Analysis of herbarium collections of sporulating *Zygodon stirtonii* suggests recognition at variety level

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During revision of *Zygodon stirtonii* from the Netherlands no intermediates between *Z. stirtonii* and *Z. viridissimus* s.s. were found, even when the taxa were growing in mixed populations. From a total number of about 100 Dutch collections of *Z. stirtonii*, 26 had sporophytes, the majority of them dating back to the 19th century. Sporulating herbarium specimens were analysed for taxonomic and sexual composition. All male, female and sporulating individuals were counted in selected coherent samples from luxurious collections. In small collections, or collections with mostly separate shoots, mostly the presence of taxa was determined. Surprisingly, collections with sporulating plants of *Z. stirtonii* often also contained *Z. viridissimus* s.s. with sporophytes. Also, in collections with sporulating plants of one taxon of *Zygodon*, sometimes only male plants of the other taxon were found intermixed. In collections with plants of both taxa with sporophytes, often male plants of only one taxon were present. It seems likely that *Z. stirtonii* and *Z. viridissimus* can cross-fertilize and produce hybrid sporophytes, with viable spores that grow into new generations of both taxa. It is proposed to recognize *Z. stirtonii* at the variety level.

Up to date maps for *Z. viridissimus* var. *stirtonii* and var. *viridissimus* in the Netherlands are presented. Also, maps of sporulating populations are provided. *Zygodon viridissimus* var. *viridissimus* and *Z. conoideus* have spread rapidly in the Netherlands in recent decades, but var. *stirtonii* has not. The lack of sporophytes for more than a century may have hampered the spread of var. *stirtonii*.

Zygodon stirtonii was originally described as a new species in 1871 by W. Ph. Schimper in Stirton (1871). There has been a long debate about its status. It was treated as a subspecies by Dixon (1896). The rank of variety for this taxon was introduced by Hagen (1908). Malta (1926), who made a revision of the genus, treated it as a forma only (*Zygodon viridissimus* fo. stirtonii). Later in the 20th century the name *Zygodon viridissimus* var. *stirtonii* (Schimp.) I. Hagen became generally accepted. However, in recent years the taxon regained species rank (Smith 2004, Meinunger and Schröder 2007, Calabrese and Muñoz 2008).

In 2010 I received a big box with unnamed *Zygodon* plants, collected by E. J. Weeda in *Sambucus nigra* L. thickets in the outer coastal dunes of the Netherlands. Several specimens proved to be *Z. stirtonii*. This induced me to revise all *Zygodon stirtonii* material for the Netherlands. A total of about 100 collections of *Zygodon stirtonii* could be traced in institutional and private herbaria. Among these

were 28 herbarium specimens with sporophytes. Two of them appeared to be wrongly identified and contained only *Z. viridissimus* (Table 1).

Is *Zygodon stirtonii* genetically different from *Z. viridissimus* s.s?

Zygodon stirtonii is perhaps exclusively characterised by its leaf tips. The costa, sometimes expanded above, is excurrent in a stout mucro of ca 0.15(0.05–0.23) mm. This costa consists of multiple cell layers, which can be deduced from the shifting of cell patterns when focussing under the microscope. Sometimes the leaf apex is slightly asymmetrical, with one margin extending further up the costa than the other. *Zygodon viridissimus* s.s. has leaf tips with the costa ending well below the one-cell-layered apex. According to Calabrese and Muñoz (2008) *Zygodon stirtonii* also has smaller upper leaf cells (5–9 μm, against 10–13 μm in

Z. viridissimus s.s.). However, this observation is based on only one collection. The protologue (Stirton 1871) also mentions 'a denser areolation' for *Z. stirtonii*. In Dutch material both taxa were found to have mid leaf cells of 7–13 μm. Many *Zygodon* species are characterized by the structure of their vegetative gemmae, but with regard to *Zygodon stirtonii* and *Z. viridissimus* s.s. this character has no distinctive value. Gemmae of both taxa are 4–6 cells long and have transverse and longitudinal septa.

