

Morphological systematics of *Serapias* L. (Orchidaceae) in Southwest Europe

C. Venhuis, P. Venhuis, J. G. B. Oostermeijer, and P. H. van Tienderen

Institute for Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam, Amsterdam,
The Netherlands

Received January 27, 2006; accepted January 18, 2007

Published online: April 30, 2007

© Springer-Verlag 2007

Abstract. We measured morphological characters and relative DNA contents to assess variation and phylogenetic relationships among *Serapias* species in three populations of each of the 10 putative taxa that occur in Southwest Europe. DNA contents indicated diploidy for most species, except for tetraploid *S. lingua* and hexaploid *S. olbia*. Multivariate (discriminant) analyses yielded two main groups: a small-flowered *S. parviflora* group and a large-flowered *S. vomeracea* group. Within the *S. parviflora* group, *S. elsae* should be considered a large-flowered variation of *S. strictiflora*. The geographically disjunct *S. gregaria* and *S. strictiflora* are probably different taxa. In the *S. vomeracea* group, analyses suggest that *S. neglecta* and *S. cordigera* are closely related. *Serapias cordigera* from the southwestern coast of the Iberian Peninsula is probably a subspecies, *S. perez-chiscanoi* was separated from all other species and *S. occidentalis* was morphologically intermediate between *S. cordigera* and *S. vomeracea*, suggesting a hybrid origin, with the latter two taxa as parents.

Key words: Mediterranean, morphology, orchids, phylogeny, self-pollination, systematics, taxonomy.

The number of recognised species in the orchid genus *Serapias* L. varies strongly among authors. Delforge (2002) recognised 26 species,

while Sundermann (1980) only mentioned three. The distribution is predominantly Mediterranean. Its range extends from the Azores and the Canaries in the west to the Caucasus in the east, and as far north as Brittany (France) (Gölz and Reinhard 1980; Perez Chiscano et al. 1991; Delforge 1995, 2002). The genus is considered monophyletic, based on genetic (Pridgeon et al. 1997; Bateman et al. 1997, 2003) and morphological differences (Delforge 1995, 2002). *Serapias* is represented in southwest Europe (Iberian Peninsula and southern France) by ten species (Delforge 2002) (Table 1). Taxa within the genus are morphologically still poorly defined. Systematic studies based on morphology have utilized both quantitative and qualitative characters (e.g. Baumann and Künkele 1989, Martine and Gerbaud 1998). However, a detailed study of the morphological variation, using multivariate analysis of many traits, has not previously been undertaken.

Serapias is also poorly studied at the molecular genetic level. Aceto et al. (1999) concluded that, although variation for floral characters and molecular markers corresponded closely, the phylogenetic patterns within the genus could not be resolved with

the molecular markers used (ITS). Pellegrino et al. (2001) isolated and characterised micro-satellite loci from *S. vomeracea* that could prove applicable to all *Serapias* species.

The aims of the present study are: (1) to determine the taxonomic status of the *Serapias* species, as described by Delforge (2002, 2004), from the Iberian Peninsula and southern France on the basis of a large number of morphological characters, (2) to determine variation in quantitative and qualitative characters within and among the putative taxa, (3) to determine the relative DNA content and thereby infer information on their ploidy level, and (4) to explore the phylogenetic relationships among the species based on morphological characters.

Materials and methods

Approach. We sampled three populations, each counting 25 specimens, of all ten presently recognised taxa by Delforge (2002, 2004) as occurring in Spain, Portugal and southern France. In addition, we sampled plants of the recently described *S. occidentalis* C. Venhuis & P. Venhuis (Venhuis et al. 2006). So, in total 75 specimens per taxon have been sampled.

Table 1. Groups and species recognised in the genus *Serapias* in southwest Europe by Delforge (2002)

Groups	Species
<i>S. parviflora</i> group	<i>S. parviflora</i> Parlatore
<i>S. vomeracea</i> group	<i>S. vomeracea</i> (Burman) Briquet
	<i>S. cordigera</i> L.
	<i>S. neglecta</i> De Notaris
	<i>S. perez-chiscanoi</i> Acedo
<i>S. lingua</i> group	<i>S. lingua</i> L.
	<i>S. olbia</i> Verguin
	<i>S. gregaria</i> Godfery
	<i>S. strictiflora</i> Welwitsch ex da Veiga
	<i>S. elsaе</i> Delforge

Morphology. A total of 23 vegetative and floral characters were recorded for each specimen. Fifteen of these characters were measured quantitatively (Table 2) and eight qualitatively (epichile -curvature, -hair distribution, -shape, -position and lamellae position, hood position, petal shape and the bract/hood length ratio), using pre-made categorical verification lists to avoid subjective and gradual changes over time in scoring procedures.

Multivariate statistical analyses. To determine whether the measured morphological characters could significantly discriminate among the taxa presented by Delforge (2002) we used a Discriminant Analysis. This approach maximises the variance among relative to within predefined groups and tests which of the measured characters contributes significantly to the discriminating functions.

DNA content. We sampled leaves of five plants per population, which were analysed with a Partec Flow Cytometer, following the standard preparation and staining procedures. Trout fluorescent erythrocytes were added to the analysed solution as a reference. The outcomes of the analyses were plotted in a frequency histogram. The histogram displays relative fluorescence intensity versus the number of cells. The results have been compared with ploidy levels based on previous chromosome counts on *S. perez-chiscanoi* (diploid, $2n = 36$) by Perez Chiscano (1988) and on *S. lingua* (tetraploid, $2n = 72$) by D'Emerico et al. (1998).

Phylogeny. To explore the putative phylogenetic relationships among the study species, we used the computer program Phylip 3.61. Within Phylip, we used the procedure CONTML, which was especially designed for continuous characters and employs the Maximum Likelihood method, based on the Brownian motion model. The analysis was based on averaged floral dimensions (length and width of bracts, hypochile and epichile, length of sepals and number of flowers) and on four qualitative flower characters (epichile position, shape of the lamellae, petal shape, and bract to hood length ratio). We used *Anacamptis palustris* (Jacq.) R.M. Bateman, Pridgeon and M.W. Chase, which is closest related to the genus *Serapias* (Aceto et al. 1999, Bateman et al. 2003), as the outgroup in our analyses. However, the long branch length subtending the outgroup makes the rooting of the tree not very reliable. It was sufficient to use one outgroup only, because *Serapias* was already known to be monophyletic.

Table 2. Pooled within-group correlations between the quantitative morphological characters and standardized canonical discriminant functions for the main analysis on all species, and the two separate analyses on each of the two main groups yielded by the first analysis. The largest absolute correlations between each variable and any discriminant function are indicated in bold.

