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Morphometric patterns in the *Nymphaea alba–candida* complex

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SUMMARY

Multivariate analyses were used to study the morphological variation in two *Nymphaea* species, *Nymphaea alba* L. and *Nymphaea candida* Presl. Three datasets consisting of qualitative, multistate discrete and continuous characters of water lily plants from 49 localities were analysed throughout The Netherlands. The analyses displayed a clear division between *N. alba* and *N. candida*. The classification of the specimens with an intermediate morphology is less stable. The important parameters that account for the differentiation of the taxa were determined, and a comparison was made with those described in the literature. Frequency analyses and multivariate techniques revealed several characters which may be used as reliable identification rules.

Key-words: cluster analysis, discriminant analysis, morphological characters, morphological variation, *Nymphaea alba–candida* complex, principal component analysis.

INTRODUCTION

Nymphaeaceae are dicotyledonous water plants (Tillich 1990) capable of clonal growth. The genus *Nymphaea* contains about 40 species with subspecies and chromosome races, and numerous forms of natural hybrids and artificially raised varieties (Gupta 1980). They can be found in the tropics as well as in the temperate zones. The species have a broad ecological amplitude and occur on sediments ranging from mineral to organic and in acid as well as alkaline fresh waters. In Europe, four *Nymphaea* species are considered to be indigenous, namely *Nymphaea alba* L., *N. candida* Presl, *N. tetragona* Georgi and *N. lotus* (L.) Willdenow (Hegi 1965; Masters 1974).

N. alba is a European species, where *N. candida* is a Euro-Siberian one (Heß *et al.* 1970) (Fig. 1a,b). The distribution of *N. candida* is consistent with a moist, continental climate with cool summers, whereas the distribution of *N. alba* seems to be restricted to a temperate maritime climate with cool summers. *N. tetragona* is also a Euro-Siberian species, but its distribution area in Europe is restricted to Russia and Finland. *N. lotus* is restricted to some hot springs in Hungary (Masters 1974).

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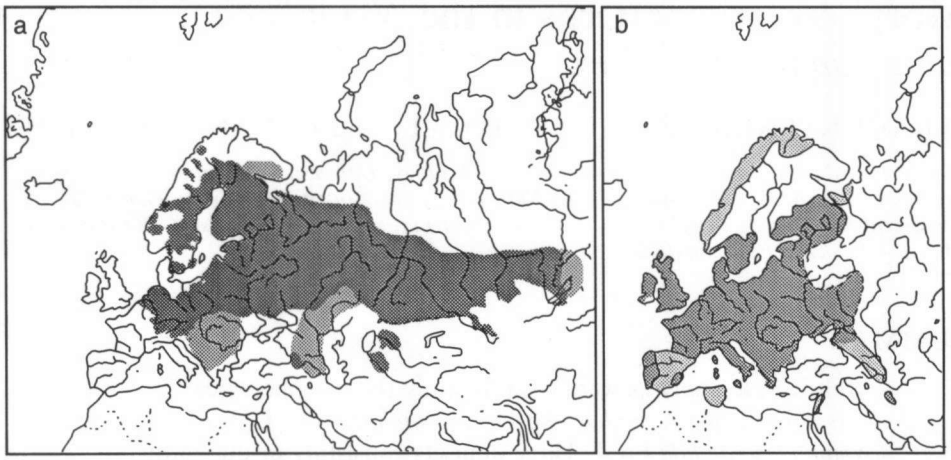

Fig. 1. (a) Distribution area of *Nymphaea candida* in Europe (■ =common; □ =less abundant). Modified after Meusel *et al.* (1965), (b) distribution area of *Nymphaea alba* in Europe (■ =common); □ =less abundant). Modified after Meusel *et al.* (1965).

It has been suggested that *N. candida* spread to the south due to the extension of the glaciers during the last glacial period (Weichsel period). With the withdrawal of the glaciers the resulting marshes and streams became connected, so that they could be colonized by this species. During this period *N. alba* survived in more southern areas and, when this glacial period ended, it spread to the north again (Conard 1905; Valle 1927). Evidence for this is the fact that the distribution of *N. candida*, in contrast to *N. alba*, becomes more incidental further south and its occurrence there can be regarded as a relic. A similar difference in biogeographic pattern is shown by two other closely related species of Nymphaeaceae, namely *Nuphar lutea* (L.) Sm. and *Nuphar pumila* (Timm) DC. (Meusel *et al.* 1965; Roweck 1988).

Both *Nymphaea* species have been studied extensively for their morphological characteristics (Caspary 1879; Conard 1905; Glück 1924; Valle 1927; Heslop-Harrison 1955; Neuhäusl & Tomšovic 1957; Beug 1961; Radic 1967; Roelofs & Van der Velde 1977; Casper & Krausch 1981; Jones & Clarke 1981). Intermediate forms of the two species were also studied (Caspary 1879; Glück 1924; Valle 1927; Neuhäusl & Tomšovic 1957). Caspary (1879) even mentioned a reduced pollen fertility and seed production of these intermediate forms, but extensive crossing and backcrossing experiments have never been carried out.

Many varieties and subspecies of *N. alba* have been described. Only two are worth mentioning here. *N. alba* var. *minor* DC. is a smaller form of *N. alba* and is thought to be restricted to colder, northern regions, like *N. candida* (Glück 1924; Hegi 1965; Casper & Krausch 1981). The second is *N. alba* ssp. *occidentalis* (Ostenfeld) Moss, also a smaller form of *N. alba* (Ostenfeld 1912; Heslop-Harrison 1955; Masters 1974; Heslop-Harrison 1975), which is found in lakes in Ireland and Scotland. However, according to Glück (1924) this subspecies is in fact the same as *N. alba* var. *minor*, except that it has spread further to the north. They are considered to be starvation forms of *N. alba*, because of the nutrient-poor conditions in the north (bogs, etc.). In any case, these varieties or formae are often mistaken for *N. candida*, thus creating further confusion.

N. candida was first found in The Netherlands in 1977 in the Haarsteegse Wiel, and its morphological characteristics have been studied, described and compared with three populations of *N. alba* (Roelofs & Van der Velde 1977). Subsequently, *N. candida* was found in several places in The Netherlands (Giesen & Van der Velde 1978; Mennema & Van Ooststroom 1979; Mennema & Holverda 1981). These locations are restricted to borders of Pleistocene glacial sand deposits (Fig. 2a). In view of three records from the past—the oldest being from 1894—based on herbarium material, *N. candida* does not seem to be a recent immigrant or introduced species in The Netherlands (Giesen & Van der Velde 1978). The localities in the north of The Netherlands are probably connected with the main distribution domain in the north of Germany (Fig. 1b). The discovery of *N. candida* in Niedersachsen and more recent findings confirm this (Tüxen 1955; Haeupler & Schönfelder 1989). Since then its occurrence in Belgium has also been confirmed (De Langhe *et al.* 1988). Morphological studies of *N. candida* and *N. alba* have been restricted to a literature survey (Conard 1905), and to specimens from Germany (Glück 1924), Finland (Valle 1927), Czechoslovakia (Neuhäusl & Tomšovic 1957), and The Netherlands (Roelofs & Van der Velde 1977). The pollen morphology of *N. alba* and *N. candida* has also been studied extensively (Glück 1924; Valle 1927; Beug 1961; Roelofs & Van der Velde 1977; Jones & Clarke 1981). Pollen grains of *N. candida* were found to be much larger than those of *N. alba*, and *N. candida* grains had projections that were much smaller. Although pollen showed a clear distinction between the two taxa, overlaps were seen in other individual characters.

Because of this overlap in morphology, the two species seem to be closely related. Hence, in a taxonomic sense, the status of *N. candida* became a controversial subject (Heß *et al.* 1970; Heukels & Van der Meijden 1990). However, there are differences which ought to be considered before accepting *N. candida* as an extreme morphological form of *N. alba*. These differences were sufficient reason to reexamine the extent to which the *Nymphaea* species differ in their morphological characteristics and ecology. Because of the great similarity between individuals of these taxa, which, with some difficulty, can be recognized by morphological characteristics—and associations between these characters—a multivariate statistical approach is used to study the overall difference between the two taxa. Frequency analyses and multivariate analyses were used to find out which characters can be used for identification.

MATERIALS AND METHODS

Plant material

Five to 10 leaves, and five to 10 flowers per water lily plant per locality were collected in the field between June and September 1983 from 49 localities in The Netherlands (Table 1, Fig. 2b). Plant material was stored in a cool box and transported to the laboratory the same day.

Before further analysis, the water lily plants were roughly identified as specimens of *N. candida* (20 plant samples) or *N. alba* (24 plant samples) by using cursory characters such as 22, 30, 31, 36–37, 38–39, 43, 44–45, 48–49, 50–51, 53, 66 and 57–58 of Table 2. None of the characters used showed distinct differences for the two taxa, all showing overlapping character values. Only five of the plant samples, found in ponds where both taxa occurred at the same time, showed this overlap in an excessive manner.

