

A STUDY OF THE *JUNCUS BUFONIUS* COMPLEX IN THE NETHERLANDS

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SUMMARY

The presence in the Netherlands of three taxa of the *Juncus bufonius* complex with different ploidy levels was established: a diploid one with $2n = 34$, a tetraploid with $2n =$ about 70, and a hexaploid with $2n = 100-110$. The taxa in question can be distinguished by several morphological characteristics, and they exhibit in addition differences in their distribution and ecology. They are here tentatively referred to *J. ranarius* ($2n = 34$), *J. minutulus* ($2n =$ c. 70) and *J. bufonius* s.str., $2n = 100-110$.

1. INTRODUCTION

Juncus bufonius s.l. is in Europe a very polymorphic complex in which an appreciable number of "species" has been distinguished (see, e.g., ASCHERSON & GRAEBNER 1904, HEGI 1939). Several workers have shown that within the aggregate also cytological variation occurs (WULFF 1937, LÖVE et al. 1971, SNOGERUP 1971).

In the Netherlands, *J. bufonius* s.l. is of common occurrence and also exhibits a great deal of variation. REICHGELT (1964) distinguishes in *Flora Neerlandica* two taxa, viz. *J. bufonius* L. ssp. *bufonius* and *J. bufonius* L. ssp. *ambiguus* (Guss.) Sch. et Thell. According to SEGAL (1962) the ssp. *bufonius* and *ambiguus* have different chromosome numbers.

The purpose of the present investigation was to establish what cytological races of the *J. bufonius* complex are found in the Netherlands, subsequently if these chromosomal races can be segregated by means of morphological features, and ultimately whether each race has its own ecological niche and phytogeographical range.

2. CYTOLOGICAL INVESTIGATION

2.1 Methods

During the summer of 1973, 31 localities where *J. bufonius* s.l. occurred were visited. At each site a population sample was taken, as a rule 50 specimens being collected. These samples are preserved in the herbarium of the Hugo de Vries-laboratorium (AMD). From three plants of each population sample young seedlings were obtained out of seeds previously subjected to a stratification

process (i.e., a cold treatment under moist conditions). These seedlings were used for the cytological investigation, after a pretreatment in the dark at 4°C for 24 hrs followed by 3 hrs of light at 25°C, by fixation in Carnoy mixture (absolute ethanol: chloroform: glacial acetic acid = 6:3:1), and staining (after a pretreatment of the seedling in 1N HCl at 60°C for 10 min.) in Schiff's reagent (Feulgen staining). The stained material was subsequently macerated in a 0.75 per cent solution of pectinase and 0.75 per cent pectinylglycosidase (Pectinol D and Rohament P, respectively) for 3 hrs at 35°C. This treatment was stopped by transferring the material to a 45 per cent solution of acetic acid in which it was left for about 30 min. The meristem of the primary leaf was subsequently teased out and used for the squashes. Squashed slides were studied under a phase-contrast microscope at a magnification of $\times 1250$ and the best ones were finally embedded in Euparal.

2.2 Results and discussion

It is usually not at all easy to obtain good chromosome counts of *Juncus bufonius* s.l. The chromosomes are not only minute (0.4–1.0 μm) and numerous, but often present in a globular arrangement or in dense clusters. For this reason observational errors are bound to occur. Over 500 counts were performed in material of 205 young seedlings. The numbers recorded lie between $2n = 27$ and $2n = 115$, and fall into three discrete groups:

- group *a*, with $2n = 27$ –37,
- group *b*, with $2n = 58$ –78, and
- group *c*, with $2n = 81$ –115.

Different counts obtained from a single seedling, and all counts of the different individual seedlings obtained from the same parent plant yielded numbers falling within the same group. The results are shown in the diagrams (see *fig. 1*).

