Morphology and genome weight of *Symphyotrichum* species (Asteraceae) along rivers in The Netherlands

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The characters used in separating *Symphyotrichum* species naturalised along rivers in Western Europe (leaf bases more or less clasping, length of outer phyllaries relative to inner ones, hairiness of leaves) hardly apply to the plants found in the wild. We present morphological and cytometric data on 88 plants of *Symphyotrichum* from riversides in The Netherlands and adjacent Germany, in order to understand better the taxonomy of these plants. Ploidy was inferred from 2*C*-values obtained by flow cytometry. Inferred tetraploid plants with relatively deeply lobed disc florets (mean lobe/limb ratio >50%), relatively few florets per head (<40), disc florets usually <4 mm, and small flowering heads are referred to *S. ontarionis* (Wiegand) G.L. Nesom. Inferred hexaploids and octoploids with relatively deeply lobed disc florets (mean lobe/limb ratio >50%) and relatively many florets per head (>50) are referred to *S.* aff. *lateriflorum* (L.) Á. Löve & D. Löve. Inferred hexaploids and octoploids with a disc floret lobe/limb ratio <50% are named *S. lanceolatum* (Willd.) G.L. Nesom. Usually these plants have large flowering heads (to 3.6 cm in diameter) with >45 florets. Putative hybrids, comprising inferred pentaploids and heptaploids, are morphologically more or less intermediate between their parent species.

Keywords: *Aster*, flow cytometry, morphometrics, naturalised, polyploidy, taxonomy

Introduction

The genus *Symphyotrichum* Nees (Asteraceae, Astereae) was split off from *Aster* L. mainly on morphological grounds (Nesom, 1994a). The large segregate genus (91 spp.) originates from North America and proves to be only remotely related to Eurasian *Aster* (Noyes & Rieseberg, 1999; Semple *et al.*, 2002). More than half of the species are polyploids (Jones, 1980; Semple & Chmielevski, 1987; Morgan & Holland, 2012). Molecular studies of relationships within subtribe Symphyotrichinae and among diploid *Symphyotrichum* species have indicated close genetic affinities between the species (Vaezi, 2008; Vaezi & Brouillet, 2009; Li *et al.*, 2012; Morgan & Holland, 2012). In Europe, species of *Symphyotrichum* occurring in the wild originate from introduced American plants.

Symphyotrichum species naturalised along rivers in The Netherlands and other parts of Western Europe show great variation in flowering time, habit, colour, phyllaries, flower, and leaf characters. This variation is insufficiently accounted for in modern European floras (van der Meijden, 2005; Sell & Murrell, 2006; Stace, 2010; Jäger, 2011; Lambinon & Verloove, 2012). The species names provided appear difficult to apply. In many atlases, *Symphyotrichum* spp. are treated as a compex of either *S. lanceolatum* (Willd.) G.L.Nesom or *S. novi-belgii* (L.) G.L.Nesom (Haeupler & Schönfelder, 1989; Benkert *et al.*, 1996; Dupont, 2001; Preston *et al.*, 2002; van Landuyt *et al.*, 2006; Boudin *et al.*, 2007; Bardet *et al.*, 2008; Toussaint *et al.*, 2008). Usually, notes to the maps report frequent failure of proper identification to species of the plants found in the wild.

Most European floras separate *Symphyotrichum* species using characters (leaf bases more or less clasping, hairiness of leaves, length of outer phyllaries relative to inner ones,) that are difficult to apply because of their great variabillity (Labreque & Brouillet, 1996; Hoffman, 1996; Haeupler *et al.*, 2003: 94). Characters of disc florets are almost neglected. For example, the lobe to limb ratio of disc florets, as introduced by Wiegand (1928, 1933), has until recently hardly been used in European identification keys (Yeo, 1976, 2011). In *Symphyotrichum*, the disc floret corolla consists of two parts: tube and limb (the widened distal part of the corolla). The limb is five-lobed. So both tube and limb constitute the disc corolla. Hoffman (1995a, b, 1996) in studying *Symphyotrichum* in Central Europe was

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the first in Europe to use the lobe/limb ratio as a diagnostic character. Jäger (2011) applied it in *Rothmaler Excursionsflora*. On the basis of a morphometric study, including disc and ray florets, Dirkse *et al.* (2014) provisionally recognised three species: *S. lanceolatum*, *S. ontarionis* (Wiegand) G.L. Nesom, and *S. lateriflorum* (L.) Á.Löve & D.Löve. The small number of investigated plants (49) and particularly the lack of cytological data limit their conclusions.