	Analysis on all species			Subanalysis <i>S. parviflora</i> group		Subanalysis <i>S. vomeracea</i> group		
	DA1	DA2	DA3	DA1	DA2	DA1	DA2	DA3
	Eigenvalue	7.01	2.71	1.32	2.90	1.68	4.45	2.94
% Variance Explained	54.5	21.1	10.2	46.9	27.2	48.4	32.0	14.4
Epichile width	0.847	0.047	0.208	0.601	-0.091	-0.260	0.538	0.355
Hypochile width	0.607	-0.140	0.080	0.407	-0.109	-0.392	0.350	0.171
Epichile length	0.535	-0.237	0.335	0.486	0.272	-0.305	0.092	0.122
Hypochile length	0.296	-0.576	0.364	0.429	0.544	-0.609	-0.068	0.362
Bract width	0.189	-0.023	0.672	-0.075	0.420	-0.026	-0.276	0.261
Plant height	0.003	0.171	0.646	-0.179	0.464	0.215	-0.140	0.516
Bract length	0.139	0.040	0.558	-0.164	0.327	0.020	-0.156	0.201
Sepal length	0.355	-0.105	0.499	0.227	0.303	-0.121	-0.062	0.315
Mean length rosette leaves	0.049	0.156	0.471	-0.184	0.300	0.132	-0.010	0.520
Number of rosette leaves	0.076	0.248	0.368	-0.065	0.147	0.241	0.047	0.353
Number of flowers	0.151	0.272	0.215	-0.186	-0.028	0.188	0.047	0.036
Number of stem leaves	0.094	0.289	0.198	-0.100	0.021	0.304	0.054	0.024
Mean width rosette leaves	0.109	0.047	0.262	-0.191	0.030	-0.031	-0.141	0.056
Ovary length	0.016	0.041	0.312	-0.166	0.288	0.010	-0.044	0.179
Petal length	0.345	-0.133	0.446	0.228	0.267	-0.151	-0.078	0.248

Results

Morphology: qualitative characters. Most qualitative morphological character states were constant within populations and species. The bract length to hood length ratio, however, varied strongly within and between populations and species. It was always less than 1 (i.e. bract shorter than the hood) for *S. lingua*, and varied within all other sampled populations. On average, most species were characterised by bracts that were shorter than the hood. Exceptions were two populations of *S. parviflora* from the Iberian Peninsula, all populations of *S. vomeracea* subsp. *vomeracea*, and two populations of *S. strictiflora*. The shape and position of the lamellae were constant within all species. *Serapias parviflora*, *S. perez-chiscanoi*, *S. vomeracea* subsp. *vomeracea* and *S. neglecta* had (almost) parallel and rather widely spaced lamellae (Fig. 1). Lamellae of *S. occidentalis* were almost parallel, but were placed much closer together. *Serapias cordigera* had

divergent lamellae, while *S. lingua* had an entire or emarginated swelling. The stigmatic surface of *S. olbia*, *S. gregaria*, *S. strictiflora* and *S. elsae* contained a deeply grooved swelling, which was slightly variable in shape and serration (Fig. 1).

Morphology: discriminant analyses. Discriminant analyses on morphometric data showed separation between two main groups:

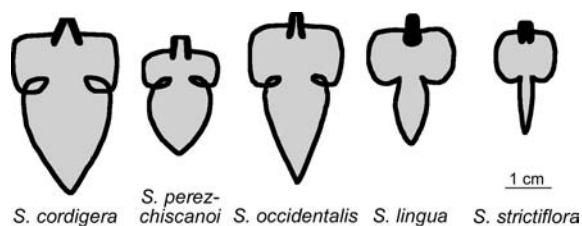


Fig. 1. Labellum and lamellae shapes of *S. cordigera*, *S. perez-chiscanoi*, *S. occidentalis*, *S. lingua* and *S. strictiflora* based on averaged measurements. The lamellae are positioned at the centre of the labellum on the upper part of the hypochile

one comprising *S. parviflora*, *S. lingua*, *S. olbia*, *S. gregaria*, *S. strictiflora*, and *S. elsae* (= *S. parviflora* group), and the other including *S. vomeracea* subsp. *vomeracea*, *S. neglecta*, *S. occidentalis*, *S. cordigera* and *S. perez-chiscanoi* (= *S. vomeracea* group) (Fig. 2a, b). Three functions, each with an Eigenvalue exceeding one, together explained 85.8 % of the total morphological variance (Table 2). The first function distinguished the groups on the width of the epichile and hypochile, and the length of the epichile (Table 2). The second function characterised the length of the hypochile, while the length and width of the bracts, the length of the sepals and plant height contributed to the third function (Table 2).

Discriminant analysis of the *S. parviflora* group separately showed a close resemblance between *S. gregaria* and *S. lingua*, and between *S. strictiflora* and *S. elsae* (Fig. 3a). To further clarify the morphological differentiation of *S. olbia*, we performed a discriminant analysis on species from the *S. vomeracea* group from southern France, combined with *S. olbia* and *S. lingua*. This analysis showed that *S. olbia* is similar to *S. vomeracea* subsp. *vomeracea* and *S. lingua* (Figure not shown).

Separate discriminant analysis on the *S. vomeracea* group showed morphological differentiation between *S. cordigera* and *S. neglecta* and between *S. vomeracea* subsp. *vomeracea* and *S. occidentalis* for the first and third function, and a close resemblance for the second function (Fig. 3b, c). *Serapias perez-chiscanoi* was clearly separated from the other taxa for most functions (Figs. 2a, 3b, c).

Relative DNA content. Analyses of all species resulted in two or three fluorescence peaks due to endoreduplication; the first peak was considered to represent the unreplicated nuclear DNA contents. The trout reference DNA showed a DAPI fluorescence peak around 20 partec, the first significant fluorescence peak of *S. perez-chiscanoi* was found at approximately 50 partec, and the first significant fluorescence peak of *S. lingua* at around 100 partec. *Serapias parviflora*, *S. vomeracea* subsp. *vomeracea*, *S. neglecta*, *S. cordigera*,

S. occidentalis, *S. gregaria*, *S. strictiflora* and *S. elsae* all showed fluorescence peaks around 50 partec, whereas *S. olbia* had the first significant fluorescence peak around 150 partec. Interpretation of the fluorescence peaks, combined with the previously known ploidy levels and chromosome numbers of *S. perez-chiscanoi* (diploid, $2n = 36$) and *S. lingua* (tetraploid, $2n = 72$), suggests that *S. parviflora*, *S. vomeracea* subsp. *vomeracea*, *S. neglecta*, *S. occidentalis*, *S. cordigera*, *S. perez-chiscanoi*, *S. gregaria*, *S. strictiflora* and *S. elsae* are all diploid, with $2n = 36$, *S. lingua* is a tetraploid with presumably $2n = 72$, and *S. olbia* is a hexaploid, with presumably $2n = 108$.