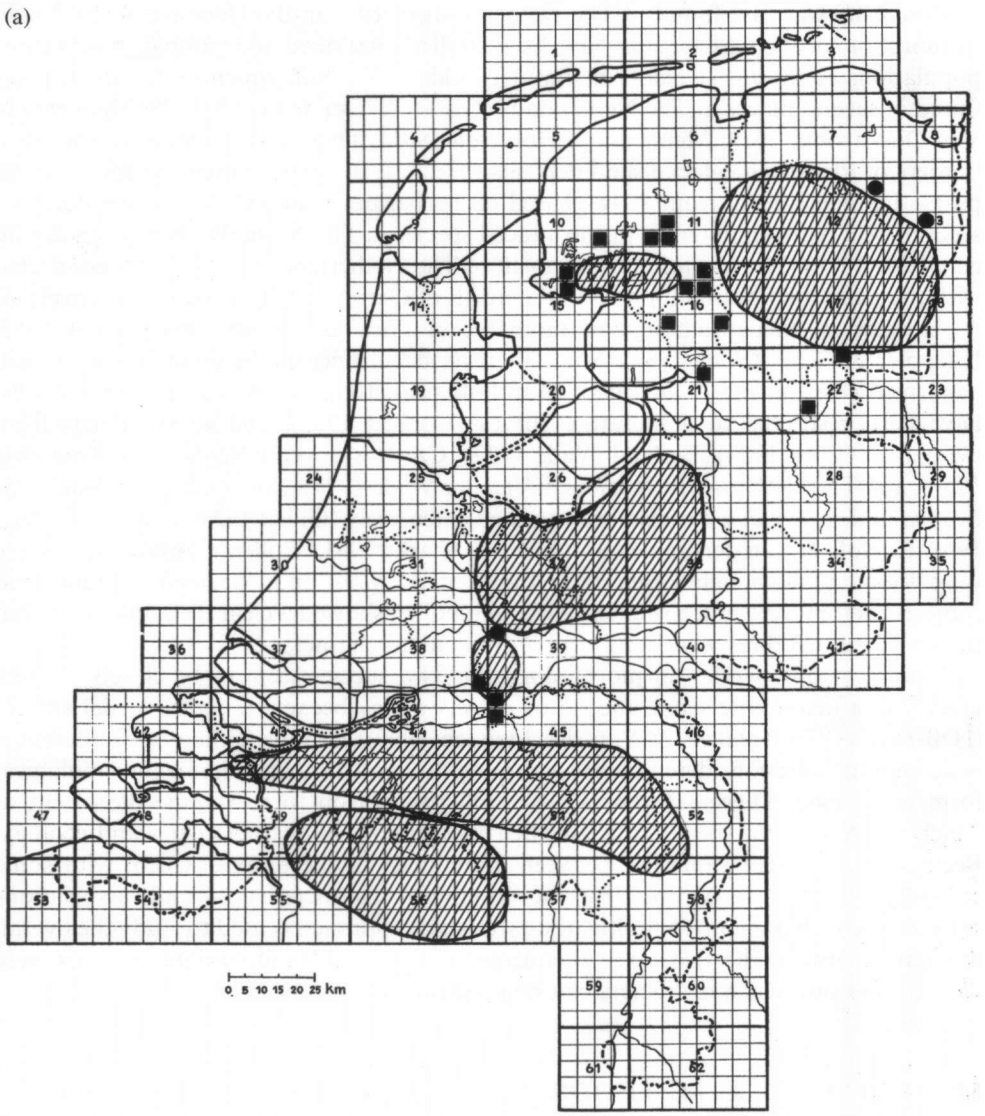

Fig. 2. (a)

The dataset

To obtain an extensive description of the morphology of the specimens a dataset was derived from their leaves, flowers and pollen (Table 2, Fig. 3, Fig 4a–b, Fig 5). The dataset was composed of three different kinds of characters, namely 21 continuous measurements, 14 multistate discrete (counts) and 23 binary coded characters, resulting in three subsets of data. The binary characters (Table 2; characters 36–58) were measured in the field in warm, sunny weather, when flowers were fully opened. The other characters were determined in the laboratory. For the continuous characters and the multistate discrete characters the average value of between five and 10 flowers and leaves were used.

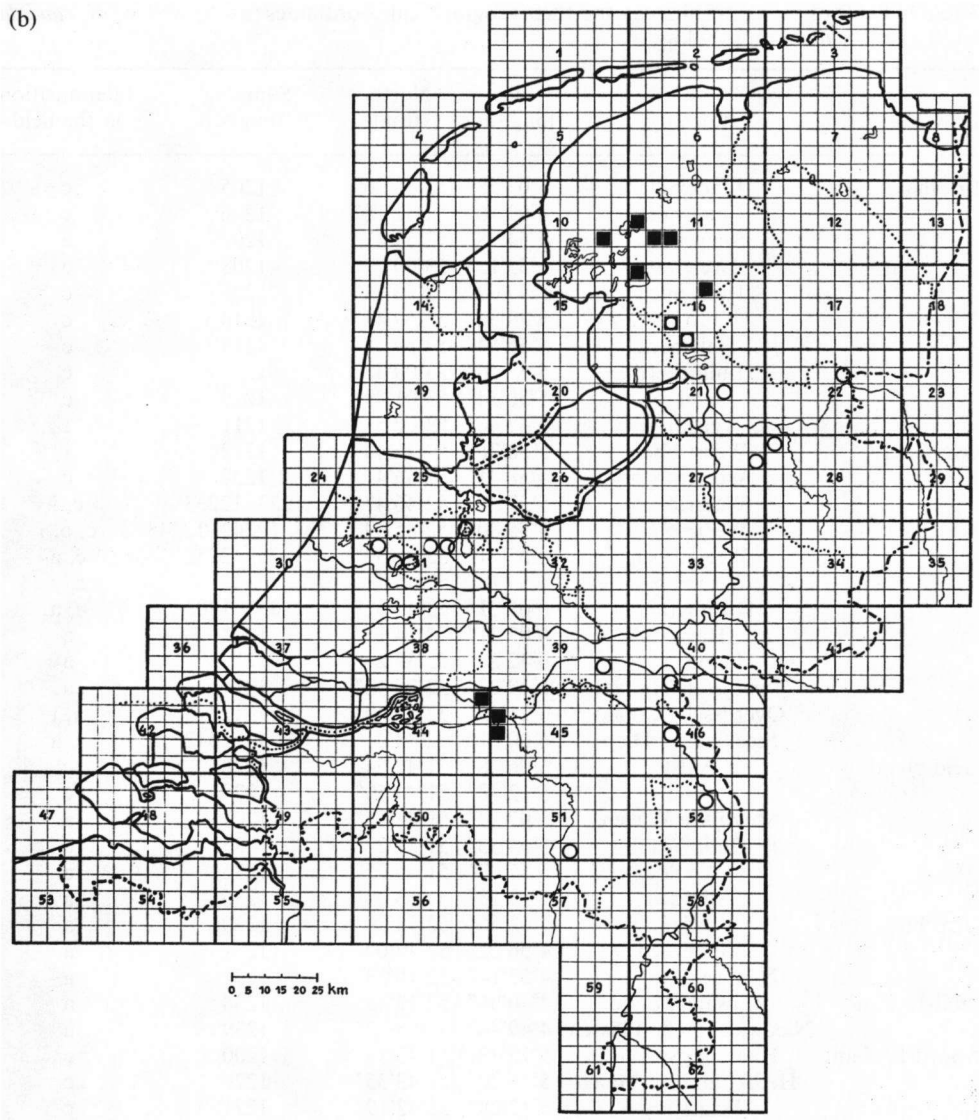

Fig. 2 (b)

Fig. 2. (a) Known distribution of *Nymphaea candida* in the Netherlands (■ = after 1950; ● = before 1950). Hatched areas: Pleistocene sandy areas, (b) *Nymphaea* populations investigated for character analysis (■ = *N. candida*; ○ = *N. alba*; □ = *N. alba* and *N. candida*). The populations are listed in Table 1.

Two to five pollen samples were collected from the two inner stamen rows of 17 *N. candida*, 20 *N. alba*, and two intermediate specimens. After air drying for several weeks, pollen samples were gold sputtered for examination under a scanning electron microscope (Philips EM 201). Pictures were taken of the whole pollen grain (2500 ×) (Fig 4a) and of an edge (11 170 ×).

Pollen used for further analysis had to satisfy two criteria. First, it must be characteristic of the whole sample and secondly, the proximal side had to be completely

Table 1. Table of the sampled areas and their geographical coordinates (a=*N. alba*, c=*N. candida* and i=intermediate morphology)

Province	Locality	East longitude	North latitude	Sampling number	Identification in the field
Friesland	Lindevallei	6°03′58″	52°52′06″	1205	c
	Lindevallei	6°03′56″	52°52′18″	1206	c
	Spitsendijk	5°54′28″	52°59′47″	1207	a
	Deelen de	5°53′15″	53°00′29″	1208	c
	Tjeukemeer	5°49′09″	52°54′43″	1209	c
	Jutrijp	5°39′36″	52°59′44″	1216	c
	Tjalleberd	5°56′54″	52°59′44″	1217	c
	Luinjeberd	5°55′56″	53°00′48″	1218	c
	Gersloot	5°56′34″	53°01′09″	1219	c
	Terhorne	5°47′46″	53°02′58″	1211	i
Overijssel	Weerribben	5°58′36″	52°46′40″	1212	i
	Weerribben	5°58′31″	52°46′47″	1222	c
	Weerribben	5°57′37″	52°46′47″	1223, 12233	c, i
	Weerribben	5°55′30″	52°45′39″	1224, 12242, 12243	c, a, i
	Weerribben	5°55′51″	52°45′39″	1225, 12252	c, a
	Weerribben	5°56′12″	52°45′48″	1226, 12262	c, a
	Dwarsgracht	6°02′21″	52°43′01″	12031, 1203	c, a
	Hasselt	6°07′31″	52°34′15″	1202	a
	Haandrik de	6°42′28″	52°37′24″	1213	a
	Overijssels Kanaal	6°19′22″	52°25′42″	1214	a
	Overijssels Kanaal	6°21′23″	52°27′49″	1227, 12273	a, i
	Steenwijker Diep	6°00′01″	52°44′41″	1204, 12042	c, a
Gelderland	Aalst	5°06′28″	51°47′54″	1228	c
	Well	5°12′38″	51°44′50″	1229	c
	Meidijkse Wielen	5°07′24″	51°48′17″	1201	a
	Oude Waal	5°53′53″	51°51′22″	1233	a
	Ooypolder	5°57′07″	51°50′37″	1234	a
	Afferden	5°38′13″	51°53′06″	1236	a
Utrecht	Vreeland	5°03′56″	52°14′29″	1230	a
	Vinkeveen	4°56′22″	52°12′00″	1231	a
	Nieuwer-Ter-Aar	4°57′14″	52°10′53″	1232	a
Zuid-Holland	Rijnsaterwoude	4°40′06″	52°12′48″	1238	a
	Nieuwkoopsche Plassen	4°49′46″	52°08′52″	1239	a
Noord-Brabant	Haarsteegse Wiel	5°12′28″	51°43′19″	1200	c
	Hedikhuizense Maas	5°11′20″	51°43′35″	1220	c
	Haarsteeg	5°12′28″	51°43′19″	1221	c
	Haps	5°53′30″	51°41′27″	1241	a
	Galgenven	5°28′43″	51°22′06″	1243	a
	Peetersven	5°28′13″	51°22′02″	1244	a
	Vilt de	5°55′28″	51°40′45″	1242	a
Limburg	Schuitwater	6°06′02″	51°30′40″	1240	a

visible. This means that the pole axis should be perpendicular to the plane of the picture. The terminology used for the projections on the pollen (i.e. baculum, verruca and gemma) is according to Beug (1961). Bacula, verrucae and gemmae were measured on the edge of the pollen, viewed in such a way that the total length of the projection was visible in sideview and could be measured,.