Most collections (95%) of *Zygodon stirtonii* from the Netherlands had been correctly identified. About 35% of the collections contained nothing but *Z. stirtonii*, while about 60% also contained *Z. viridissimus*. Some collections consisted of separate, pure patches of both taxa, while others contained mixed stands. With the study of mixed populations 'nature versus nurture' problems can be addressed. It is a widely used method, which has its drawbacks (Wyatt et al. 1982), but still can provide valuable clues to the interpretation of differences between related taxa in the absence of molecular tools. In mixed stands after moistening stems of *Z. stirtonii* and *Z. viridissimus* could always be disentangled. Main stems of *Z. stirtonii* always had lateral '*stirtonii*'-branches. In cushions of *Zygodon* leaf tip characters can often be traced down two years back using branching patterns and position of gametangia (in some 19th century collections even five years). Each stem system always had younger and older leaves of only the '*stirtonii*'- or '*viridissimus*'-type. However, one should be aware that sometimes old leaves of *Z. viridissimus* can mimic those of *Z. stirtonii* if apical laminal cells are lost by weathering. The mixed occurrence of two types of plants in one seemingly homogeneous cushion strongly indicates that their morphologic difference has a genetic base.

Can *Zygodon stirtonii* and *Zygodon viridissimus* s.s. cross-fertilize?

Zygodon viridissimus s.l. is a dioicous taxon with male and female inflorescences on separate plants. In many dioicous species sporophyte production is uncommon. Therefore it is striking that in all 19th century collections of *Z. stirtonii* in the Netherlands (n = 24, in seven 5 × 5 km squares) the plants bear sporophytes. In contrast almost all collections after 1900 are without sporophytes. This phenomenon is also reported (with less spectacular differences) for several other dioicous species in the Netherlands, e.g. *Dicranum scoparium* Hedw., *Orthotrichum lyellii* Hook. & Taylor, *Leucodon sciuroides* (Hedw.) Schwägr., *Anomodon viticulosus* (Hedw.) Hook. & Taylor and *Thuidium tamariscinum* (Hedw.) Schimp. (Touw and Rubers 1989). Maybe, 19th century bryologists preferred to collect 'complete', sporulating specimens. Nevertheless conditions for sporulation seem to have become less favourable in the 20th century. There are some reports of a recent revival of sporophyte formation (Kortselius 1995, Koopman and Meijer 1995).

Outside the Netherlands sporophytes of *Z. stirtonii* are reported from Great Britain, although occurring rarely (Smith 2004).

The expectation was to find male plants of *Z. stirtonii* between sporulating plants of *Z. stirtonii*. For most dioicous moss species it is supposed that a maximal distance of few centimetres between male and female plants is required for successful sperm dispersal (Wyatt 1994, Longton 1997). In the case of *Z. stirtonii* the composition of collections proved to be more complicated. Many collections contained both *Z. stirtonii* and *Z. viridissimus* and often both with sporophytes. And in collections with sporulating plants of one taxon, frequently male plants of the other taxon were present. In an attempt to clarify these unexpected observations, several herbarium collections were examined more thoroughly for taxonomic and sexual composition. This 'search for males' could only be executed within certain limits. Parts of the relevant collections are very old and consist of tufts of mosses carefully mounted with glue on strips of paper. In view of their museological value it is not recommendable to tear them all apart. Therefore from large specimens samples with sporophytes were selected that varied between 2 × 1 to 3 × 2 cm. All plants of these coherent patches were determined and sexed and numbers of sex-expressing individuals (i.e. stem plus innovations) were counted. In other, smaller specimens or specimens with only loose plants mainly the presence of taxa was established. Collections were examined using a 10–30× magnifying dissection microscope. Sometimes male plants of *Zygodon* with open perigonial buds at the end of the stems are easily detectable after moistening by simply visually scanning the surface of the patch. More often male plants have 1 or 2 subfloral innovations and are inconspicuous then. On individual stems the swollen male buds with short ovate leaves attract attention. Sometimes up to five generations of male buds may be found on branched stems. Frequently male plants are smaller than female ones. Female inflorescences have no eye-catching characters like enlarged bracts. However, bundles of brown archegonia and paraphyses are often easily detected by moving leaves aside with tweezers. Female inflorescences can be found terminally, but also at the base of innovations, both on young and old stems. The category 'sporulating plants' not only comprises plants with fully developed sporophytes but also plants with embryonic sporophytes, unripe capsules or broken setas. Sterile plants were mostly discarded and not counted unfortunately. In some 19th century collections sex expression was almost 100%, with all plants bearing sporophytes, or female or male gametangia. In other collections sometimes sterile plants prevailed. I may have overlooked male plants in sterile condition. However these plants are irrelevant to the present investigation, since they cannot have fertilized female plants. I can not rule out that sex expressing male plants already had died off, at the moment that sporulating plants were collected. In this case

relevant males were undetected. The results of the analysis are presented in Table 1.