These observations are clearly indicative of the occurrence of three different cytological races within the *Juncus bufonius* aggregate in the Netherlands. SNOGERUP (1963) has suggested that in the section *Tenageia* of *Juncus* (to which *J. bufonius* s.l. belongs) the (secondary) basic chromosome numbers are $x = 17$ and $x = 18$ – this in contrast to previous proposals for $x = 5, 8$, or 10 (DARLINGTON & WYLIE 1955; LÖVE & LÖVE 1961 – and on this ground we consider group *a* to represent a diploid race ($2n = 34$), group *b* a tetraploid one ($2n = c. 70$), and group *c* a hexaploid form ($2n = 100$ –110). As pointed out before, the variations in chromosome number found are largely attributable to observational errors, although, and more particularly in hexaploid plants, a true variation in number may be present. Our counts agree rather closely with the values recorded previously by other authors (see *table 1*).

Older records of chromosome numbers of $2n = 30, 32, c. 60, 80, c. 120$ (JØRGENSEN et al. 1958, LÖVE & LÖVE 1948, SEGAL 1962) are probably incorrect owing to imperfect cytological techniques and the ensuing inaccuracies and unreliable observations.

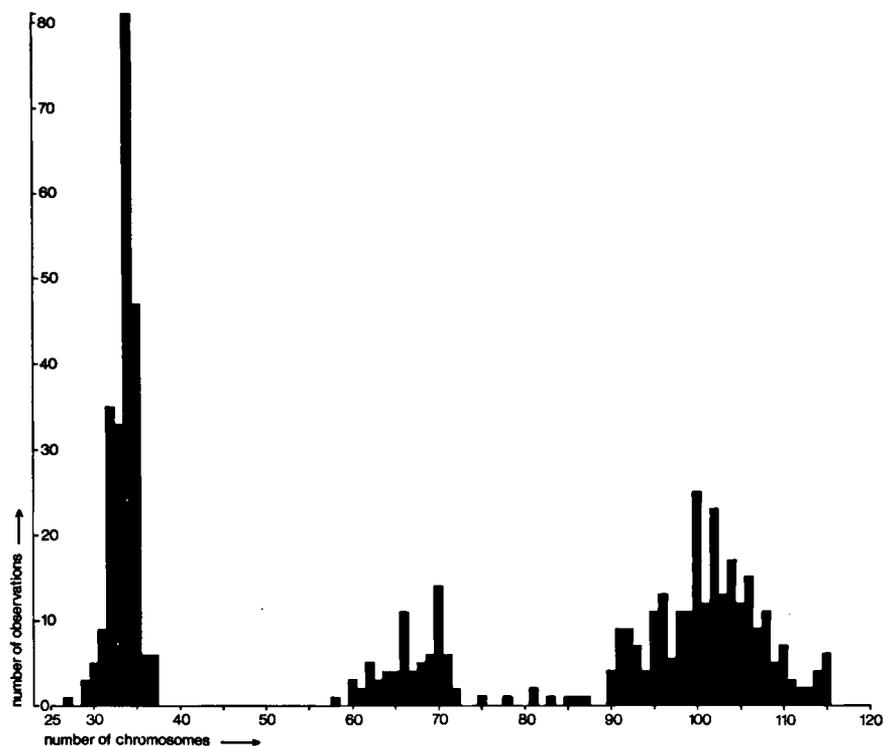


Fig. 1. Survey of the chromosome counts in the populations studied.

Table 1. Survey of recorded chromosome numbers.

Author(s)	diploid	tetraploid	hexaploid
LÖVE et al. 1971	34	—	106
SNOGERUP 1971	—	c. 72	100–110
TAYLOR & MULLIGAN 1968	34	—	—
VAN LOENHOUD & STERK 1976	34	c. 70	100–110

3. MORPHOLOGICAL STUDIES

3.1 Methods

In order to obtain an insight into the possible incidence of morphological differences between the various chromosomal races, analyses were carried out as follows.

