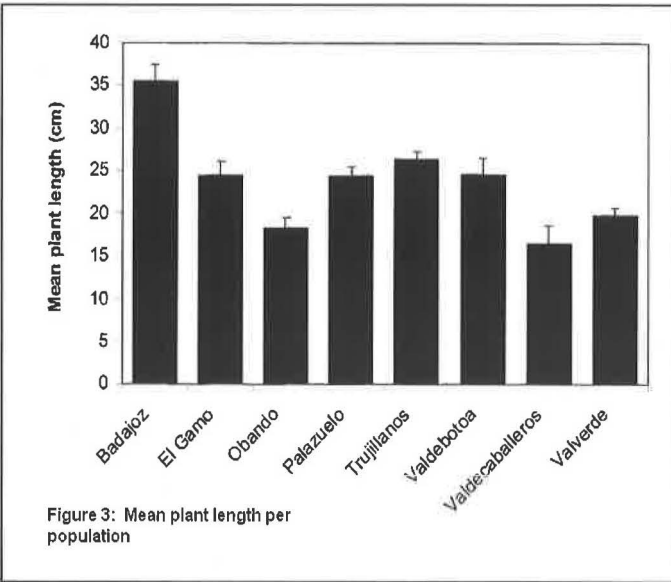


Figure 3: Mean plant length per population

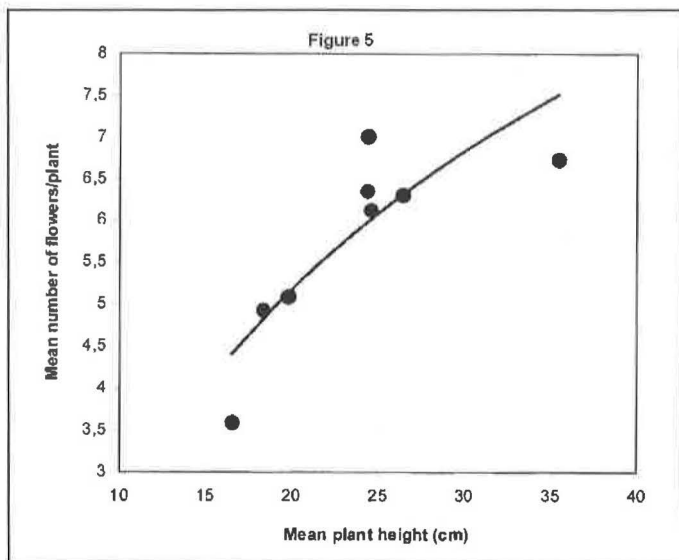
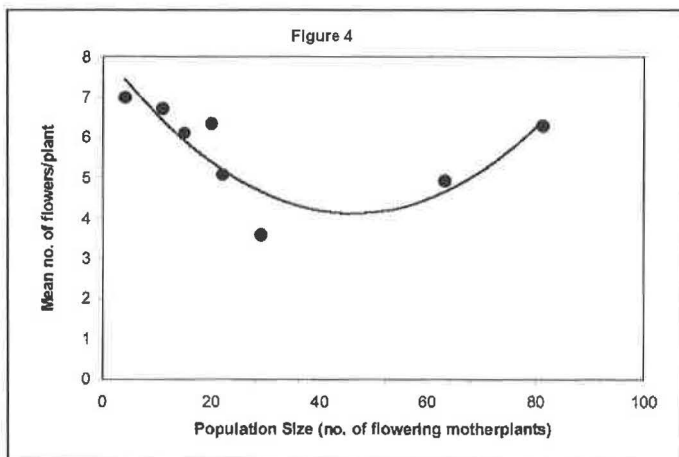
frequently results in taller plants that have to compete for light.

The results show a significant non-linear relationship, with many flowers per plant in the relatively small and large populations, and fewer flowers in the intermediately sized populations ($R^2 = 0.73$, $F_{[3, 5]} = 181.9^{***}$, Figure 4).