Non-supervised learning

As mentioned above, all individual characters showed an overlap in values. Multivariate statistical analyses, which also use between character associations expected to be different for the taxa, were used to investigate the number of clusters in the dataset, which specimens are segregated over these clusters and the characters that accounted for them. This was done with the computer program system for bioinformatic pattern analysis, BIOPAT, on the Leiden University mainframe (Hogeweg & Hesper, 1972). An analysis by eye alone of a PCA scatterplot is prone to subjectivity and misjudgements. To operationalize the grouping, a cluster analysis was also applied on the matrix with overall similarities between the specimens. For this reason the Ward average has been chosen. Ward is sensitive to clusters with a large density, i.e. a low within-cluster variability. By this sensitivity it may exaggerate the between-cluster similarities, which may lead to a misrepresentation of the clusters hierarchic ordening. In our analyses more emphasis is given to the display of clear clusters. Because there are only two taxa their hierarchic ordening is irrelevant. To estimate the association between the similarity matrix and the so-called ultrametric matrix of the dendrogram the cophenetic correlation ($C_{sim/ult}$) between both matrices was calculated (Sneath & Sokal 1973). A high cophenetic correlation means that the within- and between-cluster similarities are corresponding well between the similarity and the ultra-metric matrices, whereas a low correlation is caused by a growing exaggerating of the between-cluster similarities mentioned above, which in this two-taxon case is irrelevant. Other statistical analyses, including discriminant analyses, were done with SPSS for Windows, release 6.0 (Norusis 1993).

To obtain a classification based on shape differences, the cardinal difference between the two groups, the correlation coefficient (CORR), was used as an estimate of the overall difference between the specimens. The continuous characters were logarithmized prior to the analysis. A Ward cluster analysis was used, since this cluster criterion is not only sensitive to the average distance between the specimens of a cluster, but also to cluster density.

Prior to cluster analysis, the multistate discrete characters were ranged between zero and one. The Ward dendrogram was based on the mean character difference (MNCH). The Ward dendrogram of the MNCH reflects the simple matching coefficient between the specimens.

Discriminant analysis

The discriminant analysis develops an equation of linear combined weights and characters with maximum discriminating abilities between the groups.

For the sake of convenience, the recognition of the specimens was based only on characters which are easy to measure. Furthermore, in order to obtain an optimal discriminant equation, the characters should belong to a multivariate normal distribution of the two groups, and the two within-group covariance matrices should be equal. For that reason only continuous characters were used, which were logarithmized. To avoid the removal of specimens with missing values for certain characters from the small dataset, the missing values were replaced by the group mean for that character.

Selection of characters which maximally divided the groups was done by applying a stepwise approach. The criterion used was Wilks' lambda, which calculates the ratio between the within-group sum of squares and the total sum of squares. A small value of

Table 2. Table of the characters of leaf, flower, and pollen. The characters are subdivided into three groups of continuous, discrete multistate (counts), and binary characters (leaf and flower, only). χ^2 of a non-parametric analysis of variance are also given. In the case of the binary characters this is the χ^2 test, in the case of the multistate characters a Kruskal–Wallis average. Next to this, statistics such as sample frequencies (binary characters) and means (multistate characters) are given for *N. candida*, *N. alba* and the intermediates. χ^2 values are given as *P*-values

	χ^2	*N. cand.*	*N. alba*	Intermediate
No. continuous characters				
Leaf				
1 Leafblade length (top/leafslip) (mm)	7·96	144·8–304·0	191·7–363·0	224·0–324·7
2 Leafblade width (mm)	8·32	129·6–273·7	181·2–343·0	199·0–287·0
3 Distance leafslips (mm)	1·43	33·4–105·0	25·0–131·3	41·6–113·7
4 Diameter petiole (mm)	9·69	4·4–8·5	5·5–9·8	7·1–9·5
Flower				
5 Diameter peduncle (mm)	3·99	4·9–8·8	5·5–9·0	7·0–9·0
6 Length sepal (mm)	9·49	42·1–65·0	27·0–69·0	52·8–73·0
7 Maximum width (same) sepal (mm)	3·35	22·1–35·7	11·0–29·7	23·2–37·3
8 Width at base (same) sepal (mm)	2·12	9·7–10·0	9·0–19·3	12·4–24·7
9 Length of outer petals (mm)	8·06	40·6–59·1	27·0–68·0	51·2–72·0
10 Width of outer petals (mm)	9·54	21·4–28·0	13·0–32·0	25·4–30·0
11 Width filament of inner stamens (mm)	7·55	1·8–2·8	0·9–2·1	1·5–2·3
12 Width anthers of inner stamens (mm)	3·60	1·0–1·5	1·0–1·5	1·0–1·3
13 Width central projection (mm)	0·30	1·0–2·0	1·5–3·3	1·6–2·4
14 Length central projection (mm)	0·80	2·5–4·5	1·9–4·8	2·6–4·4
15 Diameter stigma (mm)	4·81	10·0–15·2	10·9–21·8	14·5–17·4
16 Width ovary (mm)	7·30	13·1–21·1	11·1–22·7	17·6–24·9
Pollen				
17 Highest equatorial diameter pollen (μm)	6·53	36·0–50·4	29·0–42·0	34·0–38·0
18 Lowest equatorial diameter pollen (μm)	3·00	23·0–35·0	18·5–33·7	26·0–32·0
19 Mean length bacula (μm)	6·19	0·8–1·6	1·0–3·5	1·3–2·0
20 Mean length verrucae (μm)	1·85	0·4–1·0	0·6–1·4	0·8–1·2
21 Mean length gemmae (μm)	8·85	0·7–1·3	0·8–1·8	1·2–2·5
No. discrete multistate characters (counts)				
Leaf				
22 Number of main air channels petiole	15·23	2–3	1–4	2–3
23 Number of medium sized air channels petiole	4·76	3–5	2–12	2–4
24 Number of small air channels petiole	17·91	2–8	3–13	2–10
Flower				
25 Number of main air channels peduncle	2·43	4–5	4–5	4–4
26 Number of middle air channels peduncle	1·73	2–11	7–13	8–11
27 Number of sepals	0·07	4–4	4–5	4–4
28 Number of petals	10·21	19–31	17–28	21–27
29 Number of stamens	26·80	52–85	75–131	78–100
30 Number of carpellary teeth	37·13	9–14	14–25	12–16
31 Number of grooves on inner side carpellary teeth	32·57	2–4	0–4	1–4
Pollen				
32 Number of projections/100 μm^2	21·94	23–81	6–38	4–59
33 Number of bacula/100 μm^2	11·60	5–33	1–22	12–35
34 Number of verrucae/100 μm^2	24·98	9–48	0–17	5–18
35 Number of gemmae/100 μm^2	9·22	0–12	0–6	6–7

Table 2. *Continued*

	χ^2	*N. cand.*	*N. alba*	Intermediate
No. binary characters				
Leaf				
36 Colour underside leafblade: 1=red	18·98	1·00	0·36	0·60
37 Colour underside leafblade: 1=green	15·74	0·00	0·55	0·20
0=green and red	3·19	0·00	0·09	0·20
38 Nervation (relief under) 1=pronounced	27·08	0·95	0·14	0·40
39 Nervation (relief under) 1=not pronounced	26·22	0·00	0·76	0·20
0=slightly pronounced	5·13	0·05	0·10	0·40
40 Direction main nerves leafslips 1=parallel	4·96	0·50	0·29	0·80
41 Direction main nerves leafslips 1=diverging	18·12	0·05	0·62	0·00
42 Direction main nerves leafslips 1=converging	7·15	0·40	0·10	0·00
0=in between	3·92	0·05	0·00	0·20
Flower				
43 Flower 1=on or above water surface	34·71	0·10	0·96	0·20
Flower half below water surface	34·71	0·90	0·04	0·80
44 Sepals 1=horizontal	40·58	0·00	0·91	0·00
45 Sepals 1=erect	31·92	0·90	0·04	0·40
0=in between	11·83	0·10	0·04	0·60
46 Flower 1=star shaped	44·15	0·00	0·96	0·00
47 Flower 1=cup shaped	31·92	0·90	0·04	0·40
0=in between	15·85	0·10	0·00	0·60
48 Side view flowerbase: 1=straight	36·86	0·00	0·92	0·40
49 Side view flowerbase: 1=concavely curved	36·52	0·95	0·04	0·60
0=in between	0·26	0·05	0·04	0·00
50 View underside flowerbase: 1=round	27·12	0·00	0·71	0·00
51 View underside flowerbase: 1=square	36·86	1·00	0·08	0·60
0=in between	6·87	0·00	0·21	0·40
52 Transition petals–stamens: 1=gradual	1·65	0·90	0·75	0·80
0=abrupt	1·65	0·10	0·25	0·20
53 Carpellary teeth: 1=shiny	33·64	0·00	0·88	0·40
0=dull	33·64	1·00	0·13	0·60
54 Colour carpellary teeth: 1=yellow	12·67	0·90	0·38	0·60
0=dark yellow-orange	15·55	0·05	0·63	0·40
0=red	1·48	0·05	0·00	0·00
55 Colour stigma: 1=yellow	28·09	0·10	0·88	0·20
56 Colour stigma: 1=red	31·26	0·90	0·00	0·80
0=yellow and red	1·06	0·00	0·04	0·00
57 Shape central projection: 1=rounded knob	38·28	0·00	0·88	0·00
58 Shape central projection: 1=pin shaped	33·64	1·00	0·13	0·60
0=in between	18·35	0·00	0·00	0·40

lambda indicates that a large proportion of the variation is explained by the difference between the centroids of the groups. At each step in the analysis, the character with the lowest value is entered into the discriminant equation. The significance of Wilks' lambda is based on an *F*-statistic. The minimum *F*-value for the admission of a character was set at 3·84. However, entering a character into a subset of already selected characters changes their within-group variation and also Wilks' lambda, and thus its *F*-value. Therefore, in order to maintain the optimality of the discriminant equation, characters