Several observations can be derived from this table. Collections with 'autonomous', sporulating *Z. stirtonii*, i.e. collections only with plants of *Z. stirtonii* bearing sporophytes together with male plants of *Z. stirtonii* are absent. Both *Z. stirtonii* and *Z. viridissimus* s.s. have male and female representatives. Almost 90% (21/24) of the collections with sporulating *Z. stirtonii* also contain *Z. viridissimus*. More strikingly, in about 75% of the herbarium specimens with sporulating *Z. stirtonii* (10/13 of the selected samples, 18/24 of all collections) also *Z. viridissimus* is present with sporophytes. In most of the selected samples (14/15) male plants could be found between sporulating plants. The most striking feature is that male plants of a taxon not always correspond with sporulating plants of a taxon in the same patch ('wrong' males). In collection 2 and 3 of Table 1 only male plants of *Z. viridissimus* were found between sporulating *Z. stirtonii*. On the other hand, collection 1 only contained sporulating plants of *Z. viridissimus*, but all the male plants intermixed (well-developed and with three generations of male buds) belonged to *Z. stirtonii*. Sometimes plants of both taxa bear sporophytes, but male plants of only one taxon are found (collections 4–11a). In only two collections (12, 13) male plants of the taxa were in accordance with expectations.

In my analysis the assumption is made that when sporulating plants are collected, unintentionally also male plants are gathered and that these male plants are responsible for fertilization. Of course, arguments against this hypothesis can be brought forward. Sometimes male plants seem absent in herbarium collections of sporulating *Z. stirtonii* and must have been left behind by collectors, or had died off already. For example, collection 11 in Table 1 contained a large, separate patch (split off as 11b) with numerous female and sporulating plants of *Z. stirtonii*, but not a single male plant could be identified in this sample. Also, if male plants are found in collections, these are not necessarily responsible for fertilization of female plants; perhaps male plants of another taxon outside the sample were. However, very likely female plants are fertilized by the closest male plants around. The 'wrong' male plants found in this study were often closely intermingled with sporulating plants, and were probably closer than 'unfound', other males outside the sample. Finally, male plants in herbarium collections not necessarily precede sporulating plants as fertilizing components. An example is offered by the other sample of collection 11 (11a), which contains only two old, sporulating plants of *Z. stirtonii* and one old one of *Z. viridissimus* together with numerous fresh male *stirtonii*-plants and no fresh female ones. In this case male plants may also be interpreted as having emerged from spores produced by these sporulat-

Table 1. The composition of taxa (*Z. stirtonii*, *Z. viridissimus*) and sexes (♂, ♀, ♀c.fr.) in 29 herbarium specimens of sporulating *Zygodon 'stirtonii'* in the Netherlands.

(a) Collections 1–14, with numbers of sex-expressing, or sporulating individuals counted in one coherent sample and with males found in the sample.

No.	stirtonii ♂♂	♀♀	c.fr.	viridissimus ♂♂	♀♀	c.fr.	Interpretation	Herbarium-no.	Year	Grid-cell
1	14				37	27	'♂ *stirtonii* fertilizes ♀ *viridissimus*'	L0836236	1841	30.37
2			5	4			'♂ *viridissimus* fertilizes ♀ *stirtonii*'	L0836232	1844	25.33
3		97	26	7				L0836237	1869	25.21
4		1	11	8	5	13	'♂ *viridissimus* fertilizes ♀ *stirtonii* and	L0836227	1843	30.17
5		92	53	3	26	7	♀ *viridissimus*'	L0836234	1842	30.17
6		5	10	5	8	8		L0836248	1843	30.17
7		6	12	2	3	4		L0836224	1844	25.33
8		2	12	10	5	16		L0836220	1858	25.21
9	6	25	25		17	13	'♂ *stirtonii* fertilizes ♀ *stirtonii* and ♀ *viridissimus*'	L0836231	1845	25.21
10	1		3			1		L0836258	1977	42.35
11a	24		2			1		L0836223b	1869	25.21
12	1	12	3	15	8	5	'♂ *stirtonii* or ♂ *viridissimus* fertilize ♀ *stirtonii* and	L0836225	1843	37.58
13	1	6	6	9	11	9	♀ *viridissimus*'	Weeda s.n.	1994	25.31
14	9			15	24	34	'♂ *stirtonii* or ♂ *viridissimus* fertilizes ♀ *viridissimus*'	L0836230	1842	30.17

(b) Collections 11b–28, often small specimens, or specimens only with loose plants, with no males of both taxa found. Collection 11b with counted sample. Collection 25 with ♂ *stirtonii*-plants in separate, pure patch, not coherent with capsule-bearing plants. Collection 27 with ♂ *viridissimus*-plants, but *stirtonii*-plants lacking.