Phylogeny. The morphological analyses give some insight into the possible evolutionary relationships among the studied *Serapias* species. Phylogenetic analysis of the morphological data-matrix resulted in two groups, suggesting that two distinct lineages have evolved within the genus (Fig. 4). One group contains the large-flowered species (*S. vomeracea* group) and the other four small-flowered species (*S. parviflora* group). *Serapias lingua* and *S. olbia* are placed near to the large-flowered group, most likely due to their relatively large flowers. Based on this tree, *S. cordigera* and *S. neglecta* and both *S. strictiflora* and *S. elsae* are morphologically highly similar. According to the analysis, *S. occidentalis* is intermediate between *S. vomeracea* subsp. *vomeracea* and *S. cordigera*.

Discussion

Grouping. The groups that emerged from the discriminant analyses (Fig. 2a, b) and the phylogenetic reconstruction based on the morphological characters (Fig. 4) were not concordant with the taxonomic classifications postulated by Baumann and Künkele (1989) and Delforge (1995, 2002). Instead of three groups (a *S. parviflora* group, a *S. vomeracea* group and a *S. lingua* group: Delforge 1995, 2002) or four groups (Baumann and Künkele 1989), only two main groups could be distinguished: a *S. parviflora* group and a

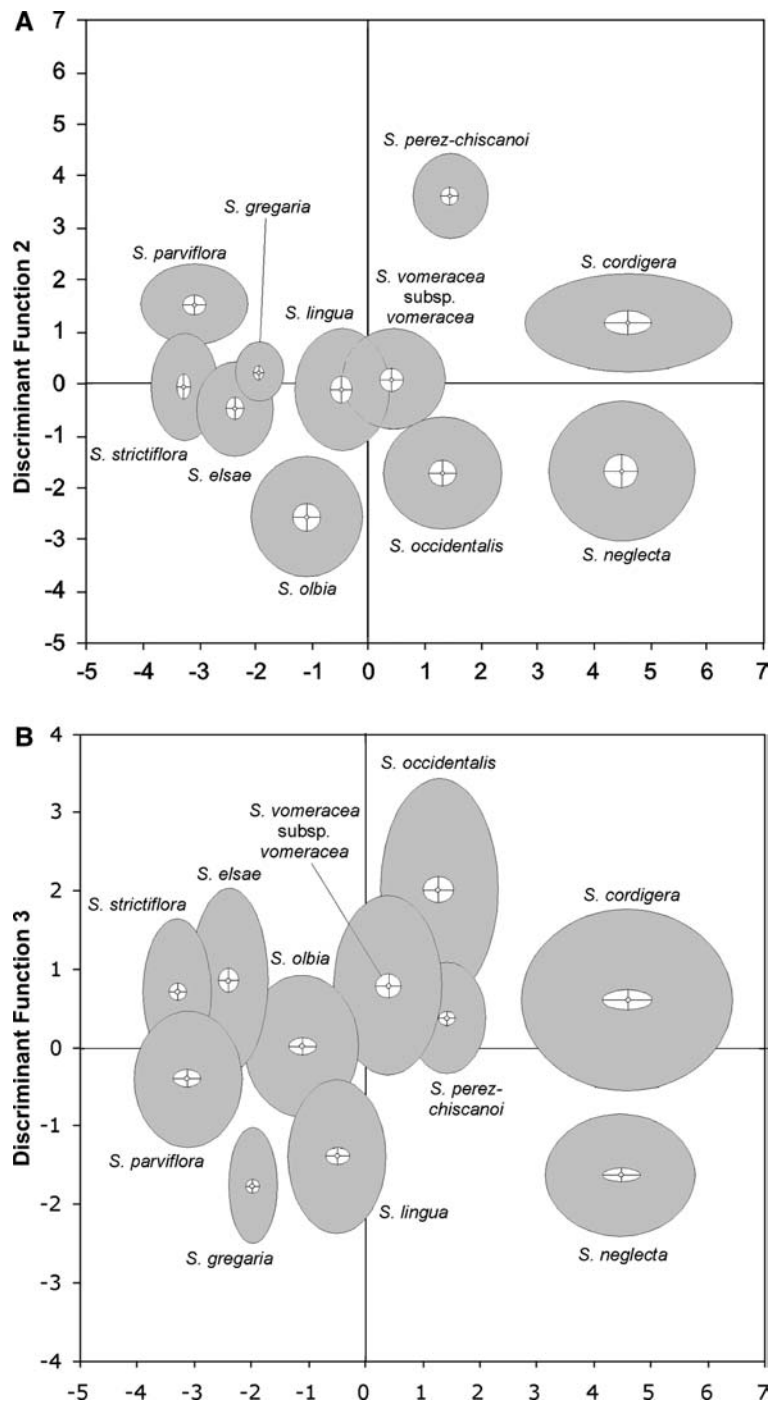


Fig. 2. Discriminant analyses with 95% confidence intervals (inner circle) and standard deviation (outer, grey circles) of: **A** all sampled *Serapias* species, first and second discriminant functions; **B** all sampled *Serapias* species, first and third discriminant functions

S. vomeracea group. These groups were predominantly separated on the basis of the epichile width (Table 2).

Below, we discuss the possible relationships between the different taxa based on our results.