While Canadian botanists have published hundreds of chromosome counts of Canadian plants (Semple & Brouillet, 1980; Semple & Brammall, 1982; Semple *et al.*, 1983a, b, 1992; Semple & Chmielevski, 1987), only two species of *Symphyotrichum* species have been counted from European material: *S. novi-belgii*, which had $2n=27$ (Tischler, 1950; Moore, 1982), and *S. lanceolatum* in which Kubešova *et al.* (2010) inferred octoploidy ($2n=8x=64$) from a 2*C*-value (5.41 pg) obtained by flow cytometry of plants from the Czech Republic. The near absence of cytological data from European plants has hampered their taxonomic treatment.

We present here morphological and cytometric data on 88 plants of *Symphyotrichum* from riversides in The Netherlands and adjacent Germany, in order to understand better their taxonomy.

Material and methods

Material

Of the 88 plants used in this study, 73 have been newly acquired, while 15 were taken from a former study (Dirkse *et al.*, 2014). Most plants were collected from sites along rivers in the eastern part of The Netherlands and four plants were collected from sites in adjacent Germany (Appendix). The collections were numbered and labelled in the field. Vouchers are kept at **NMNL**, duplicates at **L**.

Flow cytometry

For flow cytometry, leaf samples were wrapped in a moist paper tissue and put in a polyethylene bag, labelled with the collecting number of the voucher. Prior to analysis, the samples were stored in a refrigerator and analysed within 1 week of sampling. Only two cytometric measurements were conducted per plant, but this is compensated by the large number of plants measured per taxon.

Chromosome numbers as ploidy levels were inferred from 2C-values, which represent the amount of total DNA per nucleus in each somatic ($2n$) plant cell. The 2C-values were obtained by flow cytometry, following the method described by Zonneveld & van Iren (2001) and Zonneveld *et al.* (2005). A small amount of fresh *Symphyotrichum* leaf tissue was co-chopped in nuclei isolation buffer with the same amount of tissue of an internal calibration standard. The nuclei in the mixture were stained with propidium iodide (PI), a fluorochrome that stains total DNA. After incubation for 30 min, the fluorescence of about 5000 nuclei of *Symphyotrichum* and the internal standard were recorded. The 2*C*-value of the sample was calculated as the ratio of fluorescence peak size multiplied by the weight of the standard. The 2*C*-values we provide are the mean of two measurements. Some of the flow cytometry was carried out by BZ using *Agave americana* (15.9 pg) as internal standard, but about 20 analyses were conducted by Plant Cytometry Services at Schijndel, The Netherlands, using *Vinca minor* (1.51 pg) as internal standard. Their results did not significantly deviate from ours and the data were pooled.

By inference, we assigned the nine lowest 2*C*-values of our measurements to the tetraploid level ($2n=4x=32$). These 2*C*-values, divided by 4, provide nine estimates of the DNA weight of a single genome ($2n=x$). The mean of these nine estimates represents approximately the basic genome weight (Leitch & Bennet, 2004). For our tetraploid *Symphyotrichum* samples, we calculated a mean basic genome weight of 0.722 pg. Ploidy was estimated by dividing the 2*C*-values by the basic genome weight (2*C*/0.722). The inferred ploidy is the equivalent of the estimated ploidy rounded to the nearest integer.

Morphometrics

The morphometric measurements included the following:

- head diameter
- involucrum length
- length of outer phyllaries
- length of inner phyllaries
- number of ray florets
- ray floret ligule length
- ray floret ligule width
- number of disc florets
- disc floret length (corolla)
- disc floret limb length
- disc floret lobe length
- disc floret pappus length

Measurements of length were read from graph paper, under a dissecting microscope. The number of florets was counted from dissected flower heads. Means were calculated from five observations per individual. Notes were also made on plant height, leaf morphology, colour and texture.

Results

The identification of specimens to species followed Wiegand (1928, 1933), Semple & Brammal (1982), Semple *et al.* (2002), and Brouillet *et al.* (2006). The plants were 0.6–1.8 m high. In most plants, the cauline leaves were light to dark green (never bluish), rather thin to firm (never thick or fleshy), linear to lanceolate, gradually tapering from the widest part to

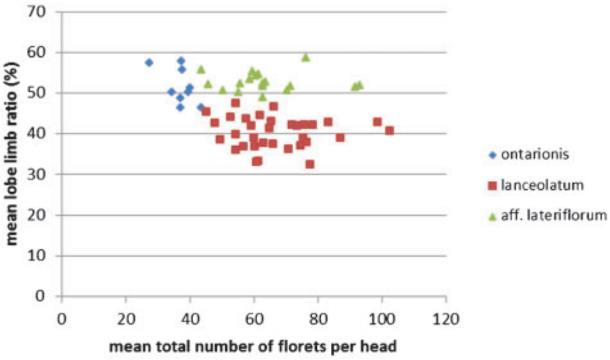

Figure 1 Mean total number of florets per head versus mean lobe to limb ratio (%) in *Symphyotrichum* species, pentaploid and heptaploid plants excluded.

the base, which was not clasping; the margins were entire or serrate, usually roughened with small curved hairs; the adaxial surface had fine scattered hairs, and the abaxial surface had very few hairs, and rarely was glabrous. Phyllaries were leafy, in several rows or almost of equal length, acute at the apex. All plants clearly belonged to *Symphyotrichum* subgen. *Symphyotrichum* sect. *Symphyotrichum* subsection *Dumosi* (Torrey & A.Gray) G.L.Nesom (Brouillet *et al.*, 2006), a complex of 12 species (Brouillet *et al.*, 2006).