The mean plant height was positively correlated with the number of flowers per plant ($R^2 = 0.729$, $F_{[1, 6]} = 16.2^{**}$, Figure 5). Taller plants produce more flowers, which suggests that small and large populations contained taller plants than populations of intermediate size. This relationship was indeed observed ($R^2 = 0.447$, $F_{[3, 5]} = 57.3^{***}$). Small populations contain many large

plants due to sub-optimal habitat characteristics (such as tall and dense vegetation), which results in low recruitment, thus very few, small (young) plants with few flowers occur. Large populations contained large plants as well, because many mature, large mother-plants are present.

We also investigated differences in fruit-setting between the populations. It was rather difficult to find all the fruiting plants of *S. perez-chiscanoi* because of the height of the surrounding grasses. Eventually, we identified 202 inflorescences out of the total of 329 observed initially. The average fruit-setting (the proportion of flowers that form a ripe fruit with matu-



re seeds) was 92%. If *S. perez-chiscanoi* is indeed 100% self-pollinating, we would expect a 100% fruit-setting. There are two reasons why not all of the flowers formed fruits. Firstly, the top flowers often withered and secondly there was some predation by caterpillars.

We compared the fruit-setting of *S. perez-chiscanoi* with that of the cross-pollinating *S. lingua*, which abundantly grows in the same region. The average fruit-setting (determined on 191 individuals in seven populations) of *S. lingua* was only 10%. To test whether the Allee-effect (i.e. small and sparse populations have more reproductive problems due to a lack of pollinator visits) applied to *S. perez-chiscanoi* and/or *S. lingua*, we investigated the correlation between population size and fruit-setting. In *S. perez-chiscanoi* we didn't expect a correlation because it is largely self-pollinating and not dependent on insect pollinators and also because it is not likely that flower withering and generalist caterpillar herbivory are correlated with population size. The analyses show indeed that there is no correlation between the parameters, so there is no evidence for an Allee-effect. In populations of *S. lingua*, we observed (1) that small populations had a relatively low fruit-setting, (2) that intermediately sized and small and linear populations showed a relatively high fruit-setting, and (3) that very large populations again had a low fruit-setting. In the large populations, we observed that more flowers were pollinated at the borders than in the interior of the population. The main reason for this probably is that unexperienced pollinators and bumblebees in search for shelter, approach the populations from the edge, visit a few plants, learn that there is no reward and then fly away. Plants in the interior will then receive much fewer visits and show an accordingly lower fruit-setting. In very large populations of the cross-pollinating *Orchis morio*, Oostermeijer *et al.* (2000) also observed a low fruit-setting, and attributed this to a relatively low population size of the pollinators (bumblebee queens) compared to that of the orchids.

In the field we observed that the relative frequencies of the two colour variations of *S. perez-chiscanoi* were related to population size. A significant correlation showed that the largest populations predominantly contained the green colour variation and the smallest populations mainly red plants.

Conservation

At this moment the species is officially protected, but hardly any political means are available to maintain the law. This can easily be discerned from the eight populations that vanished in only three years, from 1988 to 1991. We were able to establish that at least five of them vanished due to altered land-use, fertilisation and livestock grazing. Also the population of the locus classicus near the hamlet Santa Amalia disappeared due to intensified livestock grazing. All present populations are relatively small, varying from four to seventy-five plants. The areas assigned to Natura 2000 in Extremadura only contain two populations of *S. perez-chiscanoi*, of which one is the smallest of all (only four plants). On the list of protected species of Extremadura 2001, compiled by the Government of Extremadura, *S. perez-chiscanoi* is included in the category 'close to extinction', which specifies that a protection plan is necessary to prevent extinction. During the spring of 2003 a protection plan was not yet available, so we have performed a field study on this species, to obtain a better insight into its habitat, population structure and reproduction. At the end of our study we came up with several plans and ideas and these have been discussed with members of ADENEX. In the autumn of 2003, an extended and profound protection plan has been

delivered to the Government of Extremadura, in which it is advised on the best ways to conserve this species. We hope the Government will execute this protection plan in order to save the species from extinction.