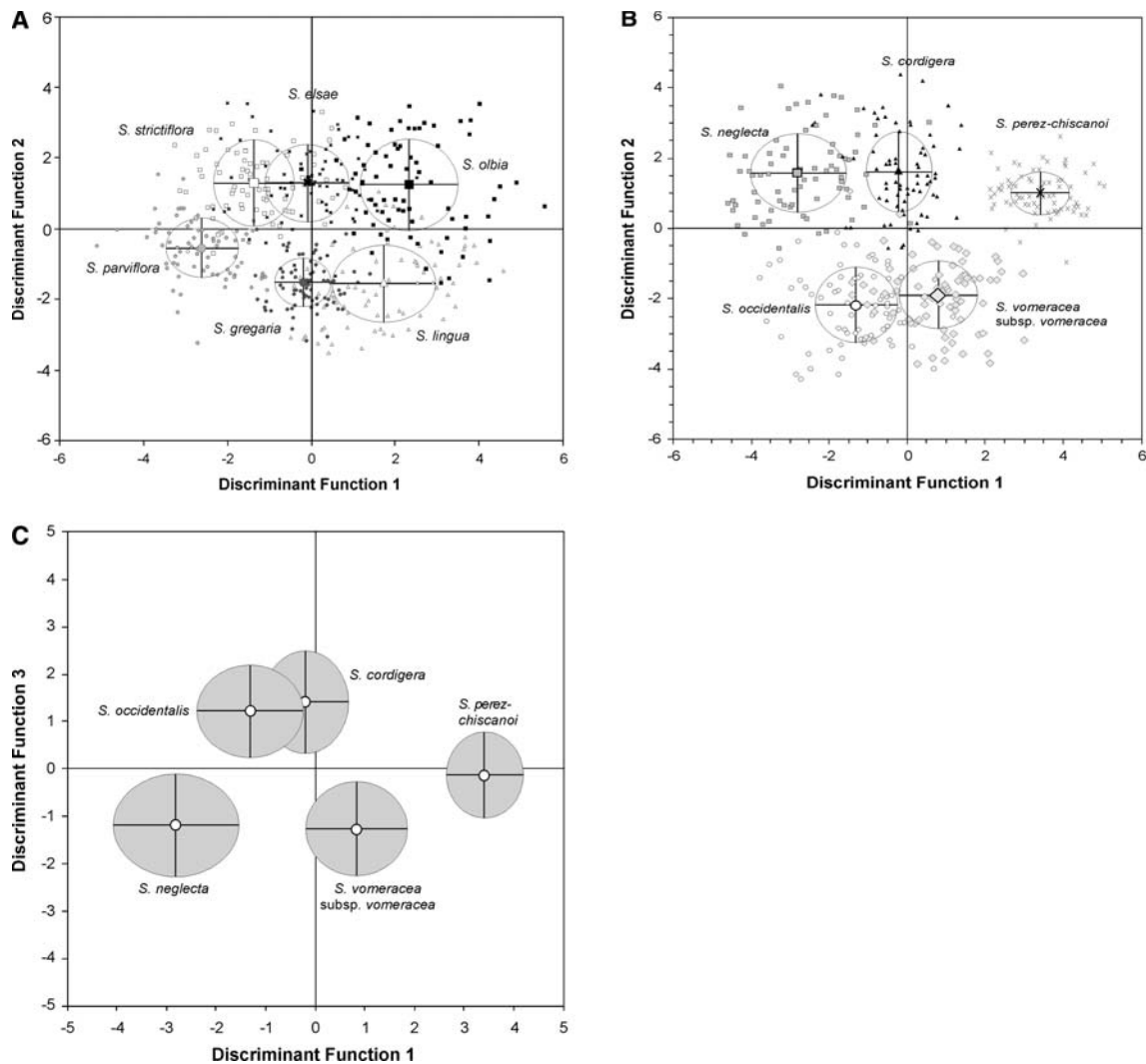


Fig. 3. Separate discriminant analyses of the two main groups, showing the scatter of the original samples and the standard deviation (indicated by the circles around the centroids of each species, centroids have the same symbol as the sample points) **A** of the *S. parviflora* group; **B** of the *S. vomeracea* group, first and second discriminant functions; **C** the *S. vomeracea* group, first and third discriminant functions (only centroids with standard deviations (grey circles) shown)

***S. parviflora* group: *S. lingua* and *S. parviflora*.** Bateman et al. (2003) suggested that *S. lingua* could be an autopolyploid derivative of *S. parviflora*. This hypothesis is not supported by our discriminant analyses (Fig. 2a, 3a). *S. parviflora* differs from *S. lingua* for two discriminant functions and has two parallel lamellae on the base of the hypochile, whereas *S. lingua* generally has a single swelling (Fig. 1). More likely candidates are *S. stricti-*

flora (which differs in only one discriminant function, and also has a single (albeit grooved) swelling on the base of the hypochile), and *S. vomeracea* (largely similar for all discriminant functions, Fig. 2a, but with two lamellae). *Serapias olbia*, *S. strictiflora*, *S. gregaria* and *S. elsaе* belong to the *S. parviflora* group, in addition to *S. lingua*.

***S. olbia*.** On the basis of the observed relative DNA contents, we conclude that

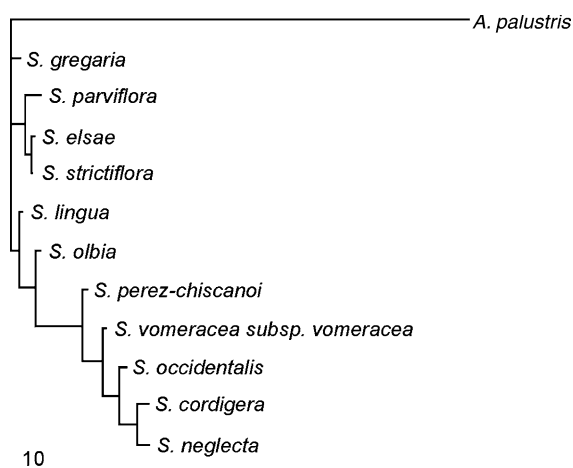


Fig. 4. Phylogenetic relationships between all sampled *Serapias* species inferred as by the CONTML algorithm (PHYLIP 3.61). Two groups can be distinguished: a *S. parviflora* group and a *S. vomeracea* group. *Serapias lingua* and *S. olbia* in between these groups, but closer to the *S. vomeracea* group, due to their relatively large flowers. Horizontal bar indicates the scaling for the branch lengths (the expected accumulated variance under the assumed model of independent, ‘Brownian’ divergence)

S. olbia has a polyploid background, and – to our knowledge – is the first hexaploid *Serapias* species. It probably has evolved by hybridization between tetraploid *S. lingua* and another, diploid, parental species. Baumann and Künkele (1989) and Delforge (1995, 2002) suggested *S. cordigera* as the diploid parent. According to Delforge (1995, 2002), *S. olbia* is a species with primitive characters, which is basal to the two main evolutionary branches, with *S. lingua* on one side and *S. cordigera* on the other. However, *S. cordigera* and *S. olbia* are morphologically dissimilar, except for the red-purple epichile colour. Our morphometric analysis rather suggests *S. vomeracea* subsp. *vomeracea* as the diploid parent species of *S. olbia* (figure not shown), in concordance with Schlechter (1923).