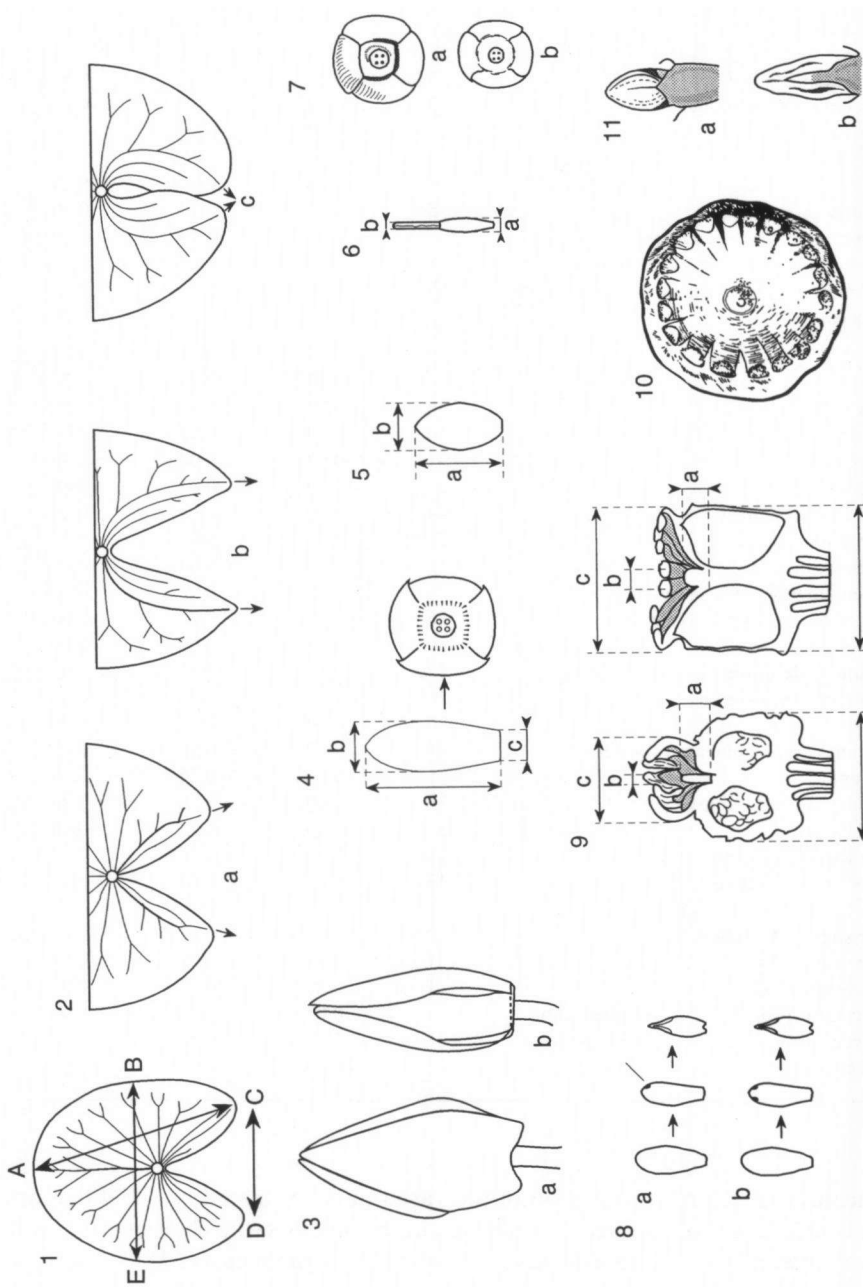

Fig. 3. Morphological characters of leaf and flower of *Nymphaea*. (1A–C) leafblade length; (1E–B) leafblade width; (1D–C) distance of leafslips; (2a) direction of main nerves diverging; (2b) direction of main nerves parallel; (2c) direction of main nerves converging; (3a) side view of flowerbase curved; (3b) side view of flowerbase straight; (4a) length of sepal (indicated by arrow); (4b) maximum width of same sepal; (4c) width at the base of the same sepal; (5a) length of petal; (5b) width of petal; (6a) width of filament of stamen; (6b) width of anthers of stamen; (7a) view of underside of flowerbase square; (7b) view underside flowerbase round; (8a) transition petals/stamens abrupt; (8b) transition petals/stamens gradual; (9a) length of central projection (left *N. candida*, right *N. alba*); (9b) width of central projection; (9c) diameter of stigma; (9d) width of ovary; (10) top view of stigma/ovary transition *N. alba*; (11a) carpellary tooth *N. alba*; (11b) carpellary tooth *N. candida*.

which obtained a maximum *F*-value of 2.71 after entering were removed from this subset.

Frequency of analysis

To establish an identification table frequency analyses were performed on all characters, along with their ratios. In order to do so, frequencies (%) were plotted for each individual character of *N. alba*, *N. candida* and the intermediates. Statistical analysis was conducted using Kolmogorov–Smirnov (PROC PAR1WAY), available in the statistical analysis system (SAS) software package (SAS Institute Inc. 1989).

RESULTS

Continuous characters

The first three main axes of a principle components analysis (PCA) of a correlation matrix of the continuous characters explained 69% of the total variation. The elements of the eigenvector of the first axis (36%) were nearly all positive (except for characters 17 and 18). Therefore this axis is considered to describe a general size factor. The specimens of *N. candida* (●) and *N. alba* (○) were distributed over the same range of this factor, mainly caused by characters 1, 2, 4 (leaf), 5, 6, 8, 9, 10 and 16 (flower) (Table 2), showing no general difference in size (Fig. 6b). A differentiation between the two taxa was mainly demonstrated by the second main axis (21%). There was an obvious gap between the taxa, which was smallest in the centre of the factor space, increasing markedly for the smaller and larger specimens (Fig. 6b). Specimens which were recognized as intermediates between the two species (▲) were situated in the scatterplot between the distributions of the two taxa or in the midst of the specimens of *N. alba*.

The differentiation between the two taxa (Fig. 6b) was primarily caused by the characters 7, 11, 12, 14 (flower) and 17 (pollen), which contrasted in loading with the characters 13, 15 (flower), 19, 20 and 21 (pollen). As the third main axis (12%) shows no differentiation between the specimens, the first two main axes sufficed to describe the characteristics of size and shape of this dataset.

The Ward dendrogram based on this correlation coefficient shows two main clusters (Fig. 6a; $C_{sim/ult}$=0·47), with the *N. alba* cluster containing all specimens with an intermediate appearance as well as a single population of *N. candida*.

Multistate discrete characters

The first principal axis of a PCA of the multistate discrete characters composed of counts explained 43% of the total variation, whereas the second and third axes explained 15 and 10%, respectively. A differentiation of the specimens of the two taxa could only be made by the first main axis (Fig. 6d). The intermediate forms were situated between both clusters. The differentiation was mainly caused by the characters 28 and 31 (flower), and 32–35 (pollen) in a positive direction, contrasting with 22, 23 (not significant), 24 (leaf), and 29 and 30 (flower) (Table 2).

The Ward dendrogram ($C_{sim/ult}$=0·70) showed two main clusters, one of *N. candida* and another of *N. alba* (Fig. 6c). The first cluster contained all 20 *N. candida* (●) and four intermediates, whereas the second cluster contained all 24 specimens of *N. alba* (○) and a single intermediate form (▲). The analysis demonstrates that the distribution of

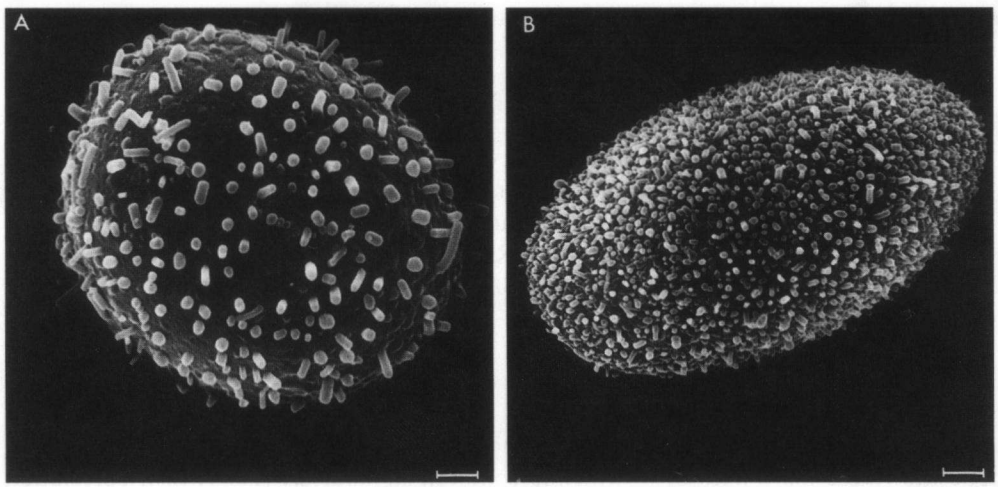

Fig. 4. (a) Pollen of (a); *N. alba* (Dwarsgracht) and (b); *N. candida*; (Steenwijker Diep). The scale bar represents 3·7 μm.

the specimens of the two taxa by their allometric differences is distinct, unlike that of the intermediate forms.

Binary characters

A PCA showed a first principal axis explaining 57% of the total variation, whereas the second and third axes explained only 7% and 6%, respectively. The gap between the two taxa was only shown by the first axis (Fig. 6f), in a similar way and more clearly than for the multistate characters (counts). The intermediate forms were situated between the two taxa, and rather close to the *N. candida* group. The distribution was mainly caused by differences in the frequency distribution of the characters 36, 38 (leaf), 45, 49, 51, 54, 56 and 58 (flower), contrasting in a negative direction with 37, 39, 41 (leaf), 44, 46, 48, 50, 53, 55 and 57 (flower) (Table 2).