Table 1 summarises the means and ranges of 2*C*-values and the most important morphological characters. Putative hybrids were grouped into two according to their inferred ploidy. Both groups show mean values more or less intermediate between those of the parent species.

We obtained 2*C*-values that indicated five levels of ploidy: tetraploid, pentaploid, hexaploid, heptaploid, and octoploid. The lowest 2*C*-values were assigned to the tetraploid level ($2n=4x$) and higher values assigned proportionately. Plants with relatively deeply lobed disc florets (mean lobe/limb ratio >50%), relatively few florets per head (<40), disc florets usually <4 mm and small flowering heads, were tetraploid and were referred to *S. ontarionis* (Wiegand) G.L.Nesom. Plants with a relatively deeply lobed limb (mean lobe/limb ratio >50%) and relatively many florets per head (>50) comprised both hexaploid and octoploid individuals and resembled *S. lateriflorum* (L.) Á. Löve & D. Löve; we refer to them as *S.* aff. *lateriflorum*. Plants with a lobe/limb ratio <50% and usually with large flowering heads (to 3.6 cm in diameter), with >45 florets per head, also comprised both hexaploid and octoploid individuals and were named *S. lanceolatum* (Willd.) G.L.Nesom. Figure 1 summarises the identification to species on the basis of the lobe/limb ratio (%) and mean total number of florets per head (i.e. disc florets plus ray florets), excluding plants of odd ploidy.

The 2*C*-values vary between 2.82 and 6.25 pg. The values increase in proportion to the estimated ploidy level (Table 1). The lowest values occur in *S. ontarionis* (up to 3.01 pg), where they cluster at the (defined) tetraploid level. *S. lanceolatum* shows the highest 2*C*-values. *S.* aff. *lateriflorum* exhibits a slightly lower mean 2*C*-value and a lower basic genome size than *S. lanceolatum* (Table 1 and Fig. 2).

Table 1 Number of sampled populations (vouchers) for *Symphyotrichum*, mean 2*C*-value, minimum 2*C*-value, maximum 2*C*-value, inferred ploidy (2*n*). Means and extreme values of most important morphological measurements

	Taxon						
Character	*lanceolatum*	*lanceolatum*	*aff. lateriflorum*	*aff. lateriflorum*	*ontarionis*	*hybrids group 1*	*hybrids group 2*
N	26	8	5	13	9	7	20
Mean 2*C*-value, pg	4.30	5.84	4.15	5.68	2.89	3.58	4.97
Range 2*C*-value	4.13–4.61	5.53–6.25	4.05–4.25	5.43–6.10	2.82–3.01	3.50–3.66	4.72–5.30
inferred ploidy (2*n*)	6*x*	8*x*	6*x*	8*x*	4*x*	5*x*	7*x*
Mean head diameter, cm	2.5	2.7	1.8	2.2	1.5	2	2.1
Range head diameter	1.5–3.6	1.6–3.4	1.2–2.3	1.9–2.8	1–2.7	1.7–2.3	1.4–3.2
Mean invol. height, mm	5.6	6.2	4.7	5.3	4.1	4.6	5.2
Range invol. height	4.0–6.5	4.6–7.6	4.3–5.2	4.9–6.1	3.7–4.6	3.8–5.2	4.2–6.4
Mean outer phyllary l, mm	3.6	3.9	3	2.7	2.1	2.6	2.7
Range outer phyllary l	2.4–5.6	2.4–6.4	2.9–3.5	1.9–4.2	1.7–2.6	2.1–3.2	1.7–4.2
Mean inner phyllary l, mm	5.1	5.3	4.4	4.6	3.7	4.3	4.7
Range inner phyllary l	3.9–6.3	3.4–6.8	3.9–5.1	4.1–5.2	3.3–4.7	4–4.7	3.5–6.2
Mean total floret no.	66.3	69	52.6	67.9	37.1	47.3	64.8
Range floret no.	45.3–98.7	54.3–102.7	53.7–62.7	55.3–93	27.4–43.7	39.5–51	44.7–82
Mean ligule l, mm	9.6	9.9	7.1	8.2	5.4	7.4	8.2
Range ligule l	5.9–14.3	7.9–12.9	4.8–8.7	7.1–10.4	4.2–6.4	4–9.1	4.6–13.8
Mean ligule w, mm	1.4	1.2	1	1.1	0.8	1	1.1
Range ligule w	0.8–1.6	0.9–1.6	0.9–1.1	0.9–1.6	0.6–1	1–1.1	0.6–1.7
Mean disc floret l	5.2	5.7	4.6	5.2	3.8	4.8	5.3
Range disc floret l	4.0–6.0	5.1–6.7	4.1–5.3	4.5–5.7	3.4–4.3	3.2–5	3.6–7.1
Mean disc lobe/limb%	40	41.5	52.8	52.4	51.4	48.2	43.6
Range disc lobe/limb%	32.5–46.7	36–47.6	50.4–55.5	48.8–58.5	46.2–57.7	42.5–54.7	25.3–60.9
Mean disc pappus l, mm	5.6	6	4.5	5.3	3.9	4.9	5.2
Range disc pappus l	4.3–6.4	5–7.4	3.9–4.9	4.5–6.4	3.4–4.3	3.8–5.7	3–6.3