Of a number of plants with known chromosome number a number of measurable characters was selected and each one was recorded. Floral characters were obtained from 2 flowers taken from each of 10 plants of each ploidy level, which

20 flowers were always taken from the same, comparable place in the compound inflorescence, boiled up in water, and examined under a binocular dissecting microscope. The seed size was determined by measuring 50 seeds taken from each of 4 plants (= 200 seeds per ploidy level) with an error of about 0.01 mm. The significance of the differences in seed size was estimated by means of the Wilcoxon test.

3.2 Results and discussion

The results of the measurements of floral and seed characters are compiled in table 2.

The data shown in this table reveal morphological differences, although in most cases there is an overlap (compare *fig. 2*). The diploid is most distinct from the other two races. Striking differences are the obtuse tip of the inner whorl of tepals (1), the number and the length of the stamens (2, 3), and the small difference in length between the inner tepals and the capsule valves (9). The tetra- and the hexaploid cannot so easily be distinguished; the tetraploid is characterised, among other things, by the shorter filaments (4), the relatively longer anthers (6), and the smaller and more acute fruit valves (7, 8).

The seed size deserves special mention. The length of the seed of the diploids and of the tetraploids is the same (10), but the seeds of the hexaploids are significantly larger. The seed breadth is the same in the diploids and the hexaploids (11), whereas the seeds of the tetraploids are significantly narrower. The mean seed dimensions, therefore, appear to provide good distinguishing characters to separate the three cytodemes.

If one attempts to identify the chromosomal races by means of the available literature (see ASCHERSON & GRAEBNER 1904, HEGI 1939, VAN OOSTSTROOM 1950, REICHGELT 1964), the diploid agrees with *Juncus ranarius* Perr. et Song. (= *J. bufonius* L. var. *halophilus* Buch. et Fern.), the tetraploid with *J. minutulus* Alb. et Jahan. (KRECHETOVIC & GONCHAROV 1935, SNOGERUP 1971), and

Table 2. Floral and seed characters of *Juncus bufonius* L. s.l. (the means and standard deviations of the measurements are given in mm).

	Diploid	Tetraploid	Hexaploid
1. Tip of inner perigone segment	obtuse	acute	acute
2. Number of stamens	6 ± 0	4 ± 2	4 ± 2
3. Length of stamen	1.6 ± 0.1	2.0 ± 0.3	2.1 ± 0.3
4. Length of filament	1.0 ± 0.1	1.1 ± 0.2	1.4 ± 0.2
5. Length of anther	0.6 ± 0.1	0.8 ± 0.4	0.7 ± 0.2
6. Ratio 4/5	1.8 ± 0.4	1.6 ± 0.8	2.2 ± 0.7
7. Length of fruit valve	3.8 ± 0.3	3.2 ± 0.5	3.5 ± 0.5
8. Tip of valve	truncate	slightly acuminate	rounded
9. Difference in length between inner perigone segment and fruit valve	0.1 ± 0.3	0.7 ± 0.5	0.6 ± 0.4
10. Length of seed	0.39 ± 0.03	0.39 ± 0.03	0.45 ± 0.04
11. Breadth of seed	0.27 ± 0.03	0.25 ± 0.02	0.27 ± 0.03