The same pattern as that shown by the PCA-scatter plot was displayed by a Ward dendrogram between the specimens ($C_{sim/ult}$=0·90): there were two main clusters. One cluster contained all but one specimen of *N. alba*, while the other main cluster was divided into two subclusters situated very close together (Fig. 6e). The first subcluster contained all 20 specimens of *N. candida*, whereas the second subcluster contained a single specimen of *N. alba* (Overijssels Kanaal) and four of the intermediate specimens, thus showing a tendency to form a separate cluster.

Cophenetic correlations

To estimate the degree of resemblance between the similarity and ultrametric matrices, the cophenetic correlations between them were calculated (Table 3). Some of the correlations between the similarity matrices (Table 3; below diagonal), proved to be rather low, but they were significantly higher for the correlations between the ultrametric matrices. This was because the similarity matrices contained all variation between the specimens, whereas the ultrametric matrices containing only the optimal structure of the dendrograms, freed from redundancies. This then showed, for all three datasets, a similar grouping.

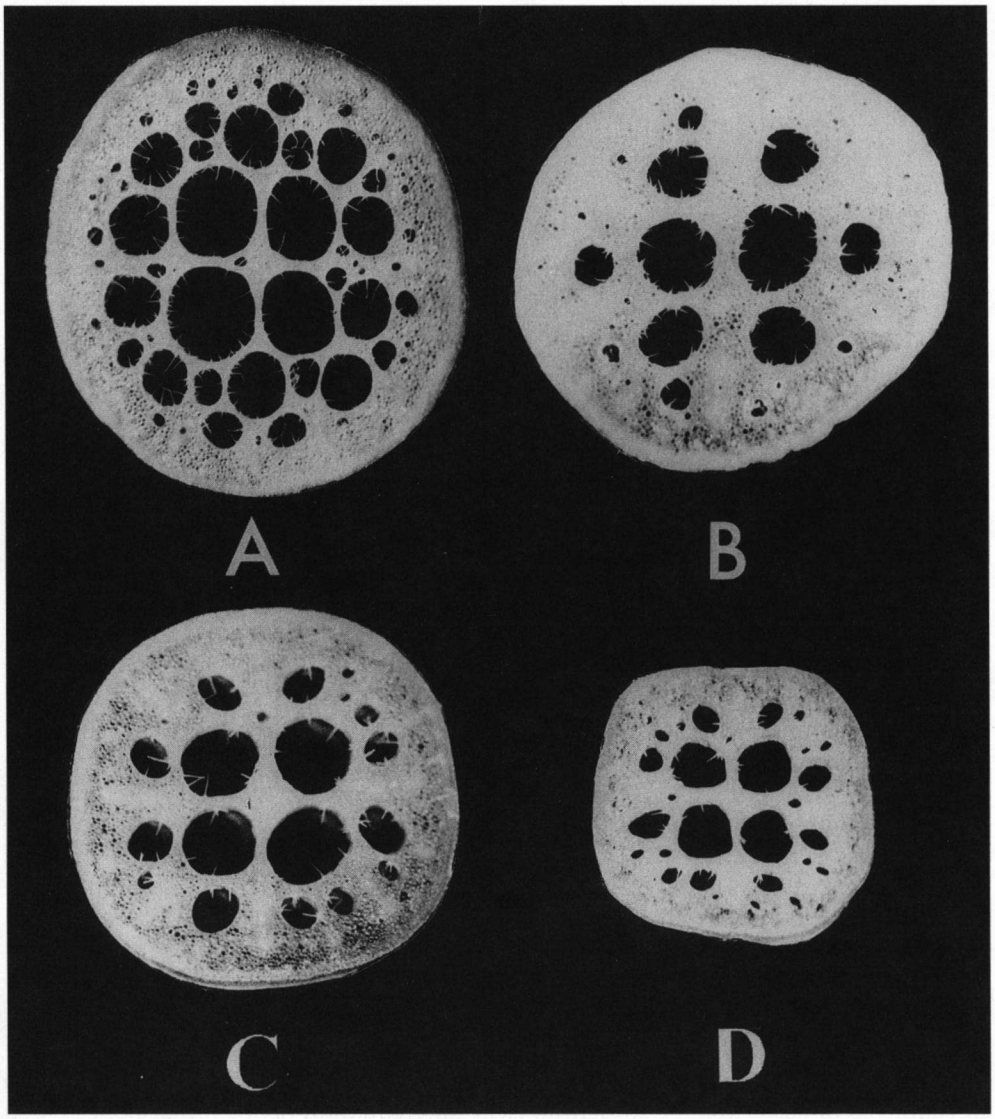

Fig. 4. (b) Numbers of main air channels in (a) peduncle *N. candida*; (b) petiole *N. candida* (Haarsteegse wiel) (c) peduncle *N. alba*; and (d) petiole *N. alba* (Oude Waal).

Discriminant analysis

Discriminant analysis of the characters revealed that five of them, all of the flowers, showed the greatest discriminating power between the groups (Table 4). The discriminant equation was able to classify all specimens of *N. alba* and *N. candida* correctly. Of the five intermediate morphs, three were classified as *N. candida*, and two as *N. alba*. The canonical correlation between the discriminant scores and the grouping of the two taxa was 0·96, whereas the eigenvalue (ratio of between-group sum of squares and within-group sum of squares) was high (12·81). Both statistics indicate the significance of the discriminant equation. To test the robustness of the equation a jack-knife method

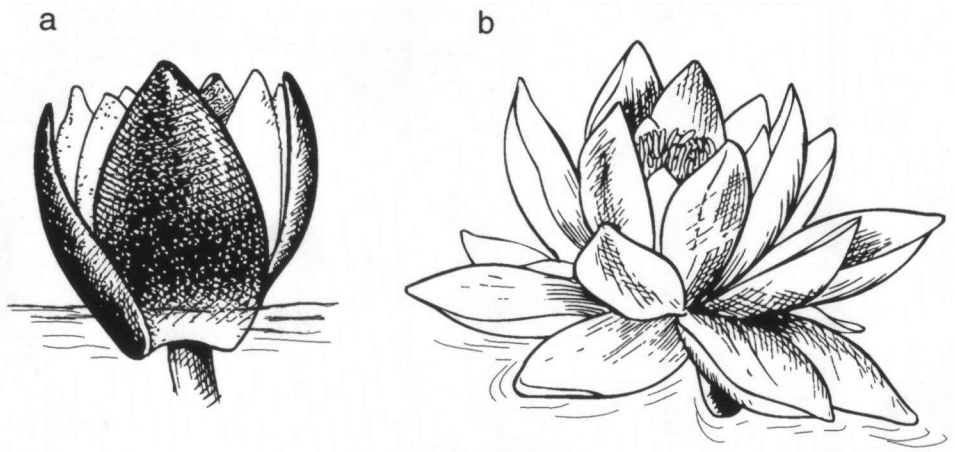

Fig. 5. Position of maximally opened flowers with respect to water surface of (a) *N. candida*, and (b) *N. alba*.

was applied to the dataset, leaving out each of the specimens in turn, calculating the equation based on the remaining n-1 specimens, and then classifying the specimen left out. The results showed that this did not influence the correct classification of the specimens.

Frequency analysis

No clear differentiation between the groups could be made on the basis of the individual characters (Table 2). However, a non-supervised multivariate approach displayed the two distinct *Nymphaea* taxa (Fig. 6a–f). For the frequency analysis all characters, including their ratios, showed overlap (data not shown). The results of the Kolmogorov–Smirnov test of useful characters are summarized in Table 5. The results of the frequency analyses and the binary characters, which proved to be important in the multivariate analysis, have been combined in an identification table (Table 6).

DISCUSSION

A univariate analysis of the variation in the characters showed a significant χ^2 for most of them. All individual continuous and multistate discrete characters showed an overlap in character values (Table 2), so that no clear differentiation between the species based on a single character could be made. This was also demonstrated by the ratios which were calculated for several continuous characters. A multivariate approach, however,

Fig. 6. Multivariate analyses of *N. alba* (○), *N. candida* (●), and intermediate forms (▲), on the basis of continuous characters. (a) Ward dendrogram showing the optimal structure in the similarity matrix of shape differences, estimated by means of the correlations between the specimens. (b) Scatterplot of the specimens according to the first two main axes of a PCA. Loadings of the characters for these two axes are displayed by means of arrows. For the sake of convenience the loadings have been multiplied by 10. The characters are indicated by their number (Table 2). (c) Ward dendrogram showing the optimal structure in the similarity matrix of mean character differences based on ranged characters. Symbols and features are similar to those in (a). (d) Scatterplot of the specimens according to the first two main axes of a PCA. Character 27 is not used because it is too invariant. Symbols and features are similar to those in (b). (e) Ward dendrogram showing the optimal structure in the similarity matrix of mean character differences. Symbols and features are similar to those in (a) and (c). (f) Scatterplot of the specimens according to the first two main axes of a PCA. Symbols and features are similar to those in (b) and (d).