***S. strictiflora* and *S. gregaria*.** Delforge (1995, 2002) considered *S. strictiflora* and *S. gregaria* as true species, that probably both evolved from hybrids between tetraploid *S. lingua* and diploid *S. parviflora*. Interpretation of the relative DNA content suggests that

both species are diploid, which therefore excludes the tetraploid *S. lingua* as a likely parent. Martine and Gerbaud (1998) argued that *S. strictiflora* and *S. gregaria* should be considered conspecific, but their analyses used only three floral characters. Our discriminant analyses (Fig. 3a) show that *S. strictiflora* differs from *S. gregaria* in plant height (212 versus 113 mm, respectively), hypochile length (9.1 versus 7.8 mm), sepal length (19.8 and 14.8 mm) and epichile width (4.7 versus 5.4 mm). The analyses show that *S. gregaria* more closely resembles *S. lingua* and that *S. strictiflora* is more similar to *S. parviflora*. Our phylogenetic analyses also show that *S. strictiflora* is very similar to *S. parviflora*, whereas *S. gregaria* is placed closer to *S. lingua*. No single character, however, discriminates one species from the other. Separate distributions (*S. gregaria* is restricted to southern France, whereas *S. strictiflora* is restricted to the southwest part of the Iberian Peninsula and regions of northern Africa), combined with morphometric and phylogenetic analyses, lead us to hypothesize that these two species presumably originated independently. Their treatment by Kreutz (2004) as *S. strictiflora* subsp. *strictiflora* and as *S. strictiflora* subsp. *gregaria* therefore seems premature.

***S. elsae*.** Maire and Stephenson (1930) described *S. stenopetala* from Algeria, which was renamed *S. lingua* subsp. *stenopetala* by Maire and Weiller (1959). Delforge (1995, 2002) also recognised this taxon from the southwestern part of the Iberian Peninsula, but under the name *S. stenopetala*. He described plants as having pale flowers, bracts exceeding the hood and a grooved stigmatic surface. Based on his field observations, he concluded that it is not a variety of *S. lingua* and that it has evolved into a species with deviant characters, rather than an occasional hybrid. Baumann and Künkele (1989), Venhuis et al. (2004), Presser (pers. comm., 2004) and Tyteca (pers. comm., 2004) have all questioned the taxonomic status of this species. On the basis of recent photos of *S. lingua* subsp. *stenopetala*

from Algeria, Delforge (2004) also recognised that the plants from Algeria were not morphologically similar to *S. stenopetala* from the Iberian Peninsula, and hence described the latter as a distinct species, *S. elsae*.

Our observations on plants of *S. elsae* from the Iberian Peninsula show that: (1) the majority of the flowers were red, and only rarely pale, (2) in each analysed population only a few plants had bracts that exceeded the hood, and (3) in all populations of *S. elsae*, the majority of specimens closely resembled *S. strictiflora*. Delforge (2004) hypothesised that *S. elsae* originated from a hybrid between *S. strictiflora* and *S. lingua*. Our analysis of the relative DNA content showed that *S. elsae* is most likely diploid, and therefore could not have originated from hybridisation between tetraploid *S. lingua* and diploid *S. strictiflora*. In addition, our morphometric and phylogenetic analyses showed that *S. elsae* more closely resembled *S. strictiflora* than *S. lingua* (Figs. 2a, b, 3a, 4). We also found that all qualitative characters corresponded with *S. strictiflora*. On this basis, we conclude that *S. elsae* is only a large-flowered variant of *S. strictiflora*, which we propose to name *S. strictiflora* var. *elsae*. We did not find this taxon in southern Andalusia, where *S. strictiflora* appears to grow in pure populations, but only in the Algarve up to Mafra and further inland to Evora, and the westernmost part of Extremadura (Spain). The description of the new variety is given below.

***Serapias strictiflora* var. *elsae* (Delforge)**

**C. Venhuis & P. Venhuis, comb. et stat. nov.
Fig. 5A, B**

Description: Plants with 2–3, subglobose or ovoid tubercles. Stem straight and cylindrical, 100–300 mm high, green and often spotted red. Leaves linear-lanceolate, 4–7, 3–5 basal-leaves 40–160 mm long and 6–14 mm broad, and 1–2 bract-like cauline leaves above. Inflorescence lax, bearing 2–7 slender flowers. Bracts oval-lanceolate, grey to red with reddish veins, 25–50 mm long and 10–18 mm wide,

normally shorter than the hood. Sepals and petals form a pointed hood, which is normally (sub-)horizontally positioned. Sepals oval-lanceolate, 15–25 mm long, green to red coloured with pinkish to purple veins. Petals usually 3 mm shorter than sepals, from a purple, orbicular base acuminate into a slender, tapering, green to red apical part. Labelum by a constriction divided into hypochile and epichile. Hypochile 0.8 – 1.2 cm long and 1.1 – 1.7 cm wide. Lateral lobes hidden inside, or slightly emerging from the hood, purple with a pink to red coloured centre. Hypochile with a deeply grooved stigmatic surface, deep purple. Epichile lanceolate, 12–20 mm long and (5-) 6–10 mm wide, yellowish, pinkish, bright red to purple and very short yellowish to reddish hairs. Ovary cylindrical, 10–25 mm.

***S. vomeracea* group. *S. cordigera* and *S. neglecta*.** *Serapias cordigera* and *S. neglecta* are morphologically rather similar (Figs. 2a, b, 3b). They nevertheless have clear discriminating characters. *Serapias neglecta* is distinguished from *S. cordigera* by its low stature, lateral hypochile lobes that far exceed the hood and a salmon-pink flower colour, and can therefore be regarded as a different species.