No.	*stirtonii* ♂♂	*stirtonii* ♀♀	*stirtonii* c.fr.	*viridissimus* ♂♂	*viridissimus* ♀♀	*viridissimus* c.fr.	Interpretation	Herbarium-no.	Year	Grid-cell
11b		50	14				with sporulating *stirtonii*	L0836223a	1869	25.21
15		x	x					L0836221	1844	25.33
16			x					L0836242	1869	25.21
17			x			x	with sporulating *stirtonii* and sporulating	L0836228	1843	30.17
18			x			x	*viridissimus*	L0836254	1869	25.21
19			x		x			L0836226	1869	25.21
20			x		x			L0836253	1864	25.21
21			x		x			L0836222	1869	25.11
22			x		x			L0836246	1870	31.48
23			x		x			GRO s.n.	1843	30.17
24			x		x			GRO s.n.	1869	25.21
25	x				x	x	with ♂ *stirtonii* and sporulating *viridissimus*	L0836235	1863	30.17
26		x	x		x		with sporulating *stirtonii* and ♀ *viridissimus*	L0836229	1844	25.33
27				x		x	with ♂ *viridissimus* and sporulating ♀ *viridissimus*	L0836245	1843	37.58
28						x	with sporulating *viridissimus*	L0836247	1841	48.28

Some details of sporophytes (with reference to collection-number in table):
1. Sporophytes on *viridissimus*-plants: theca (length × width) ca 1.3 × 0.6 mm; spores round, papillose, with content, 14–17 μm, sometimes old thecas with numerous germinated spores
2. On *stirtonii*-plants: thecas (unripe?) sometimes 'inflated' (1.4 × 0.9 mm), with the lid still on; theca empty or with young spores in tetrads
3. On *stirtonii*-plants: thecas (unripe?) ca 1.5 × 0.8 mm, with the lid still on; with young spores in tetrads
4. On *stirtonii*-plants: unripe thecas, with the lid still on
5. On *stirtonii*-plants: thecas ca 1.2 × 0.6 mm, sometimes with round, light brown, papillose spores of 14–17 μm (no germinated spores seen), sometimes with angular, colourless, empty spores of 9–14 μm
On *viridissimus*-plants: with round, light brown, papillose spores of 16–18 μm
6. On *stirtonii*-plants: with broken setas
7. On *stirtonii*-plants: with closed, swollen thecas
8. On *stirtonii*-plants: with young sporophytes
9. On *stirtonii*-plants: with broken setas
10. On *stirtonii*-plants: with broken setas
11a On *stirtonii*-plants: with young sporophytes
12. On *stirtonii*-plant: old theca, 1.2 × 0.6 mm, spores irregular, ca 14 μm
13. On *stirtonii*-plants: with young sporophytes or broken setas
14. On *viridissimus*-plants: with young sporophytes
11b On *stirtonii*-plants: with young sporophytes or with broken setas
15. On *stirtonii*-plants: with broken setas
16. On *stirtonii*-plants: theca with the lid still on, spores in tetrads

ing plants. It must be stated that only in collections 1–14, plants of both taxa must have grown on the same tree, as they were found in one coherent cushion. In other cases (collections 17–26) this is not certain, because it cannot be excluded that sometimes plants of both taxa may have been gathered from different trees and ended up in the same packet.

All variation of the distribution of taxa and sexes in the samples can be explained by assuming that the two taxa (as done in recent literature) are genuine, separate species that are reproductively completely isolated. In this case the presence of a sporophyte on a female plant of one taxon necessarily means that a male plant of that same taxon must have been within gamete dispersal distance, whether it was present in the sample or not. Also, male plants in the sample belonging to another taxon than the plants with sporophytes are only present there by coincidence and have nothing to do with fertilization. However, an alternative and simpler explanation would be that *Z. stirtonii* and *Z. viridissimus* s.s. can cross-fertilize and produce hybrid sporophytes. Hybridization was inferred in the same manner for a (small and shrivelled) sporophyte

on a female plant of *Rhytidiadelphus subpinnatus* (Lind.) T.J.Kop. by Holyoak (2001). Here the only available male plants in the vicinity that could have fertilized female plants belonged to *R. loreus* (Hedw.) Warnst.