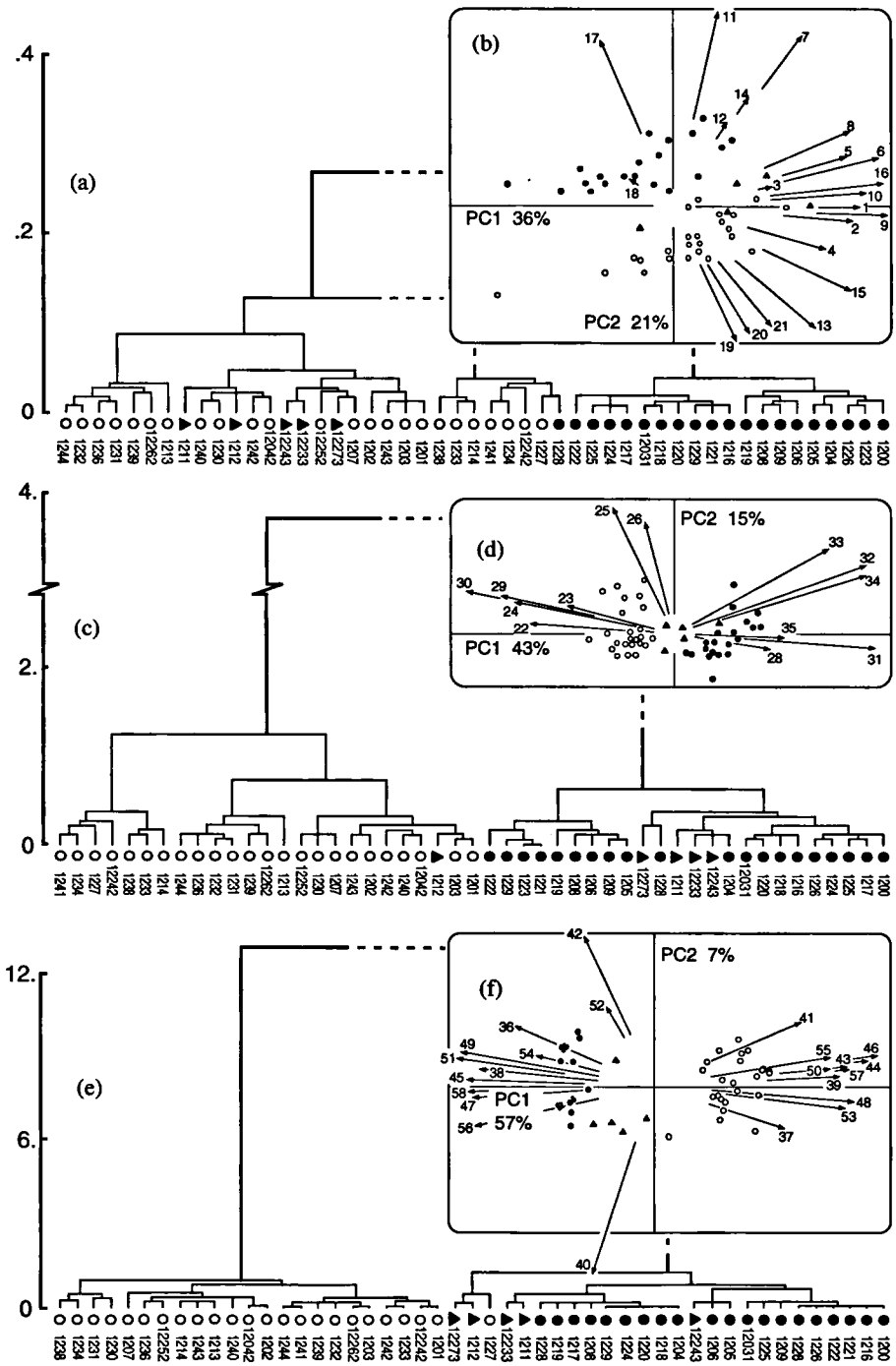
(a)
(b)
(c)
(d)
(e)
(f)
PC1 36%
PC2 21%
PC2 15%
PC1 43%
PC2 7%
PC1
57%

Table 3. Cophenetic correlations between the similarity matrices (below diagonal), and between the ultrametric matrices (above diagonal) for three different kinds of datasets

	Dataset	*N. candida* (continuous)	*N. alba* (multistate)	Intermediate (binary)
N. candida	Continuous	1·00	0·88	0·78
N. alba	Multistate	0·37	1·00	0·84
Intermediate	Binary	0·49	0·65	1·00

Table 4. Characters selected by discriminant analysis. For the characters see Fig. 3. Coefficient of the discriminant equation are also given. The score of the discriminant equation was derived by multiplying coefficient A by the logarithmized (ln) character value. Next all products were summed, and the constant was added. If the score has a negative value the specimen belongs to *N. candida*, if it is positive it belongs to *N. alba*

No.	Description	Coefficient A
7	Maximum width sepal	− 10·2436
9	Length of outer petals	7·1405
13	Width central projection	2·6980
14	Length central projection	− 3·0117
15	Diameter stigma	6·4676
	constant	− 10·6555

which through its simultaneous use of the characters in combination with their individual discriminating power also incorporates differences in within-group character correlations, resulted in differentiation between the two *Nymphaea* taxa (Fig. 6a–d).

Most of the differentiation occurred in allometric differences displayed by the second component, which led to a grouping of the specimens in two main clusters (Fig. 6a, b). The gap between the taxa and their distinctness improved when discrete multistate characters and binary characters were used (Fig. 6c–f). Since in a PCA based on these character types this gap was shown by the first principal axis only, it indicates a simpler pattern of loadings than in the PCA of the continuous characters.

In the dendrograms the intermediate forms, which were identified as intermediates, were grouped in various ways. In the dendrogram based on shape differences in the continuous characters they were distributed over the main clusters of *N. alba* and *N. candida* (Fig. 6a). In the dendrogram using discrete multistate characters they were nearly all grouped with *N. candida* (Fig. 6c), whereas in the dendrogram using binary characters they were grouped with *N. candida* only (Fig. 6e).

Continuous characters

The width of the sepals was greater in *N. candida*. Valle (1927) found the same, but presented much lower values for populations in Finland. The width of the filament of the stamens in the most inner row was greater in *N. candida*, whereas the width of the anthers was the same in both species. It is the shape of the stamens that is important

here. In *N. candida* the filament was 1·5 times wider than the anthers. For *N. alba* the width was the same for both anthers and filament. This results in an elliptic stamen in *N. candida* and a lancelot one in *N. alba* (Tutin 1964; Heß *et al.* 1970). The inner stamens seemed to have more primitive character states for *N. candida*. The centrifugal stamen/petal progression in *N. candida* is accompanied by the loss of fertile tissue (Moseley 1958). This implies that in this respect *N. candida* has more primitive characters than *N. alba*. It is also characteristic that the filaments of the anthers of *N. alba* are bent down at acute angles, whereas those in *N. candida* are gently curved over the stigmatic disk (Van der Velde 1986). The central projection in *N. alba* was longer than in *N. candida*. A character whose loading contrasts with this feature was the width of the central projection, which was greatest in *N. alba*. Glück (1924) mentions similar observations. The diameter of the stigma was much greater in *N. alba*. Neuhäusl & Tomšovic (1937) and Roelofs & Van der Velde (1977) found the same in their plants, but they measured the ratio of stigma diameter to ovary diameter. Glück (1924) also found a higher value for *N. alba*.

The greatest equatorial diameter of the pollen was larger in *N. candida* than in *N. alba* (Fig. 4a). The same result was found in the literature (Conard 1905; Glück 1924; Valle 1927; Neuhäusl & Tomšovic 1937; Beug 1961; Tutin 1964; Roelofs & Van der Velde 1977). However, the shape of the pollen differed from those found in the literature, which range from elliptic to round. These analyses were made using different pollen treatments, so care must be taken in their interpretation. In some cases, the pollen treatment is not even mentioned, although it is considered an important factor contributing to the shape and size of the pollen. Nevertheless, one might expect a certain consistency in shape regardless of any treatment. The pollen grains in our samples were more elliptic in *N. candida*, which is also mentioned by Valle (1927) and Roelofs & Van der Velde (1977). The mean length of bacula, verrucae and gemmae was greater in *N. alba*, whereas the total number of projections, and the numbers of each of the types of projections, were higher in *N. candida*. Roelofs & Van der Velde (1977) also found closely spaced verrucae and bacula in *N. candida*, and a more scattered pattern of larger bacula and verrucae in *N. alba*. No literature data were available on the percentage and length of gemmae.

Multistate discrete characters

The number of main, medium sized, and small air channels in the petioles in *N. alba* was higher than in *N. candida*. However, it is difficult to make a clear distinction between small and medium sized air channels. Future research could restrict itself to counting the number of main air channels, which was two in *N. candida* and four in *N. alba*. Valle (1927) and Kostyniuk (1970) found four main air channels in both taxa, whereas Roelofs & Van der Velde (1977) and Bukowiecki & Furmanowa (1964) also found two in *N. candida*. The number of stamens was much higher in *N. alba*. Roelofs & Van der Velde (1977) and Conard (1905) found the same, although the latter found 64–100 stamens for *N. alba*, while we found 75–131. The number of grooves on the carpellary teeth was 2–4 in *N. candida*, and 0–4 in *N. alba*. In the literature, Valle (1927) and Roelofs & Van der Velde (1977) found a value of 0–1 for *N. alba*, and 3 for *N. candida*. Conard (1905) also found 3 grooves for *N. candida*, but found 1–5 for *N. alba*. The number of petals was higher for *N. candida*. Glück (1924) and Tutin (1964) found the opposite. However, this character does not seem to be useful, because of considerable

Table 5. Kolmogorov–Smirnov two-sample test for several significant variables. EDF=empirical distribution function; Ks=Kolmogorov–Smirnov statistic; Ksa=asymptotic Kolgorov–Smirnov statistic; D=two-sample Kolmogorov–Smirnov statistic

	Width of filament	Number of carpellary teeth	Number of stamens	Diameter stigma	Number of grooves in carpellary teeth
EDF at maximum for *N. candida*	0·0875	0·9756	0·9506	0·9593	0·0385
Deviation from mean	−3·3410	4·2225	2·7056	2·6521	−4·0470
EDF at maximum for *N. alba*	0·8649	0·0253	0·3418	0·3291	0·9863
Deviation from mean	3·4738	−4·3019	−2·7396	−2·6854	4·1832
EDF at maximum for total	0·4610	0·5093	0·6530	0·6313	0·4967
Value at maximum deviation	1·8	13·0	84·0	15·2	2·0
Ks	0·3884	0·4751	0·3044	0·2984	0·4737
Ksa	4·8198	6·0279	3·8504	3·7743	5·8204
D	0·7774	0·9503	0·6088	0·5968	0·9478
Probability>Ksa	0·0001	0·0001	0·0001	0·0001	0·0001

	Length of central projection	Width of central projection	Number of petals	Number of projections	Numer of verrucae
EDF at maximum for *N. candida*	0·8765	0·3333	0·1266	0	0
Deviation from mean	3·1639	−1·3875	−1·5751	−1·6216	−1·8889
EDF at maximum for *N. alba*	0·1646	0·6456	0·4810	0·7143	0·8500
Deviation from mean	−3·2037	1·4050	1·5751	1·4155	1·6895
EDF at maximum for total	0·5250	0·4875	0·3038	0·4054	0·4722
Value at maximum deviation	1·8	3·2	21·0	19·5	9·4
Ks	0·3560	0·1561	0·1772	0·3539	0·4224
Ksa	4·5026	1·9746	2·2276	2·1525	2·5342
D	0·7120	0·3122	0·3544	0·7143	0·8500
Probability>Ksa	0·0001	0·0008	0·0001	0·0002	0·0001