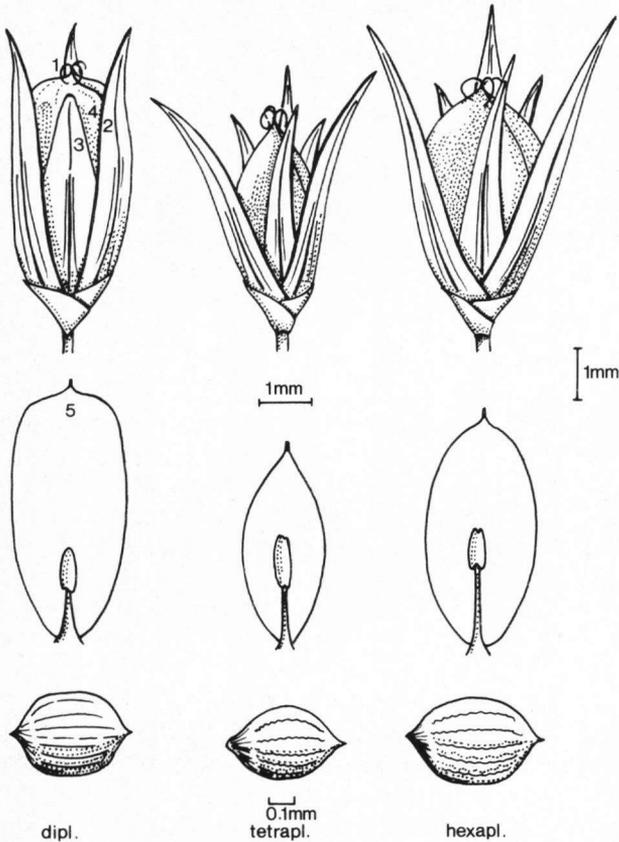


Fig. 2. The morphology of flowers, fruitvalves with stamens and seeds of diploid (*J. rana-rius*), tetraploid (*J. minutulus*) and hexaploid (*J. bufonius*) plants. 1 = stigma. 2 = outer perigone leaf. 3 = inner perigone leaf. 4 = fruit. 5 = fruit valve.

the hexaploid with *J. bufonius* L. s. str. For the time being we accept these names for the three chromosomal races within the *J. bufonius* complex which occur in the Netherlands.

4. PHYTOGEOGRAPHICAL AND ECOLOGICAL STUDIES

4.1 Methods

In order to establish the distribution of each cytodeme in the Netherlands, the Dutch specimens present in the Rijksherbarium Leyden (L) and the herbaria of Utrecht University (U), the University of Amsterdam (AMD) and of the Catholic University of Nijmegen (584 sheets in all) were identified by means of the abovementioned differentiating characters and placed in one of the three taxa (see 3.2).

Some insight in the respective ecological requirements was obtained by recording, at each mass collection site, brief descriptions of habitat and soil type, and relevés made according to the methods of the Braun-Blanquet approach (see WESTHOFF & VAN DER MAAREL 1973). These relevés were combined in a table for each polyploid race, and from these three tables the spectrum was calculated of the vegetation classes (highest syntaxonomical units) distinguished in the Netherlands (WESTHOFF & DEN HELD 1969). To this end, for each characteristic species of every class represented in the table the following quotient was calculated:

$$Q = \frac{a + 2b}{\Sigma (a + 2b)}$$

in which a stands for the number of cases in which the species in question was recorded in the combined estimate (i.e. abundance and cover estimate) as "r", "+", or "I", and b for the number of cases in which this species appeared with the qualification "2", "3", "4" or "5" in the combined estimate, Σ being the total number of all characteristic species occurring in the table. By adding up the Q values of all characteristic species of each class a number is obtained which yields, after multiplication by 100, the percentage of representation of the class in the table (see table 3).

4.2 Results and discussion

When the herbarium sheets were being named, it appeared that *Juncus ranarius* is easily recognisable. *J. minutulus* and *J. bufonius* are not so readily distinguishable but their segregation by means of the seed characters proved to be possible in all cases. With these herbarium records in hand it was possible to draw up distribution maps of the species in the Netherlands, as shown in the figs. 3, 4 and 5.

J. ranarius proves to be of common occurrence in a broad zone along the coast, along the IJsselmeer and in the large reclamation areas (polders), but is rarer in the interior along the great rivers (fig. 3). In *Flora Neerlandica* (REICHGELT 1964) this taxon is said to be "not rare along the coast and the IJsselmeer, of more sparse occurrence more inland". The inland localities appear to be mainly restricted to the area of the great rivers. Reichgelt states that "*J. bufonius* L. ssp. *bufonius*" (which taxon, in his circumscription, most probably includes both the tetra- and the hexaploid) is common throughout the country. Fig. 4 shows, however, that the tetraploid (*J. minutulus*) is the rarest of the three and almost restricted in its occurrence to the Pleistocene sands in the interior. *J. bufonius* is indeed wide-spread (fig. 5) but prefers moist habitats.