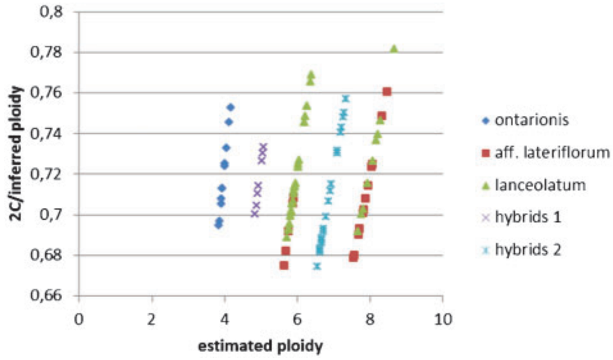

Figure 2 **2*C*-value (picogram) versus estimated ploidy in *Symphyotrichum* species.**

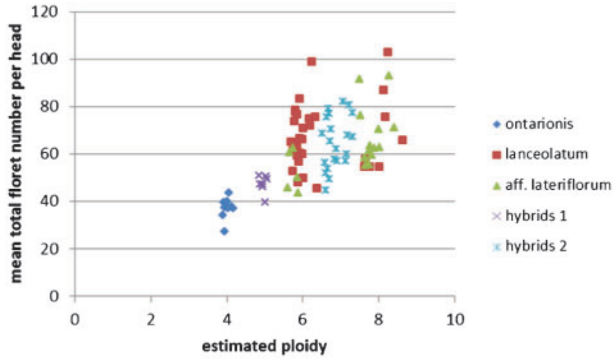

Figure 4 **Mean number of florets per head versus estimated ploidy in *Symphyotrichum* species.**

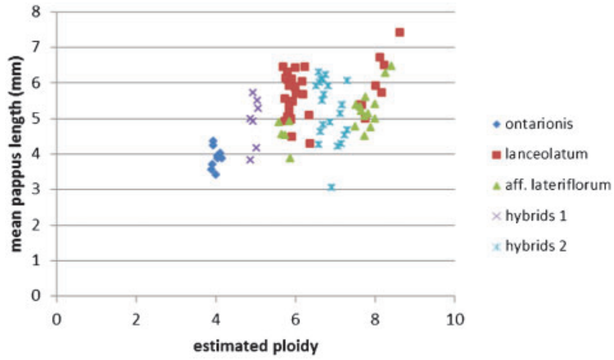

Figure 5 **Mean pappus length (mm) versus estimated ploidy in *Symphyotrichum* species.**

The basic genome weight (2*C*/inferred ploidy) remains constant over the levels of estimated ploidy (Fig. 2). The even levels of ploidy represent species, although hybrids between plants with the same ploidy cannot be ruled out. The odd levels of ploidy indicate putative hybrids between plants with different ploidy levels. Morphologically, these hybrids may look like *S. ontarionis*, *S. lanceolatum* or *S.* aff. *lateriflorum*. Pentaploids (hybrids 1) with a basic genome weight above 0.72 pg could derive from hybridisation between *S. ontarionis* and hexaploid *S. lanceolatum*. Those with a basic genome below 0.72 pg could derive from *S. ontarionis* and hexaploid *S.* aff. *lateriflorum* or hexaploid *S. lanceolatum*. Heptaploids (hybrids 2) may have originated in many ways. Speculating on their specific origins is beyond the scope of our paper.

Most of the morphological characters vary positively with ploidy level, with octoploids having the highest values and tetraploids the lowest (Table 1 and Figs. 3–6). As such, they contribute more to ploidy level distinctions rather than species distinctions. There is, however, one exception. The disc floret lobe/limb ratio appears to be independent of ploidy level and therefore of great use in diagnosing species rather than ploidy levels. Above the tetraploid level, and apart from heptaploid hybrids, the lobe/limb ratio consistently shows a discontinuity at *c.*50% (Fig. 3). This value separates *S.* aff. *lateriflorum* from *S. lanceolatum*, irrespective of whether the plants are hexaploid or octoploid.