Serapias cordigera is normally an obligate outcrosser (Perez Chiscano et al. 1991, Baumann and Baumann 1999). Like Tyteca (1997), we observed that flowers of *S. cordigera* from the Algarve are predominantly self-pollinated and even cleistogamous, and that some specimens are (semi-)hypochromatic. Plants with pale hypochromatic flowers from this region had already been given names such as *S. cordigera* var. *leucantha* Guimarães and *S. cordigera* var. *leucoglottis* Welwitsch, *S. nurrica* Corrias and *S. viridis* Perez Chiscano (Guimarães (1887), Wallenwein and Breier (1992), Salkowski (1993), Kreutz (1995) and Tyteca (1997)). Plants from the studied population from the Algarve (Cotifo) had slightly smaller flowers than individuals of *S. cordigera* from Extremadura and southern France. The flowers of *S. cordigera* from Cotifo were all red-purple but several specimens had white lines along the margins of the epichile (thus resem-

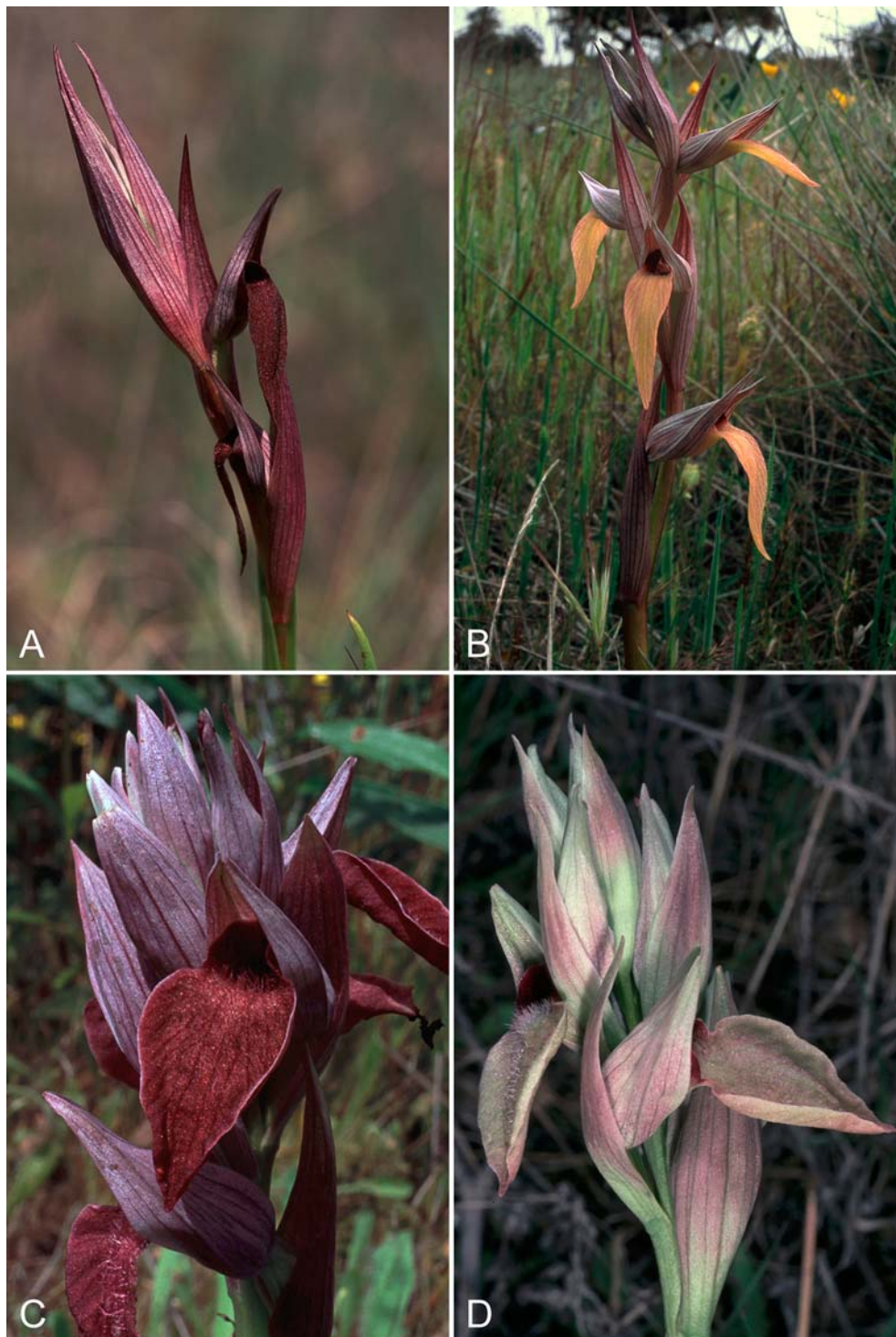


Fig. 5. **A** *S. strictiflora* var. *elsae*, Barão de São Miguel, Algarve, 15-04-2005; **B** *S. strictiflora* var. *elsae*, Cabrela, Alentejo, 11-04-2004; **C** *S. cordigera* subsp. *gentilii*, Cotifo, Algarve, 16-04-2005; **D** *S. cordigera* subsp. *gentilii*, Barranco do Velho, Algarve, 09-04-1994. The taxon names given here are those proposed in this paper on the basis of our analyses. Photographs A–C by C. Venhuis, D by C. A. J. Kreutz

bling *S. nurrica*, a species we did not study). Our observations showed that the pollinia of *S. cordigera* from the Algarve were pulverulent, quickly disintegrating onto the stigmatic surface. Tyteca (1997) concluded that flowers of *S. cordigera* from the Algarve were similar to those of *S. perez-chiscanoi* as described by Keitel (1991). Given the small flower size of the latter species, this implies that the *S. cordigera* flowers measured by Tyteca were smaller than those measured by us. Though only a small percentage of the flowers were green, yellow, pink or white-lined, they differed in more aspects from “normal” *S. cordigera*.

Our morphometric analyses, combined with observations of Perez Lara (1886) and Tyteca (1997), showed that plants in the Algarve populations had slightly smaller flowers with a less heart-shaped epichile than those from the remainder of its distribution area. Baumann and Baumann (1999) described a coastal subsp. *cretica* of *S. cordigera* that also has a slender epichile, but these plants were cross-pollinated and purple. The population of *S. cordigera* from the Algarve is, nevertheless, clearly a member of the *S. cordigera* cluster. On the basis of its morphometric discrimination, a disjunct distribution, deviant reproductive behaviour, and to prevent confusion with respect to the multiple names that exist for specimens of *S. cordigera* from the coastal regions of the Algarve and southwest Spain, we propose to rename this taxon *S. cordigera* subsp. *gentilii*.

***Serapias cordigera* subsp. *gentilii* C. Venhuis, P. Venhuis & Kreutz subsp. nov.** Holotypus: Portugal, Algarve, Cotifo, 24-04-06. C. Venhuis & J. G. B. Oostermeijer (AMD123443). Dedicated to Antonio Gentil Cabrilla; contemporary Spanish biologist working for the nature conservation organization ADENEX. Fig. 5C, D.