A comparison of the habitat ecology and the soil conditions reveals that all three species are mostly found on moist, sandy soils which usually exhibit some superficial compaction and water stagnation, and where the vegetation cover is rather open owing to a certain amount of disturbance. The diploid prefers more or less natural sites which are usually more or less brackish and have a reduced

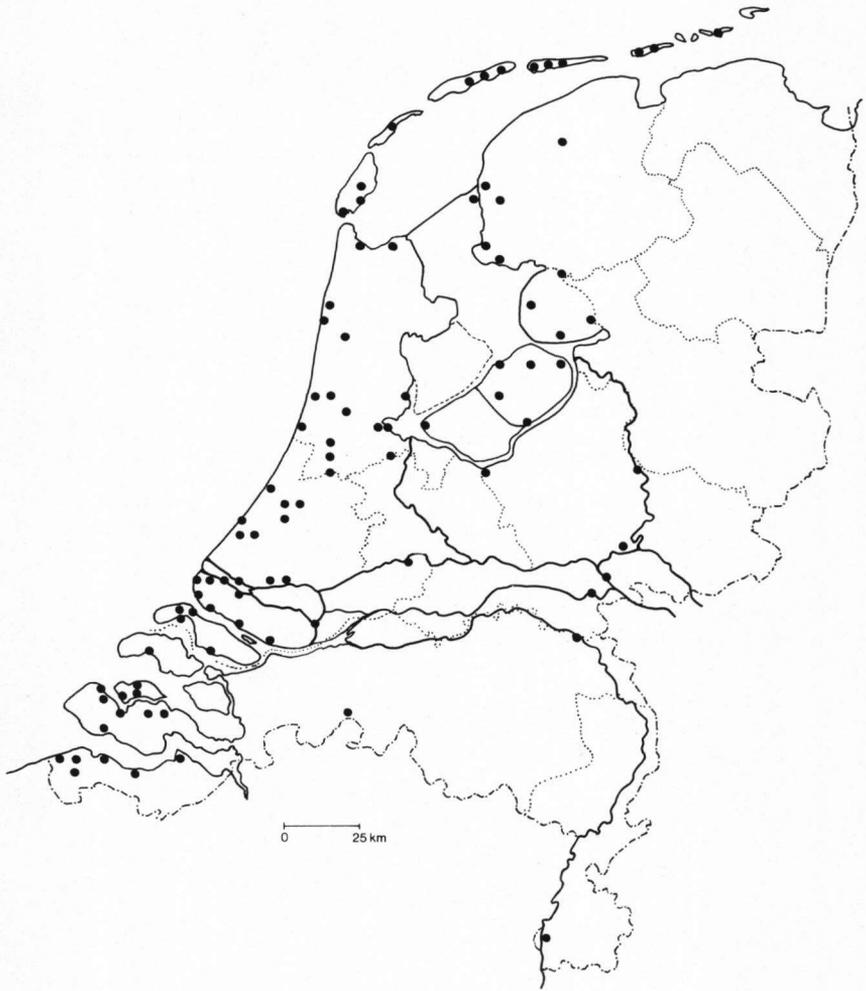


Fig. 3. Distribution of *J. ranarius* in the Netherlands.

or undeveloped soil. The tetraploid is more restricted to relatively little disturbed, oligotrophic and not extreme habitats, whereas the hexaploid is especially encountered in anthropogenic, strongly disturbed places which tend to be ruderal.

In brackish, ruderal localities diploids and hexaploids are often found growing together. When the three polyploid races are arranged in a sequence according to their degree of environmental dynamics, the tetraploid manifestly prefers the least dynamic, and the hexaploid the most dynamic environments, the diploid taking up an intermediate position.

Table 3 shows the contribution (expressed in percentages) of the characteristic species of various vegetation classes which were sympatric with *Juncus bufonius*



Fig. 4. Distribution of *J. minutulus* in the Netherlands.

s.l. The classification of WESTHOFF & DEN HELD (1969) is followed here; for the calculation, see p. 198.