There are recent reports that the maximum fertilization distance in mosses can be more than a few centimetres as was previously thought. In that case the study of herbarium collections of dioicous taxa with sporophytes may be unsuited for testing paternity. It was demonstrated in a transplantation experiment that the distance can be up to 34 cm in the large pleurocarpous *Rhytidiadelphus triquetrus* (Hedw.) Warnst. (Bisang et al. 2004). In species of *Polytrichum* with splash cups the distance can even reach up to 2 m (van der Velde et al. 2001). Microarthropods may also carry sperm cells over longer distances (Cronberg et al. 2006). However, I also tested herbarium collections of another species of *Zygodon*, *Z. conoideus* (Dicks.) Hook. & Taylor for presence of male plants. So far, it has been found fifteen times with sporophytes in the Netherlands. In twelve 'autonomous' collections male plants, all belonging to *Z. conoideus*, were indeed present within millimetres or centimetres of sporulating plants. Therefore I think for species of *Zygodon* a maximum fertilization distance of a few centimetres is a realistic one.

In the samples the diploid sporophytes on plants of *Zygodon* may have grown from a zygote that originated from haploid gametes of two *stirtonii*-parents (hereafter called SS-sporophytes), or from gametes of two *viridissimus*-parents (VV-sporophytes), or – assuming cross-fertilization taking place – from a gamete of a *stirtonii*-parent and a gamete of a *viridissimus*-parent (SV-hybrid sporophytes). In the Netherlands so far no unambiguous SS-sporophytes (in collections that contain nothing but sporulating plants of *Z. stirtonii* and male plants of *Z. stirtonii*) seem to have been collected. Always also plants of *Z. viridissimus* were found to be present. However, SS-sporophytes very likely occur on *stirtonii*-plants in collections 9, 10 and 11a, as only male plants of *Z. stirtonii* are present. Sporophytes on *viridissimus*-plants in these collections will be SV-hybrid. All sporophytes in collections 1–3 (with male and female plants belonging to different taxa) are probably SV-hybrid. In collections 4–11a sporophytes will be SV-hybrid on plants of the taxon with no male representative. Collection 27 (and many other collections with sporulating plants of *Z. viridissimus* outside the scope of this study) probably has VV-sporophytes. Sporophytes on *viridissimus*-plants in collections 4–8 can also be looked upon as VV-sporophytes.

No ripe SS-sporophytes have been found so far in this study. In collections 9, 10 and 11a (with presumed SS-sporophytes) *stirtonii*-plants only had broken-off setas or very young sporophytes. Ripe sporophytes of presumed SV-hybrid nature were found in some collections. In some cases I found no differences between presumed SV-sporophytes and VV-sporophytes, both having ellipsoid, brown capsules, without a peristome, with eight weak ribs and a short neck with phaneropore stomata, and round, papillose spores of ca 14–17 μm. Spores of such SV-capsules appeared normal in structure and size. In other cases I found capsules of presumed SV-sporophytes that only contained colourless, angular spores of ca 9–14 μm. These spores are probably of a non-viable nature. Sometimes capsules of presumed SV-nature were remarkably swollen and contained spores in tetrads. It is unclear to me whether these should be considered unripe stages in a normal succession or abnormal hybrid forms.

Hybrid sporophytes of *Zygodon stirtonii* and *Z. viridissimus* s.s. may produce viable offspring