Discussion

The taxa provisionally recognised here belong to a complex of twelve species that are known to hybridise in their native North America (Brouillet *et al.*, 2006). In Europe, current names for the plants in focus include (usually as *Aster*) *S. lanceolatum*, *S. novi-belgii*, *S. tradescanti* (L.) G.L. Nesom, *S. parviflorum* (Nees) Greuter, and *S.* × *salignum* (Willd.) G.L. Nesom (*S. lanceolatum* × *S. novi-belgii*) (Wagenitz, 1964–1979; Jovet & Vilmorim, 1975; Yeo, 1976, 1998, 2011; van der Meijden, 2005; Stace, 2010; Jäger,

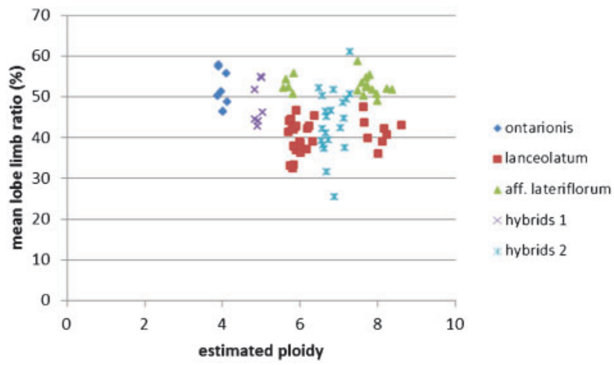

Figure 3 **Mean lobe limb ratio (%) versus estimated ploidy of *Symphyotrichum* species.**

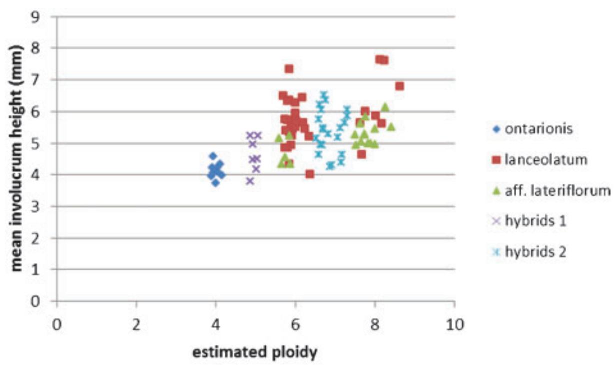

Figure 6 **Mean involucrum height (mm) versus estimated ploidy in *Symphyotrichum* species.**

2011). Our taxonomic treatment deviates from those encountered in current European floras, and we provide explanations below.

Tetraploid plants: S. ontarionis

On the basis of their morphology, we refer the tetraploid plants to *S. ontarionis*, a species described from eastern North America (Wiegand, 1928). *S. ontarionis* is not included in modern European floras (e.g. Wagenitz, 1964–1979; van der Meijden, 2005; Stace, 2010; Jäger, 2011; Lambinon & Verloove, 2012), and the identification of Dutch plants as this species requires explanation. The plants that we call *S. ontarionis* are small in all parts: involucrum height 3.7–4.6 mm, disc floret lobe/limb ratio 46–58%, total number of florets per head 27–44, and disc floret length 3–4 mm. The values for involucrum height, total floret number, disc floret legth, and ligule length are similar to Canadian plants. Yet Canadian plants differ a little in having a slightly lower lobe/limb ratio (39–51%) (Semple & Brammal, 1982; Bouchard, 1994). The available data do not suggest an explanation for this seemingly subtle difference. A more obvious and possibly important difference between the plants treated here as *S. ontarionis* and those identified as this species by Canadian botanists is the absence or near-absence in Dutch plants of hairs on leaves and stem. Canadian plants have these hairs, particularly on the abaxial surface of the cauline leaves. We did not see the same pubescence in our plants. Our plants have the leaves almost glabrous on both sides: at most, the leaves have a few, fine scattered hairs on the adaxial surface, directed to the apex, and hardly visible to the naked eye. According to Bouchard (1994), Canadian *S. ontarionis* sometimes also lack hairs. These glabrous plants are referred to *S. ontarionis* var. *glabratum* (Semple) Brouillet & Bouchard. So the absence of hairy leaves does not preclude the application of *S. ontarionis* to the glabrous Dutch plants.

For the plants referred here to *S. ontarionis*, Hoffman (1995a, b, 1996; Jäger, 2011) proposed the name *S.parviflorum* (as *Aster*). However, since *S. parviflorum* lacks a type, a proper set of identification parameters (Hoffman 1995a), and could be considered a synonym of *S. tradescanti* (L.) G.L. Nesom, we prefer to use *S. ontarionis* (Dirkse *et al.*, 2014).