Other *Serapias*-species in Extremadura

***Serapias parviflora* PARLATORE**

Serapias parviflora is rather abundant in Extremadura. It sometimes grows together with *S. perez-chiscanoi* and needs a slightly drier habitat than other *Serapias*-species. We found very little variation in this species.

***Serapias cordigera* L.**

Serapias cordigera is much rarer than *S. lingua* and *S. parviflora* in Extremadura. We found this species at several locations and it has a very uniform appearance. It can be distinguished from other species due to its robust appearance. It is a large plant with large, red, hairy flowers, a broad labellum and broad leaves. We also found several peculiar plants in a population without any other *Serapias*-species near Obando; these plants had rather large flowers, an intense red but more slender labellum. These plants were probably of hybrid origin between *S. cordigera* and another *Serapias*-species, and correspond with the specimens described by Perez Chiscano et al. (1991) under the name *S. vomeracea* subsp. *vomeracea*.

***Serapias lingua* L.**

Serapias lingua is the most abundant species in Extremadura and can be found wherever undisturbed and humid pastures occur. This species is extremely variable in colour as well as in size. It is characterised by one single swelling on the hypochile. We observed plants with completely white and yellow flowers, pink flowers, pink flowers with red veins and red flowers. We also found a strange plant of *S. lingua* of which all flowers contained two labellae.

***Serapias lingua* L. var. *distenta* PRESSER**

S. lingua var. *distenta* does not have a normally formed hood because it has a huge deformation of the sepals, giving the flowers a very strange appearance (page 72, photo's 3 and 4). The deformed sepals are a combination of a normally formed sepal and half a part of the hypochile; sometimes even fifty percent of the grooved swelling is visible. Because of this deformation, the hood that is so characteristic for the *Serapias*-species does not exist, giving these plants a rather graceful appearance. In Extremadura, we found this feature in three very distinct populations (La Roca de la Sierra, Trujillanos and Obando). The population near La Roca de la Sierra contained precisely thirty plants, which varied from ten to thirty cm in length. The plants were present in an area of about 3 m². The inflorescences contained three to six striking flowers, which is slightly more than the normal for *S. lingua*, which generally has two to four, sometimes five flowers in the same region of Extremadura. The population in Obando contained about fifteen and in Trujillanos about fifty plants. We also found this deformation in *S. stenopetala* and in *S. strictiflora* (like the original description by Presser) in mid-east Portugal.

***Serapias strictiflora* WELWITSCH ex VEIGA**

Serapias strictiflora is very rare in Extremadura. As shown above, it probably originated by hybridisation between *S. lingua* and *S. parviflora*. We found this species near *S. lingua* but it can be distinguished by a very slender labellum and a deeply grooved swelling on the hypochile. The bracts are rather short (17 to 35 mm) and the sepals and petals very long (16 to 25 mm) (Delforge, 1995, 2002). The long sepals and petals cause a bend just where the lateral lobes end. We only found a couple of plants in two different populations. The first population is located in the centre of Extremadura near Valverde de Merida, where we only found two plants. The second population is located in the east of Extremadura near Obando, where we found three individuals. All plants were characterised by a very lax inflorescence containing one to three flowers, like Delforge (1995, 2002) who describes one to four (-five) flowers.

***Serapias stenopetala* MAIRE & STEPHENSON**

We found *S. stenopetala*, though rare, in Extremadura together with *S. strictiflora*. We have also seen this species more abundantly in several populations in Portugal (near Lisbon and Evora), where it also grew together with *S. strictiflora*. It can be distinguished from other *Serapias*-species by its rather slender epichile (though broader than *S. strictiflora*) and a deeply grooved swelling on the hypochile. The sepals and petals are 14 to 21 mm long and the bracts can be large, up to 50 mm (DELFORGE, 1995, 2002). The inflorescence contains more flowers than *S. strictiflora*; DELFORGE (1995, 2002) describes three to eight (-thirteen) flowers per inflorescence. We also found many plants that contained characteristics of *S. strictiflora* as well as *S. stenopetala*, which makes distinguishing between these species sometimes very difficult. Some specimens also showed similarities to *S. vomeracea*, which we did not find in Extremadura and Portugal.