The most important point of correspondence between the three spectra is the large representation of the species of the *Plantaginetea majoris* (about 25%), which is, generally speaking, indicative of the disturbed nature of stands with *Juncus bufonius* s.l. The species in question are usually found in contact zones of the *limes convergens* type (WESTHOFF & VAN DER MAAREL 1973). The diploid distinguishes itself by the substantial contribution by species of halophytic vegetation types (*Thero-salicornietea*, *Cakiletea* and *Asteretea*) and of the *Bidentetea*,



Fig. 5. Distribution of *J. bufonius* s.str. in the Netherlands.

which suggests nitrophily and a changing water table. The tetraploid is characterised by the small amount of elements belonging to vegetation types of nutrient-rich communities and relatively greater representation of communities of oligotrophic habitats, as compared to the diploids and hexaploids. Of frequent occurrence are the species belonging to the *Secalietea*, *Koelerio-Corynephoretea*, *Parvocaricetea*, and *Nardo-Callunetea*, which is indicative of a more acid, more sandy, and more stable character of the localities where the tetraploids were found. The hexaploid seems to prefer nutrient-rich and more disturbed sites as may be deduced from the large proportion of species of the *Bi-*

Table 3. Vegetational spectra of the habitats (relevés) recorded, arranged according to the ploidy level of the *Juncus* species present. The numbers indicate the percentages of the characteristic species per vegetation class.

	diploid	tetraploid	hexaploid
Plantagine ^t ea majoris	23.9	25.9	25.3
Thero-Salicornietea	2.7	–	0.5
Cakiletea maritimae	2.4	–	2.0
Asteretea tripolii	18.9	–	5.8
Bidentetea tripartiti	16.2	6.0	9.9
Phragmitetea	11.4	5.2	9.6
Chenopodietea	7.7	8.7	10.4
Isoeto-Nanojuncetea	7.7	8.7	8.9
Molinio-Arrhenatheretea	4.0	8.7	9.6
Parvocaricetea	1.1	5.2	6.1
Koelerio-Corynepherea	0.3	6.9	1.0
Nardo-Callunetea	–	8.7	3.3
Secalietea	–	12.1	1.5
Other classes	3.7	3.8	6.0

dentetea, *Chenopodietea* (ruderal communities), *Phragmitetea*, and *Arrhenatheretea*.

Table 3 shows also, that the hexaploid has the widest ecological amplitude. There are not so many publications dealing with the ecology of the species complex. Of old *Juncus ranarius* has been reported to be halophilous (ASCHERSON & GRAEBNER 1904, KIRCHNER et al. 1934, VAN OOSTSTROOM 1950), which is confirmed by our findings.

As far as the synecology is concerned, *J. bufonius* ssp. *bufonius* has been considered to be a characteristic species of the *Isoeto-Nanojuncetea* (WESTHOFF & DEN HELD 1969). This class is not so abundantly represented in our table, however, but this may be attributable to the fact that communities belonging to this class become increasingly rarer owing to agricultural practices interfering with the original landscape structure.

5. CONCLUSIONS

In the Netherlands three cytological races occur within the *Juncus bufonius* complex which can by means of constant morphological differences be identified with previously described taxa: the diploid ($2n = 34$) with *J. ranarius* Perr. et Song., the tetraploid ($2n = 70$) with *J. minutulus* Alb. et Jahan, and the hexaploid ($2n = 100-110$) with *J. bufonius* L. s. str.. The different chromosome numbers render hybridisation between the three races highly improbable; in other words there is an effective genetical barrier. Each of the three races has, in addition, its characteristic distribution and habitat preference, so that the conclusion seems to be warranted that the taxa with the different ploidy levels represent discrete biological species (biospecies). Whether they can also be accepted as "good"

(morphologically separated) taxonomical species remains to be seen considering the relatively restricted area and rather few individuals studied. The diploid differs in several respects from the other two, e.g., in its floral morphology, seed size, seed shape and seed sculpturing, inflorescence, and habit, and accordingly deserves recognition as a separate species; it also differs from the other two in the predominance of allogamy (the other two show a strong tendency towards consistent autogamy). The differences between the tetra- and the hexaploid races, on the other hand, are mostly of a quantitative nature (the hexaploid is larger and more robust as a rule).