It is possible that plants with the lowest 2*C*-values could represent *S. lateriflorum* rather than *S. ontarionis*. However, in *S. lateriflorum*, disc floret lobe/limb ratios of less than 50% hardly occur and the total number of florets rarely exceeds 30 (Semple & Brammal, 1982). Therefore, we consider *S. lateriflorum* is not applicable to our tetraploid plants. *S. tradescanti*, which is diploid, would not apply because of its low lobe/limb ratio (<40%). In addition, *S. tradescanti* would require plants smaller than 0.6 m (Bouchard, 1994; Brouillet *et al.*, 2006). In all, our plants with the lowest 2*C*-values, most likely represent glabrous *S. ontarionis*.

Hexaploid and octoploid plants: S. lanceolatum

We refer plants with a lobe/limb ratio 32.5–46.7% to *S. lanceolatum*. These plants have a mean total number of 45.3–98.7 florets per head, involucra 4.0–6.5 mm high, ray floret ligules 5.9–14.3 mm long, and mean disc floret pappi 4.3–6.4 mm long. According to Semple & Chmielewski (1987), *S. lanceolatum* represents a single, highly variable polyploid ($2n=4x$–$8x$) species, in which five varieties are recognised (Brouillet *et al.*, 2006). Overall, the morphology of the Dutch and German plants corresponds rather well with North American plants (Semple & Brammall, 1982; Semple & Chmielevski, 1987; Brouillet *et al.*, 2006). Notable discrepancies concern the mean number of florets per head and the mean length of disc floret pappi, which are 51 florets and 4.2 mm in North American plants. These values are less than those we found in our material (Table 1). The high number of ray florets in our material is caused by a double row of these. Our plants would look more showy than American ones and a preference by Dutch gardeners for showy plants might explain this trait in the plants examined.

Apart from *S. lanceolatum*, European floras (e.g. Wagenitz, 1964–1979; Stace, 2010; Jäger, 2011; Lambinon & Verloove, 2012) report the occurrence along rivers of both *S. novi-belgii* and, especially, *S.* × *salignum*. We did not recover these taxa in our study. According to Labrecque & Brouillet (1996), *S. novi-belgii* applies to hexaploid plants only. Among our hexaploid plants, or plants of other ploidy levels, none has been found fitting the concept of *S. novi-belgii*. The identification parameters of *S. novi-belgii* (Labrecque & Brouillet, 1996) including more or less fleshy leaves, distinctly clasping leaf bases, large involucra (6.0–9.0 mm high), and a low lobe/limb ratio (15–20%) are well beyond our observations. *S.* × *salignum* could apply to specimens of *S. lanceolatum* having relatively long outer phyllaries as compared to the inner ones. Yet we interpret these plants as phenotypes of *S. lanceolatum*; see also Hoffman (1995a) and Semple & Cmielevski (1987). Data from a broader geographical area are needed to understand the difference between the views expressed in our paper and those in the Floras cited above.

Hexaploid and octoploid plants: *S.* aff. *lateriflorum*

We refer all hexaploid and octoploid plants with a lobe/limb ratio>50% to *S.* aff. *lateriflorum*. The plants share: disc florets 53–93 per head, involucrum height 4.3–6.1 mm, disc florets 4.1–5.7 mm long, disc floret pappi 3.9–4.9 mm long, and ray floret ligule 4.8–10.4 mm long. The plants resemble small *S. lanceolatum* (Table 1 and Figs. 1—6), but differ in the larger lobus limbus ratio. As indicated, the identification is tentative because North American plants differ in being smaller in most parts. North American plants exhibit fewer florets (18–32), larger involucra (5.2–8.1 mm high), shorter ray floret straps (5.0–6.4 mm), shorter disc florets (3.5–4.4 mm), and shorter disc floret pappi (3.3–3.9). In addition, North American plants usually have hairy leaves with abaxial a hairy midvein. The plants we investigated have twice as much florets and glabrous leaves and midveins. To these plants, var. *tenuipes* Wiegand could apply but this needs further investigation. *S. lateriflorum* is an even more variable polyploid (2n=2x—8x) than *S. lanceolatum* (Wiegand, 1928; Bouchard, 1994; Chmielewski & Semple, 2001; Brouillet *et al.*, 2006). The identification of our plants as *S.* aff. *lateriflorum* is provisional because the plants that we investigated do not fit the current concept of *S. lateriflorum* as it is used in North America (Brouillet *et al.*, 2006). Since the taxonomy of *S. lateriflorum* is not fully clear (Bouchard, 1994; Brouillet *et al.*, 2006), we are unable to interpret the morphological discrepancies between the plants we investigated and the species concept as applied in North America. In addition, the inconsistent use of both *S. lateriflorum* and *S. tradescanti* is worth noting (Gray, 1882; Fernald, 1933; Jones, 1984; Hoffman, 1995a, 1996; Brouillet *et al.*, 2006; Jarvis, 2007).