Description: Plants with 2 subglobose or ovoid pseudotubercles. Stem straight and cylindrical, 100–260 mm high, green and often spotted red. Leaves linear-lanceolate, 5–11: 4–8 basal-

leaves 80–150 mm long and 7–16 mm broad, and 1–3 bract-like cauline leaves above. Inflorescence dense, with 3–11 large flowers. Bracts oval-lanceolate, green to grey-lilac with greenish to reddish veins, 26–47 mm long and 13–20 mm wide, normally shorter than the hood. Sepals and petals form a pointed hood, which is normally (sub-)horizontally positioned. Sepals oval-lanceolate, 18–26 mm long, green to grey-lilac coloured with greenish to purple veins. Petals usually 3 mm shorter than sepals, from a pink to purple, orbicular base acuminate into a slender, tapering, green to red apical part. Labellum divided by a constriction into a hypo- and epichile. Hypochile 9–13 mm long and 16–24 mm wide. Lateral lobes hidden inside, or slightly emerging from, the hood, red to purple with a pink to red-coloured centre with two diverging lamellae, deep purple. Epichile slightly heart-shaped, often with divergent edges, 15–24 mm long and 13–20 mm wide, yellowish, pinkish to purple and with large yellowish to reddish hairs. Ovary cylindrical, 10–20 mm.

***S. vomeracea* subsp. *vomeracea* and *S. occidentalis*.** *Serapias vomeracea* is distributed across most of the European Mediterranean zone (Landwehr 1977; Perez Chiscano et al. 1991; Delforge 1995, 2002) and is commonly divided into three subspecies: *S. vomeracea* subsp. *vomeracea*, *S. vomeracea* subsp. *longipetala* (Tenore) Baumann & Künkele and *S. vomeracea* subsp. *istriaca* (Perko) Kreutz. *Serapias vomeracea* subsp. *vomeracea* probably occurs in the western (Benito Ayuso and Tabuenca Marraco 2001) and central part of the Mediterranean zone (Baumann and Künkele 1989). *Serapias vomeracea* subsp. *longipetala* occurs further eastwards, from Italy to Greece (Baumann and Künkele 1989), and *S. vomeracea* subsp. *istriaca* occurs in Istria (Delforge 2002). Benito Ayuso and Tabuenca Marraco (2001) suggested that individuals on photographs of *S. vomeracea* subsp. *vomeracea*, shown for populations in Extremadura by Pérez Chiscano et al. (1991) to some extent differ from *S. vomeracea* from the northeastern part of Spain. Confusion further

increases by the fact that Tyteca (1997), Kreutz (pers. comm., 2004), Benito Ayuso (pers. comm., 2004) and Venhuis et al. (2004) postulated that the individuals depicted on these photos display morphological similarities with *S. cordigera*, which occurs on the entire Iberian Peninsula (Landwehr 1977). On the basis of morphological data, Venhuis et al. (2006) concluded that the putative *S. vomeracea* from Extremadura probably originated from hybridisation between *S. vomeracea* subsp. *vomeracea* and *S. cordigera* and described this taxon as a new species: *S. occidentalis*. Our discriminant analyses show that function 1 distinguishes *S. occidentalis* from *S. vomeracea* subsp. *vomeracea* (Fig. 3b). This discriminant function demonstrates that *S. occidentalis* has a significantly longer and wider hypochile. Function 2 distinguishes *S. occidentalis* from *S. cordigera* on the basis of a smaller epichile and a relatively lax inflorescence. For the first and third discriminant functions, *S. occidentalis* clusters with *S. cordigera* (Fig. 3c) which is distinguished from *S. vomeracea* subsp. *vomeracea* on the basis of taller plants and a larger sepal size. In contrast with *S. cordigera*, which has two divergent lamellae, *S. occidentalis* is characterised by two more or less parallel lamellae that are positioned closely together. On the basis of morphometric- and phylogenetic data, *S. occidentalis* is thus intermediate between *S. vomeracea* subsp. *vomeracea* and *S. cordigera*. This could indeed indicate a hybrid origin.

***S. perez-chiscanoi*.** Most authors describe *S. perez-chiscanoi* as a close relative of *S. vomeracea* (Perez Chiscano 1988; Baumann and Künkele 1989; Perez Chiscano et al. 1991; Delforge 1995, 2002). It seems, however, that *S. vomeracea* does not occur in southwest Spain and Portugal (Benito Ayuso and Tabuenca Maracco 2001, Venhuis et al. 2004). The absence from southern Spain and Portugal of *S. vomeracea* does not necessarily imply that *S. perez-chiscanoi* could not be closely related to *S. vomeracea*, as it could have speciated allopatrically. Kreutz (1995) described yellow-flowered *Serapias* plants from

the Algarve (Portugal) and concluded, like Jansen (1993), that they were different from *S. perez-chiscanoi* in Extremadura. His assumption was based on the yellow colour of the flowers, the intensely red epichile, the pinkish bracts, sepals and petals, and a different growth habitat to that of *S. perez-chiscanoi* in Extremadura. Tyteca (1997) argued that “*S. perez-chiscanoi*” plants from Extremadura, as well as the *S. cordigera* plants from the Algarve, represent degenerate forms of *S. cordigera*, and so at most merit the status of form or variety. Based on our morphological analyses on material from Extremadura, we agree that *S. perez-chiscanoi* resembles *S. cordigera*, but consider it to be a distinct species for the following reasons: (1) discriminant analyses (Figs. 2a, 3b, c) showed that *S. perez-chiscanoi* is morphologically clearly separated from all other species; (2) *S. perez-chiscanoi* has separate, parallel lamellae, contrasting with the divergent lamellae of *S. cordigera*; (3) *S. perez-chiscanoi* is autonomously self-pollinating, whereas *S. cordigera* is generally an obligate outcrosser (Delforge 1995, 2002); (4) in the field we observed that the habitat of *S. perez-chiscanoi* is different from that of *S. cordigera*. *S. perez-chiscanoi* seems to prefer water-retaining soils in depressions, whereas *S. cordigera* occurs on drier slopes. Additionally, we never observed both taxa co-occurring at the same site. Finally, flower colour differs considerably between the two taxa, although this is not necessary a relevant character because hypochromatic individuals occur in many *Serapias* species found in Spain and Portugal. Several of the above-mentioned characters are also applicable to *S. cordigera* subsp. *gentilii*. It may be that this small and often pale-flowered taxon is characterised by a selfing breeding system, as some taxa from other orchid genera (Catling 1990) also show this tendency.

We thank José Luis Pérez Chiscano, Rob Poot, Karel Kreutz, Helmut Presser, Olivier Gerbaud, Noel Kerremans, Kees Jager, Rien Schot and Frank Verhart for their kind help in providing

localities of the species. Francisco María Vázquez Pardo is gratefully acknowledged for his assistance in providing literature from the past 150 years about the genus *Serapias* in Spain.