In order to elucidate the taxonomic status of the cytodesmes within the *J. bufonius* aggregate it will be necessary to extend the biosystematic studies to the whole of Europe, since, particularly in the Mediterranean area and in eastern Europe, a number of presumably related taxa have been described (see, e.g., HUSNOT 1908, KRECHETOVIC & GONCHAROV 1935). As long as such a more extensive survey has not been made, the assignment of a taxonomic status to the tetra- and the hexaploid forms remains arbitrary, but for the time being, also on account of the morphological, cytological and phytogeographical differences, SNOGERUP (1971) is followed and the species level is accepted for these two taxa.

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REFERENCES

- ASCHERSON, P. & P. GRAEBNER (1904): *Synopsis der Mitteleuropäischen Flora* II, 2: 420–423.
 DARLINGTON, C. D. & A. P. WYLIE (1955): *Chromosome atlas of flowering plants*. Aberdeen.
 HEGI, G. (1939): *Illustrierte Flora von Mitteleuropa* II, 2. Aufl.: 192–193.
 HUSNOT, T. (1908): *Joncées*. Cahen par Athis (Orne).
 JØRGENSEN, C. A., TH. SØRENSEN & M. WESTERGAARD (1958): The flowering plants of Greenland. A taxonomical and cytological survey. *Biol. Skr. Dan. Vid. Selsk.* 9 (4):47.
 KIRCHNER c.s. (1934): *Lebensgeschichte der Blütenpflanzen Mitteleuropas* I, 3: 85–195.
 KRECHETOVIC, V. I. & N. F. GONCHAROV (1935): Juncaceae in: *Flora of the U.S.S.R.* III (V. L. KOMAROV ed.) English edition, Jerusalem, 1964: 400–455.
 LÖVE, A. & D. LÖVE (1948): *Chromosome numbers of northern plant species*. Reykjavic.
 — & — (1961): Chromosome numbers of Central and Northwest European plant species. *Op. Bot.* 5: 1–581.
 — & B. M. KAPOOR (1971): Cytotaxonomy of Rocky Mountain orophytes. *Arctic and Alpine research* 3 (2): 139–165.
 OOSTSTROOM, S. J. VAN (1950). Aanwinsten der Nederlandse flora in 1945, 1946 en 1947. *Ned. Kruidk. Arch.* 57: 218–219.
 REICHGELT, W. (1964). In S. J. VAN OOSTSTROOM et al. (eds.): *Flora Neerlandica* I 6: 166–169.
 SEGAL, S. (1962): Het soortsbegrip bij het *J. bufonius* complex. *Jaarb. Kon. Ned. Bot. Ver.*, 1962: 58–59.

- SNOGERUP, S. (1963): Studies in the genus *Juncus* III. *Bot. Not.* **116**: 142–156.
- (1971): Juncaceae. In K. H. RECHINGER (eds.): *Flora Iranica* lit. **75**. Graz.
- TAYLOR, R. L. & G. A. MULLIGAN (1968): *Flora of the Queen Charlotte Islands* (2). Ottawa.
- WESTHOFF, V. & A. J. DEN HELD (1969): *Plantengemeenschappen in Nederland*. Zutphen.
- & E. VAN DER MAAREL (1973): The Braun Blanquet approach. In R. H. WITTAKER (ed.): *Handbook of vegetation science*, Part V. *Ordination and classification of vegetation*. Den Haag.
- WULFF, H. D. (1937): Karyologische Untersuchungen an der Halophytenflora Schleswig-Holsteins. *Jahrb. Wiss. Bot.* **84**: 812–840.