References

- Acedo, C. 1990. *Serapias perez-chiscanoi*, nom. nov. Anales del Jardin Botanico de Madrid 47 (2): 510.
- Baumann, H. & S. Kunkeler. 1989. Die Gattung *Serapias* L.- eine taxonomische Übersicht. Mitteilungsblatt Arbeitskreisen Heimische Orchideen, Baden-Wurttemberg, Jahrgang 21 (3), 701-946.
- Da Conceição Vellozo, F.J.M. 1825. Orchidaceae *Serapias viridis* VELL. (*Spirantheos* sp.). Florae Fluminensis (9), table 49.
- Delforge, P. 1995. Orchids of Britain & Europe. Harper Collins Publishers, London.
- Delforge, P. 2002. Guia de las Orquideas de Espana y Europa. Lynx Edicions, Barcelona.
- Jansen, H. 1993. *Serapias viridis* PEREZ CHISCANO in Portugal!. Ber. Arbeitskreisen Heimische Orchideen, Jahrgang 10 (1), 50-53.
- Keitel, C. 1991. *Serapias viridis* PEREZ CHISCANO, *Dactylorhiza maculata* ssp. *caramulensis* VERMEULEN und andere interessante spanische Orchideearten. Mitteilungsblatt Arbeitskreisen Heimische Orchideen, Baden-Wurttemberg, Jahrgang 23 (1), 107-121.
- Kapteyn den Boumeester, D.W. 2001. Variabiliteit van *Serapias lingua* op een standplaats bij Grazalema (Andalusië, Spanje). Eurorchis 13: 41-44.
- Kreutz, C.A.J. 1995. Enkele groeiplaatsen van *Serapias* in Portugal en Spanje. Eurorchis 7, 90-96.

- Landwehr, J. 1977. Wilde Orchideeën van Europa. Deel 2. Vereniging tot Behoud van Natuurmonumenten in Nederland, 's-Graveland.
- Oostermeijer, J.G.B., S.H. Luijten, T. Petanidou, M. Kos, A.C. Ellis-Adam & J.C.M. den Nijs. 2000. Pollination in rare plants: is population size important? Det Norske Videnskaps-akademi. I. Matematisk Naturvidenskapelige Klasse, Skrifter, Ny Serie 39, 201-213.
- Perez Chiscano, J.L. 1988. Nueva especie de *Serapias* L. en Extremadura (España). Monografías del Instituto Pirenaico de Ecología, Homenaje a Pedro Montserrat, Jaca y Huesca, 305-309.
- Perez Chiscano, J.L., J.R. Gil Llano & F. Duran Oliva. 1991. Orquídeas de Extremadura. Fonda Natural, Madrid.
- Presser, H. 1997. Eine interessante *Serapias*-Sippe in Portugal. Ber. Arbeitskreisen Heimische Orchideen, Jahrgang 14 (1), 75-76.
- Tyteca, D. & B. 1986. Orchidees de Portugal – 11. Esquisse systematique, chlorologique et cartographique. Les Naturalistes Belges 67, 163-192.
- Tyteca, D. 1997. The orchid flora of Portugal. Journal Europäischer Orchideen, Vol. 29 (2/3), 267-271 and 356-359.

Acknowledgements

We would like to thank Marisela Cornado Garcia for her help with translating our final report and the summary of this article from English to Spanish, and for being such an excellent interpreter during our meetings with members of the 'Junta de Extremadura' and ADENEX. Dr. Hans den Nijs kindly commented on an earlier version of this article.

Correspondence address:

Caspar Venhuis
 3e Goudsbloemdwardsstraat 21
 1015 KA Amsterdam
 The Netherlands
 0031-020-3301837
 casvenhuis@hotmail.com