References

- Aceto S., Caputo P., Cozzolino S., Gaudio L., Moretti A. (1999) Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Molec. Phylogenet. Evol.* 13: 67–76.
- Bateman R. M., Hollingsworth P. M., Preston J., Luo Y.-B., Pridgeon A. M., Chase M. W. (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Bot. J. Linn. Soc.* 142: 1–40.
- Bateman R. M., Pridgeon A. M., Chase M. W. (1997) Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 2. Infrageneric relationships and reclassification to achieve monophyly of *Orchis* sensu stricto. *Lindleyana* 12: 113–141.
- Baumann B., Baumann H. (1999) Ein Beitrag zur Kenntnis der *Serapias cordigera*-Gruppe (A contribution to the knowledge of the *Serapias cordigera* group). *J. Eur. Orch.* 31: 495–521.
- Baumann H., Künkele S. (1989) Die Gattung *Serapias* L. Eine taxonomische Übersicht. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.* 21: 701–946.
- Benito Ayuso J., Tabuenca Marraco J. M. (2001) Apuntas sobre orquídeas Ibéricas. *Estud. Mus. Cienc. Nat. Alava* 16: 67–87.
- Catling P. M. (1990) Auto-pollination in the Orchidaceae. In: Arditti J. (ed.) *Orchid biology, reviews and perspectives*. V. Timber Press, Oregon, pp. 121–158.
- Delforge P. (1995) *Orchids of Britain & Europe*. Harper Collins Publishers, London, UK.
- Delforge P. (2002) *Guía de las Orquídeas de España y Europa*. Lynx Ediciones, Barcelona, Spain.
- Delforge P. (2004) Le Sérapias d'Elsa. *Nat. Belg.* 85: 103–109.
- D'Emérico S., Galasso I., Pignone D. (1998) Karyomorphological considerations on some taxa of the genus *Serapias* L. (Orchidaceae). *Inf. Bot. Ital.* 29: 311.
- Gözl P., Reinhard H. R. (1980) *Serapias* (Orchidaceae) Ergebnisse statistischer und chorologischer Untersuchungen. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.* 12: 123–189.
- Gravendeel B., Vogel E. F. de (2002) Revision of *Coelogyne* section *Moniliformes* (Orchidaceae) based on morphology, plastid and nrDNA ITS sequences. *Blumea* 47: 409–462.
- Guimarães J. (1887) *Orchideographia portugueza*. *Bol. Soc. Broteriana* 5: 17–84.
- Jansen H. (1993) *Serapias viridis* Perez Chiscanoi in Portugal. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.* 10: 50–53.
- Keitel C. (1991) *Serapias viridis* Perez Chiscano, *Dactylorhiza maculata* ssp. *caramulensis* Vemeul. und andere interessante spanische Orchideenarten. *Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ.* 23: 107–121.
- Kreutz C. A. J. (1995) Enkele groeiplaatsen van *Serapias* in Portugal en Spanje. *Eurorchis* 7: 90–96.
- Kreutz C. A. J. (2004) *Catalogue of European Orchids*. Kreutz Publishers, Landgraaf: p. 239.
- Landwehr J. (1977) *Wilde Orchideeën van Europa*. Vereniging tot Behoud van Natuurmonumenten in Nederland, deel I + II: p. 575.
- Maire A., Stephenson T. (1930) Sur quelques plantes nouvelles ou peu connues de l'Algérie orientale. *Bull. Soc. Hist. Nat. Afr. Nord* 21: 48–50.
- Maire R. C. J. E., Weiller M. (1959) *Flore de l'Afrique du Nord* 6. Lechevalier, Paris, pp. 317–319.
- Martine P., Gerbaud O. (1998) Considérations sur quelques *Serapias* varois. *L'Orchidophile* 130: 10–22.
- Pellegrino G., Cafasso D., Widmer A., Soliva M., Musacchio A., Cozzolino S. (2001) Isolation and characterization of microsatellite loci from the orchid *Serapias vomeracea* (Orchidaceae) and cross-priming to other *Serapias* species. *Molec. Ecol. Notes* 4: 279–280.
- Perez Chiscano J. L. (1988) Nueva especie de *Serapias* L. en Extremadura (España). Homenaje a Pedro Montserrat, Jaca y Huesca: 305–309.
- Perez Chiscano J. L., Gil Llano J. R., Duran Oliva F. (1991) *Orquídeas de Extremadura*. Fonda Natural, Madrid, p. 223.
- Pérez Lara J. M. (1886) *Florula gaditana*. Pars prima. *An. R. Soc. Esp. Hist. Nat.* 1: 349–475.
- Pridgeon A. M., Bateman R. M., Cox A. V., Hapeman J. R., Chase M. W. (1997) Phylogenetics of subtribe Orchidinae (Orchidoideae, Orchidaceae) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis* sensu lato. *Lindleyana* 12: 89–109.

- Salkowski H. E. (1993) *Serapias nurrica* Corrias in der Provinz Algarve/Südportugal. Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ. 10: 28–29.
- Schlechter R. (1923) Mitteilungen über europäische und mediterrane Orchideen. In: Fedde F. Repertorium Europaeum et Mediterraneum 36: 561–573.
- Sundermann H. (1980) Europäische und Mediterrane Orchideen. 3rd ed. Brücke-Verlag Kurt Schmiersow, Hildesheim.
- Tyteca D. (1997) The orchid flora of Portugal. Jour. Eur. Orch. 29: 267–271, 356–359.
- Venhuis C., Venhuis P., Ellis-Adam A. C. (2006) A new Tongue-orchid (Orchidaceae) in southwest Spain: *Serapias occidentalis*. Anales Jard. Bot. Madrid. 63: 131–143.
- Venhuis C., Oostermeijer J. G. B., Tonk J. Th. P. (2004) Conservation biology of *Serapias perez-chiscanoi* Acedo in the Guadiana river basin in Extremadura (Spain). Eurorchis 16: 48–63.
- Wallenwein F., Breier W. (1992) Bemerkungen zu einigen Arten der Gattung *Serapias* L. aus Spanien. Mitt. Bl. Arbeitskr. Heim. Orch. Baden-Württ. 24: 115–120.

Address of the authors: C. Venhuis, P. Venhuis, J. G. B. Oostermeijer (e-mail: oostermeijer@science.uva.nl), and P. H. van Tienderen, Institute for Biodiversity and Ecosystem Dynamics, Universiteit van Amsterdam, Kruislaan 318, 1098 SM, Amsterdam, The Netherlands.