	Maximum diameter of pollen	Length of bacula	Length of verrucae	Max. diam. of pollen/ Min. diam. of pollen	Number of stamens/ Number of carpellary teeth
EDF at maximum for *N. candida*	0·1321	0·9623	0·6981	0·1698	0·0247
Deviation from mean	−2·7139	2·0092	1·6564	−2·0857	−3·0965
EDF at maximum for *N. alba*	0·9000	0·3878	0·2245	0·7600	0·7215
Deviation from mean	2·7941	−2·0896	−1·7227	2·1474	3·1355
EDF at maximum for total	0·5049	0·6863	0·4706	0·4563	0·3688
Value at maximum deviation	37·0	1·7	0·7	1·3214	5·0000
Ks	0·3838	0·2870	0·2366	0·2950	0·3484
Ksa	3·8951	2·8989	2·3898	2·9936	4·4068
D	0·7679	0·5745	0·4736	0·5902	0·6968
Probability>Ksa	0·0001	0·0001	0·0001	0·0001	0·0001

overlap. The number of carpellary teeth was always higher in *N. alba*. This was also found by Conard (1905), Glück (1924), Valle (1927), Neuhäusl & Tomšovic (1957) and Roelofs & Van der Velde (1977). This is a useful character, because little overlap is seen between the two species.

Table 5. *Continued*

	Max. width of sepal/ width of petal	Length of sepal/ Max. width of sepal	Width of filament/ Width of anthers	Length of centr. proj.-/Width of centr. proj.	Diameter of stigma/ Diameter of ovary
EDF at maximum for *N. candida*	0·1728	0·8902	0·1625	0·9753	0·8125
Deviation from mean	−3·3382	3·3932	−2·7864	4·0528	2·6545
EDF at maximum for *N. alba*	0·9241	0·1266	0·8108	0·0633	0·2152
Deviation from mean	3·3802	−3·4570	2·8971	−4·1038	−2·6712
EDF at maximum for total	0·5438	0·5155	0·4740	0·5250	0·5157
Value at maximum deviation	1·0370	2·1500	1·5000	0·5143	0·8333
Ks	0·3756	0·3818	0·3239	0·4560	0·2986
Ksa	4·7507	4·8441	4·0196	5·7677	3·7658
D	0·7512	0·7637	0·6431	0·9120	0·5973
Probability>Ksa	0·0001	0·0001	0·0001	0·0001	0·0001

	Width of sepal/ Width at base sepal	Length of petal/ Width of petal
EDF at maximum for *N. candida*	0·1644	0·8272
Deviation from mean	−1·7599	1·5382
EDF at maximum for *N. alba*	0·6129	0·4810
Deviation from mean	1·9097	−1·5575
EDF at maximum for total	0·3704	0·6563
Value at maximum deviation	1·7500	2·1923
Ks	0·2235	0·1731
Ksa	2·5970	2·1891
D	0·4485	0·3461
Probability>Ksa	0·0001	0·0001

Binary character

The colour of the underside of the leafblade was always red or purple for *N. candida*. Roelofs & Van der Velde (1977) found the same, whereas Glück (1924) found the red colour only on the edge of the underside of the leafblade. Care has to be taken in the interpretation of this leafblade colour, because young leaves of *N. alba* also have this red appearance. The overall nervation of *N. candida* leafblades is always pronounced, as was also reported by Conard (1905), Glück (1924), Valle (1927) and Roelofs & Van der Velde (1977).

The main nerves in the leafslips of *N. candida* were never diverging. This is probably related to the leafslip overlap in this species (Glück 1924; Roelofs & Van der Velde 1977), but the leafslip-overlap can also occur in *N. alba*. Hegi (1965) describes the main nerves of the leafslips of *N. alba* as parallel or slightly bent at the ends, and as converging for *N. candida*.

The flowers of *N. alba* floated entirely on the water surface, whereas those of *N. candida* were partially submerged (Fig. 5). This was also found by Roelofs & Van der Velde (1977). In warm and sunny weather the sepals of *N. alba* were always horizontal on the water surface, while those of *N. candida*, were erect, as is also mentioned in the

Table 6. Identification table for *Nymphaea alba* and *N. candida*

Character	*N. candida*		*N. alba*	
	Range	Mean	Range	Mean
Continuous				
Maximum width sepals	22·1–35·7	27·8	11·0–29·7	23·3
Width filament inner stamens	1·8–2·8	2·2	0·9–2·1	1·5
Length central projection	2·5–4·5	1·4	1·9–4·8	2·3
Width central projection	1·0–2·0	3·4	1·5–3·3	3·1
Diameter stigma	10·0–15·2	12·4	10·9–21·8	16·8
Highest equatorial diameter pollen	36·0–50·4	43·6	29·0–42·0	34·1
Mean length bacula	0·8–1·6	1·4	1·0–3·5	2·0
Mean length verrucae	0·4–1·0	0·7	0·6–1·4	0·9
Mean length gemmae	0·7–1·3	1·0	0·8–1·8	1·4
Counts				
Number of main air channels petiole	2–3	2	1–4	3
Number of stamens	52–85	71	75–131	91
Number of carpellary teeth	9–14	10	15–25	18
Number of grooves on inner side carpellery teeth	3–4	3	0–3	1
Number of petals	19–31	25	17–28	22
Number of projections pollen/100 μm^2	23–81	48	6–38	17
Number of verrucae pollen/100 μm^2	9–48	25	0–17	6
Ratios				
Max. width sepal/width petal	0·8–1·5	2·1	0·5–1·2	0·9
Length sepal/max. width sepal	1·5–2·5	1·9	1·8–3·4	2·4
Width sepal/width at base sepal	1·6–3·0	2·1	1·2–2·5	1·8
Length petal/width petal	1·7–2·7	2·0	1·6–2·8	2·2
Width filament inner stamens/width anthers inner stamens	1·0–2·8	1·8	0·8–2·3	1·3
Length central projection/width central projection	0·3–0·6	0·4	0·5–1·6	0·8
Diameter stigma/diameter ovary	0·6–1·2	0·8	0·6–1·5	0·9
Max. diam. pollen/min. diam. pollen	1·1–2·1	1·5	1·0–2·3	1·3
Number of stamens/number of pistil-teeth	5·2–10·3	7·0	4·0–6·7	5·2
Binary				
Shape of the central projection	pin-shaped		round knob	
Colour underside leafblade	always red		red or green	
Nervation leafblade	pronounced		not pronounced	
Direction main nerves leafslips diverging	never		usually, or parallel	
Position flower on/half below water surface	always half below		on	
Vertical/erect sepals	always		never	
Shape of flower (maximally opened)	cup-shaped		star-shaped	
Side view flowerbase	concavely curved		straight	
View underside flowerbase	square		round	
Carpellary teeth shiny/dull	dull		shiny	
Colour carpellary teeth	yellow		mostly orange–dark yellow	
Colour stigma	red		yellow	

literature (Conard 1905; Valle 1927; Roelofs & Van der Velde 1977). This also implies that the flowers of *N. candida* are never star-shaped, but show a square shape (Giesen & Van der Velde 1983). *N. candida* flowers are less colourful in the ultraviolet region of the spectrum, as seen by flower-visiting insects, especially bees, syrphids and ephydrid

flies. This could make these flowers less attractive to these insects (Giesen & Van der Velde 1983). The side view of the underside of the flowerbase was always concavely curved in *N. candida*, while in *N. alba* it was straight or rounded. This was also mentioned by Conard (1905), Valle (1927) and Roelofs & Van der Velde (1977). The view of the underside of the flowerbase was square in *N. candida* and circular in *N. alba*, which is also in agreement with results found in the literature (Glück 1924; Valle 1927; Neuhäusl & Tomšovic 1937; Roelofs & Van der Velde 1977). The flowers of *N. candida* were more rigid than those of *N. alba*. When the flowers of *N. candida* were pulled underneath the water surface they became filled with water, in contrast with *N. alba* flowers which closed gently under such circumstances and remained free of water when released, which means that pollination in *N. candida* can be hindered by wave action. This would make *N. candida* less successful in sexual reproduction under such circumstances. It is interesting to note that in general the petioles and peduncles of *N. alba* were stiffer than those of *N. candida*, which seemed to be flexible. This, together with the pronounced nervation at the underside of the floating leafblades, can be considered as adaptations to wave action in *N. candida*. The fact that *N. candida* has been found in deeper and more wave exposed waters than *N. alba* confirms this.

The carpellary teeth were always dull in *N. candida* but shiny in *N. alba*, as was also found by Roelofs & Van der Velde (1977). Furthermore, they always had a yellow colour in *N. candida* while being mostly dark yellow to orange in *N. alba*. The stigma was often red in *N. candida* and nearly always yellow in *N. alba*, in accordance with the findings of Valle (1927) and Glück (1924). Although no *N. alba* specimens with a red stigma were found, this characteristic does not seem to be very useful for identification, because it is variable in *N. candida*. The central protection was always pin-shaped in *N. candida*. This is confirmed by the observations of Conard (1905), Valle (1927), Glück (1924, Neuhäusl & Tomšovic (1937) and Roelofs & Van der Velde (1977). This feature is also concealed in the continuous characters 13 and 14 (Table 2).

General

Nymphaea candida in The Netherlands appears to differ from the descriptions in the literature in a number of characters. First, the number of main air channels of the petiole was found to be two for *N. candida* in The Netherlands, while specimens described in some of the literature had four (Valle 1927; Kostyniuk 1970). Secondly, the number of petals was larger in *N. candida* from The Netherlands. Of the flowers investigated 90% possessed 20 petals or more, with a maximum of 32. The literature reports much lower numbers, which is probably due to poor conditions in the areas where they were found. Finally, the ovary of *N. candida* from The Netherlands was covered entirely with stamens, whereas Conard (19805), Glück (1924) and Valle (1927) found that the part under the stigma was free of stamens. It is interesting that the differentiation of *N. candida* by Presl was originally based on this character (Conard 1905). However, this character has also been described in the subspecies *N. alba* ssp. *occidentalis* as described by Ostenfeld (1912) and Heslop-Harrison (1955, 1975). This subspecies is often mistaken for *N. candida*, thus creating further confusion.