Although the determination of the plants as circumscribed above is not straightforward, the presence of a species in addition to *S. ontarionis* and *S. lanceolatum* is strongly suggested by associated differences in the lobe to limb ratio and the genome weight (Figs. 2 and 3).

Pentaploids and heptaploids: putative hybrids

The intermediate levels of estimated ploidy indicate the occurrence of hybrids. The hybrid origin of the plants is supported by their intermediate morphology (Table 1). In several places, individuals of different genome weight grow close to each other (Appendix). Plants of odd ploidy could be direct descendents from the original introductions but may as well have originated locally. Although authors have mentioned hybridisation as an explanation for the confusing taxonomy of *Symphyotrichum* (Yeo, 1975; Wagenitz, 1964–1979; Nesom, 1994b; Hoffmann, 1995a), only a few cases have been documented (Semple & Brammal, 1982). The number of hybrids that we found, especially heptaploids, is unexpectedly high. Among the collected plants, almost as many heptaploid hybrids occur (20) as hexaploid *S. lanceolatum* (26). This we cannot explain.

Parentage of the hybrids differs among individuals. Most likely, pentaploids represent interspecific hybrids between the tetraploid *S. ontarionis* and the hexaploid *S. lanceolatum* or *S.* aff. *lateriflorum*. Three plants having a disc floret lobe/limb ratio >50% probably represent hybrids between *S. ontarionis* and hexaploid S. aff. *lateriflorum* (1b). Four other pentaploids with a basic genome weight above 0.72 pg most likely originate from a cross between *S. ontarionis* and hexaploid *S. lanceolatum* (1a). Those with a basic genome below 0.72 pg could derive from S. ontarionis and hexaploid *S.* aff. *lateriflorum* or hexaploid *S. lanceolatum*. Semple & Brammal (1982) documented wild hybrids between *S. lanceolatum* and *S. lateriflorum* and compared the morphological traits of these hybrids to those of the parental species and *S. ontarionis*. Most pentaploid hybrids had a disc floret lobe/limb ratio of 30–50% and looked like glabrous *S. ontarionis*.

Heptaploid plants may derive from intraspecific or interspecific crosses. The intraspecific crosses probably involve hexaploid and octoploid *S. lanceolatum* or hexaploid and octoploid *S.* aff. *lateriflorum*. A disc floret lobe/limb ratio>50% would be expected from an intraspecific cross between plants of *S.* aff. *lateriflorum* with different ploidy levels. A low lobe/limb ratio (<40%) would indicate a hybrid between plants of *S. lanceolatum* with different ploidy levels (Semple & Brammall, 1982). Heptaploid plants having a lobe/limb ratio 45–50% most likely result from interspecific crosses between *S.* aff. *lateriflorum* and *S. lanceolatum*, one hexaploid and the other octoploid.

Conclusions

Our data on the morphology and genome weight of *Symphyotrichum* species along rivers in The Netherlands and adjacent Germany suggest the occurrence of three species and a number of hybrids, existing at five levels of ploidy (2n=4x–8x). The presence of *S. lanceolatum* is beyond reasonable doubt. The presence of *S. ontarionis* is strongly suggested, but small differences from American plants allow for a slight doubt. The presence of *S. lateriflorum*, is not convincingly indicated, yet we have been unable to propose a better name. Therefore, pending further studies, we use *S.* aff. *lateriflorum*. The occurrence of hybrids is suggested by odd levels of inferred ploidy and intermediate morphology. The application of names is necessarily tentative and, moreover, requires not only further study but also caution when applied elsewhere.

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Appendix

Table A1 Provenance of material used in the study, including voucher identification number, year of collection, locality, and Dutch grid reference

Voucher no.	Year	Locality	Dutch grid reference
9061	2013	IJssel, Lathum	202.143–447.225
9065	2013	IJssel, Lathum	198.613–445.511
9069	2013	IJssel, Lathum	198.099–445.518
9075	2013	IJssel, Lathum	197.916–445.39
9057	2013	IJssel, Rheden	200.085–446.368
9073	2013	IJssel, Rheden	200.120–446.597
9077	2013	IJssel, Rheden	200.127–446.605
9081	2013	Maas, De Hamert	209.231–391.203
9082	2013	Maas, De Hamert	207.448–392.568
9085	2013	Maas, De Hamert	209.268–391.144
9086	2013	Maas, De Hamert	207.835–392.306
9090	2013	Maas, De Hamert	207.860–392.282
9094	2013	Maas, De Hamert	208.501–391.984
9060	2013	Maas, Well	203.230–395.627
9083	2013	Maas, Well	203.500–395.624
9087	2013	Maas, Well	203.340–395.627
9091	2013	Maas, Well	203.334–395.624
9121	2013	Pannerdensch Kanaal	196.816–435.581
9122	2013	Pannerdensch Kanaal	197.077–435.392
9123	2013	Pannerdensch Kanaal	197.085–435.381
9124	2013	Pannerdensch Kanaal	197.082–435.381
9127	2013	Pannerdensch Kanaal	196.814–435.591
9128	2013	Pannerdensch Kanaal	196.803–435.597
9113	2013	Rhein Salmorth Nordrhein-Westfalen	207.109–428.638
9114	2013	Rhein Salmorth Nordrhein-Westfalen	206.405–428.86
9115	2013	Rhein Salmorth Nordrhein-Westfalen	208.347–428.225
9116	2013	Rhein Salmorth Nordrhein-Westfalen	206.426–428.809
8591	2012	Waal, Bemmel	190.470–431.85
9037	2013	Waal, Bemmel	189.721–431.762
9038	2013	Waal, Bemmel	190.555–431.847
9039	2013	Waal, Bemmel	190.456–431.839
9040	2013	Waal, Bemmel	190.429–431.836
9063	2013	Waal, Bemmel	189.72–431.76
8121	2011	Waal, Beuningen	179.753–432.446
8122	2011	Waal, Beuningen	179.765–432.445
8123	2011	Waal, Beuningen	179.483–432.614
8124	2011	Waal, Beuningen	179.398–432.647
9079	2013	Waal, Beuningen	179.814–432.439
9080	2013	Waal, Beuningen	197.838–432.502
9084	2013	Waal, Beuningen	179.217–482.863
9088	2013	Waal, Beuningen	197.207–432.8
9092	2013	Waal, Beuningen	179.714–432.486
9093	2013	Waal, Beuningen	180.674–432.263
8129	2011	Waal, Eerlecom	194.954–429.31
8130	2011	Waal, Eerlecom	194.958–429.328
8131	2011	Waal, Eerlecom	194.611–429.433
8132	2011	Waal, Eerlecom	194.542–429.905
8133	2011	Waal, Eerlecom	194.55–429.429
9074	2013	Waal, Eerlecom	194.535–429.512
9076	2013	Waal, Eerlecom	194.969–429.389
9078	2013	Waal, Eerlecom	194.599–429.449
9059	2013	Waal, Erlecom	194.955–429.298
9062	2013	Waal, Erlecom	194.688–429.427
9066	2013	Waal, Erlecom	194.733–429.376
9070	2013	Waal, Erlecom	194.964–429.394
9019	2013	Waal, Gendt	194.257–431.258
8586	2012	Waal, Groenlanden	191.206–431.641
8587	2012	Waal, Groenlanden	191.314–431.646
8588	2012	Waal, Groenlanden	191.265–431.441
8597	2013	Waal, Groenlanden	191.431–431.357
8598	2013	Waal, Groenlanden	191.235–431.461
8601	2013	Waal, Groenlanden	192.387–431.508
9125	2013	Waal, Hulhuizen	197.036–532.636
9126	2013	Waal, Hulhuizen	197.027–432.653
9041	2013	Waal, Klompenwaard	198.002–432.964

Table A1 Continued

Voucher no.	Year	Locality	Dutch grid reference
8125	2011	Waal, Nijmegen	189.33–429.318
8126	2011	Waal, Nijmegen	189.508–429.351
8127	2011	Waal, Nijmegen	189.755–430.979
8128	2011	Waal, Nijmegen	189.524–430.725
8589	2012	Waal, Nijmegen	189.096–429.333
8590	2012	Waal, Nijmegen	188.461–429.119
9023	2013	Waal, Nijmegen	189.334–429.321
9067	2013	Waal, Nijmegen	188.557–429.096
9071	2013	Waal, Nijmegen	189.309–429.323
9129	2013	Waal, Nijmegen	188.344–429.11
9130	2013	Waal, Nijmegen	188.323–429.126
9132	2013	Waal, Nijmegen	188.273–429.135
9133	2013	Waal, Nijmegen	188.213–429.14
9134	2013	Waal, Nijmegen	188.186–429.129
9135	2013	Waal, Nijmegen	188.216–429.132
9136	2013	Waal, Nijmegen	188.213–429.14
8134	2011	Waal, Ooij	193.684–430.256
8135	2011	Waal, Ooij	193.656–430.216
9020	2013	Waal, Ooij	193.998–431.188
9021	2013	Waal, Ooij	194.145–431.21
9022	2013	Waal, Ooij	194.148–431.211
9025	2013	Waal, Ubbergen	189.982–429.46
9131	2013	Waal, Weurt	184.882–430.86