Size of leaves and flowers, as well as the number of plant parts (e.g. stamens, petals, sepals and carpellary teeth), depend on (1) the nutrient availability in the soil in which the plant is rooted (Glück 1924; Roelofs & Van der Velde 1977), and (2) the age of the plant (Glück 1924). This is why several ratios used in the analyses showed considerable

overlap and, as a multivariate approach gave more significant results, were therefore excluded from multivariate analyses. The numbers of petals, stamens and carpellary teeth are positively correlated with flower size, which in turn depends on nutrient availability (Glück 1924). The distinction between small and medium sized air channels depends on the objectivity of the researcher, but this is not a problem, because only the main air channels are important for the distinction between the taxa. The difference between verrucae and gemmae, however, can become a problem, which can only be solved by practice.

No north–south gradient was seen in either of the separate cluster analyses (Fig. 6a,c,e). However, the specimens classified as intermediate in the cluster analysis were always found where both taxa meet. These plants were collected in the Weerribben and the Overijssels Kanaal, areas where both *N. candida* and *N. alba* occur (Giesen & Van der Velde 1978).

CONCLUDING REMARKS

In conclusion, it has become clear by multivariate analysis that *N. candida* differs from *N. alba* in its morphological characteristics. The fact that the discriminant equation was able to classify all specimens of *N. alba* and *N. candida* correctly, together with its statistics (Tables 3 and 4), demonstrates that it is very effective. However, both analyses are based on characters that are more or less dependent on the nutritional conditions of the soil. Furthermore, discriminant analyses may not be practical for identification in the field. In order to avoid problems originating from these allometrically associated environmental factors, we also offer the results of a frequency analysis. Although ratios were not used in multivariate analyses they are given here, thus creating an extra dataset describing the plant shape. The results of theses frequency analyses, combined with those of the discriminant function analyses, provide a classification table shown in Table 6. Although there was considerable overlap in some characters, the combination of characters are useful for identification (Table 5) for *N. alba*, *N. candida* and the intermediates. Since no information was available on crossing and backcrossing experiments, however, it is also clear that further research needs to be done. Because *N. candida* has never been found in acidic waters in The Netherlands, whereas *N. alba* has been found in both acidic and alkaline waters (Van der Velde *et al.* 1986), it would be interesting to analyse the association of morphological and environmental parameters. Moreover, it would be of interest to study these taxa at a molecular level by means of isozyme and DNA electrophoresis.

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REFERENCES

Beug, H.-J. (1961): *Leitfaden der Pollenbestimmung für Mitteleuropa und angrenzende Gebiete*, p. 21, Gustav Fischer Verlag, Stuttgart.

Bukowiecki, H. & Furmanowa, M. (1964): Ecological anatomy of waterlilies, *Nymphaea candida* Presl. *Acta Poloniae Pharmaceutica.* **21:** 113–119.

Caspary, R. (1879): Hvilken utbredning hafva Nymphaeaceerne i Skandinavien? *Botaniska Notiser*: **3:** 65–93.

Casper, S.J. & Krausch, H.-D. (1981): *Süßwasserflora von Mitteleuropa, Band 24*, pp. 461–465, Gustav Fischer Verlag, Stuttgart, New York.

Conard, H.S. (1905): *The Waterlilies. A monograph of the genus Nymphaea*, The Carnegie Institution of Washington, Publication No. 4, pp. 172–179.

De Langhe, J.E., Delvosalle, L., Duvigneaud, J., Lambinon, J. & Van den Berghen, C. (1988): *Flora van België, het Groothertogdom Luxemburg, Noord-Frankrijk en de aangrenzende gebieden (Pteridofyten en Spermatofyten)*. 2e druk, pp. 48–51, Uitgave van het Patrimonium van de Nationale Plantentuin van België, B-1860 Meise, Ceuterick n.v.

Giesen, Th.G. & Van der Velde, G. (1978): De verspreiding van *Nymphaea candida* Presl en *Nymphaea alba* L. in Nederland. *Gorteria* **9:** 61–66.

Giesen, Th.G. & Van der Velde, G. (1983): Ultraviolet reflectance and absorption patterns in flowers of *Nymphaea alba* L., *Nymphaea candida* Presl and *Nuphar lutea* (L.) Sm. (Nymphaeaceae). *Aquat. Bot.* **16:** 369–376.

Glück, H. (1924): *Biologische und Morphologische Untersuchungen über Wasser-und Sumpfgewächse, IV. Teil: Untergetauchte und Schwimmblattflora*, pp. 328–459, Gustav Fischer Verlag, Jena.

Gupta, P.P. (1980): Cytogenetics of aquatic ornamentals VI: evolutionary trends and relationships in the genus *Nymphaea*. *Cytologia* **45:** 307–314.

Haeupler, H. & Schönfelder, P. (1989): *Atlas der Farn- und Blütenpflanzen der Bundesrepublik Deutschland*, pp. 177–178, Verlag Eugen Ulmer GmbH & Co, Stuttgart.

Hegi, E. (1965): Familie Nymphaeaceae. *Illustrierte Flora von Mitteleuropa, Band III*, pp. 1–29, Carl Hansen Verlag, Wien.

Heslop-Harrison, Y. (1955): *Nymphaea* L. em. Sm. (nom. conserv.). *J Ecol.* **43:** 719–734.

Heslop-Harrison, Y. (1975): *Nymphaea* L., *Nuphar* Sm. In: Stace, C.A. (ed.): *Hybridization and the Flora of the British Isles*, pp. 131–132. Academic Press, London.

Heß, H.E., Landolt, E. & Hirzel, R. (1970): *Flora der Schweiz, Band 2*, pp. 7–9, Birkhäuser Verlag, Basel/Stuttgart.

Heukels, H. & Van der Meijden, R. (1990): *Flora van Nederland*, p. 144, Wolters-Noordhoff, Groningen.

Hogeweg, P. & Hesper, B. (1972): *BIOPAT, Program system for biological pattern analysis*. Theor. Biol. Group, Univ. of Utrecht, Utrecht (internal publication).

Jones, M.R. & Clarke, G.C.S. (1981): Nymphaeaceae. In: Punt, W. & Clarke, G.C.S. (eds): *The Northwest European Pollen Flora, III*, pp. 56–67, Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York.

Kostyniuk, M. (1970): Comparative leaf anatomy and alkaloid content in the Nymphaeaceae Bentham and Hooker. *Monog. Bot.* **31:** 1–56.

Masters, C.O. (1974): *Encyclopedia of the Water-lily*, T.F.H. Publications, Inc., USA.

Mennema, J. & Van Ooststroom, S.J. (1979): Nieuwe vondsten van zeldzame planten in Nederland, hoofdzakelijk in 1978. *Gorteria* **9:** 347–364.

Mennema, J. & Holverda, W.J. (1981): Nieuwe vondsten van zeldzame planten in Nederland, hoofdzakelijk in 1980. *Gorteria* **11:** 189–213.

Meusel, H.E., Jäger, E. & Weinhert, E. (1965): *Vergleichende Chorologie der Zentraleuropäischen Flora*, VEB Gustav Fischer Verlag, Jena.

Moseley, M.F. (1958): Morphological studies of the Nymphaeaceae I; the nature of the stamen. *Phytomorphology* **8:** 1–29.

Neuhäusl, R. & Tomšovic, P. (1957): Die Gattung *Nymphaea* (L.) Smith in der Tschechoslowakei. *Preslia* **29:** 225–249.

Norusis, M.J. (1993): *SPSS for Windows, Professional Statistics, release 6.0*. SPSS Inc., USA.

Ostenfeld, C.H. (1912): The international phytogeographical excursion in the British Isles. VI. Some remarks on the floristic results of the excursion. *New Phytol.* **11:** 114.

Radics, F. (1967): A revision of the *Nymphaea* material in Hungarian Natural History Museum. *Pars Bot.* **59:** 135–145.

Roelofs, J.G.M. & Van der Velde, G. (1977): *Nymphaea candida* Presl, een waterlelie nieuw voor Nederland. *De Levende Natuur.* **80:** 170–186.

Roweck, H. (1988): Ökologische Untersuchungen an Teichrosen. *Arch. Hydrobiol. Suppl.* **81:** 103–358.

SAS Institute Inc. (1989): *SAS/STAT User's Guide. Version 6, Vol. 2*, 4th edn, pp. 1195–1203, SAS Institute Inc., Cary, NC, USA.

Sneath, P.H.A. & Sokal, R.R. (1973): *Numerical Taxonomy*. W.H. Freeman & Co., San Francisco.

Tillich, H.J. (1990): Die Keimpflanzen der Nymphaeaceae—Monocotyl oder Dicotyl? *Flora* **184:** 169–176.

Tutin, T.G. (1964): Nymphaeaceae. In: Tutin, T.G. *et al.* (eds.): *Flora Europaea*, vol. 1, pp. 204–205, Cambridge University Press, Cambridge.

Tüxen, R. (1955): *Nymphaea candida* Presl in Niedersachsen. *Mitt. flor.-soz. Arbeitsgem. N.F.* **5:** 113.

Valle, K.J. (1927): Suomen *Nymphaea*-Lajit (Referat: über die *Nymphaeae*-arten Finnlands). *Ann. Soc. Zool. Bot. Fenn. Vanamo.* **7:** 203–309.

Van der Velde, G. (1986): Developmental stages in the floral biology s.l. of Dutch Nymphaeaceae (*Nymphaea alba* L., *Nymphaea candida* Presl,

Nuphar lutea (L.) Sm.). *Acta Bot. Neerl.* **35**: 111–113.

Van der Velde, G., Custers C.P.C. & de Lyon, M.J.H. (1986): The distribution of four nymphaeid species in the Netherlands in relation to selected abiotic factors. *Proceedings EWRS/AAB 7th Symposium on Aquatic Weeds*, pp. 363–368, Loughborough.