Cross-fertilization combined with viability of the hybrid progeny would also explain the high frequency (10/15 of the samples) of co-sporulation of *Z. stirtonii* and *Z. viridissimus*. If this is not the case (as in recent literature with the concept of two separate species that are reproductively completely isolated) frequently diaspores of four different mates of *Zygodon* (♂ and ♀ *stirtonii*, and ♂ and ♀ *viridissimus*) must have independently colonized a site, often an isolated tree. Via air transport, I think chances for this must be very small. In case of cross-fertilization and viability of the progeny, the independent establishment of three (♀ *stirtonii*, ♀ *viridissimus* and ♂ *stirtonii* or *viridissimus*) or even two mates (♀ *viridissimus* and ♂ *stirtonii* or ♂ *viridissimus* and ♀ *stirtonii*) would suffice. If a *viridissimus*- and a *stirtonii*-plant of opposite gender colonize a tree (via spores or gemmae) they can produce hybrid sporophytes. After meiosis these F_1-generation sporophytes can produce via recombination four types of F_2-generation spores (♂ and ♀ *stirtonii*, ♂ and ♀ *viridissimus*). Applying classical genetics, two preconditions seem necessary to make this increase of variation possible. First, the genes that determine gender and the *stirtonii-viridissimus*-trait should probably be on different chromosomes. Otherwise only two types of F_2-spores can be formed after meiosis in the F_1-hybrid capsules, since the alleles for gender and the *stirtonii–viridissimus*-trait would be linked. Of course, with linked alleles four different combinations would still be possible via crossing over. Secondly, all genes for the *stirtonii–viridissimus*-trait should be on one chromosome (perhaps even on one locus in view of crossing over-events). Otherwise recombinant F_2-spores would contain different 'amounts' of *stirtonii*-genes. In that case in the progeny arising from F_2-spores, intermediate phenotypes between *Z. stirtonii* and *Z. viridissimus* might occur, yet these have not been found in this study. I estimate that chances are high that these four types of spores can interdependently end up on the same tree, when capsules are washed out by rainwater. Next generation individuals can probably develop inside parental plants. Perhaps this would occur via decaying old capsules, since occasionally numerous germinating spores can be found in old cap-

sules of *Zygodon*. In this way, after one generation of *Z. viridissimus* or *Z. stirtonii* with hybrid sporophytes, a next generation of both taxa with sporophytes can develop at the same site.

I have found indications that hybridisation is only valid in one direction and the term SV-hybrid sporophytes should be better divided into two separate categories: sv-hybrid sporophytes, with ♀ *stirtonii*-plants fertilized by ♂ *viridissimus*-plants, and vs-hybrid sporophytes, with ♀ *viridissimus*-plants fertilized by ♂ *stirtonii*-plants. So far I only found viable, germinating spores in the category of presumed vs-hybrid capsules (in collection 1, with numerous germinating spores in several old capsules on *viridissimus*-plants), and not yet in presumed sv-hybrid sporophytes. So maybe ♀ *viridissimus* has to meet ♂ *stirtonii* for successful co-sporulation of both taxa in the next generations. If ♀ *stirtonii* and ♂ *viridissimus*-plants meet, this may not be the case. Also in other genera, like *Physcomitrium*, *Funaria*, *Sphaerocarpos*, *Polytrichum* and *Weissia*, taxa (though on the species level) have been reported to intercross more easily in one direction than the other (Natcheva and Cronberg 2004). Of course, my theory of viability of hybrid *Zygodon*-spores needs to be tested by experiments.

Zygodon stirtonii back to variety level

Zygodon stirtonii was raised to the species level in the most recent European checklist (Hill et al. 2006, based on Smith 2004). I propose to recognize *Zygodon stirtonii* at the variety level. The morphological differences with *Z. viridissimus* s.s. seem genetically based, but are small and probably only concern leaf tips. In the Netherlands autonomous populations of sporulating *Z. stirtonii* have never been detected so far. The study of Dutch herbarium collections strongly indicates that plants of both taxa can cross-fertilize. The frequent co-occurrence of plants of both taxa with sporophytes, even on isolated, inland sites (e.g. near Utrecht and Heerjansdam), also indicates that hybrid sporophytes can produce viable offspring of both taxa. I therefore think that *Z. stirtonii* is not reproductively isolated from *Z. viridissimus* s.s. and that both taxa belong to one species, *Z. viridissimus* s.l. In van Zanten and Hofman (1994) cross-fertilization was also an argument for lowering the status of the newly described *Hypnum heseleri* Ando & Higuchi. They could even demonstrate the viability and nature of hybrid spores via culture experiments.

The distribution range of var. *stirtonii*, which inhabits mainly coastal parts of northwestern Europe from northern Spain, to the middle of Norway, southern Sweden and western Poland, also falls within the distribution range of var. *viridissimus* (Meinunger and Schröder 2007, Smith 2004, Stebel et al. 2007, Calabrese and Muñoz 2008, Hassel 2010).

The rank of variety is proposed for *Z. stirtonii*, as only one morphological character (concerning the leaf tip) seems to be involved and the geographic range coincides with *Z. viridissimus* s.s. The rank of subspecies would be more appropriate if multiple, independent characters and different geographic ranges were at hand.

According to the protologue (Stirton 1871) capsules of *Z. stirtonii* have 'a more rounded outline' than those of *Z. viridissimus*. Whether this is an additional distinguishing character between var. *stirtonii* and var. *viridissimus* is questionable. On plants of *Zygodon viridissimus* s.l. ripe capsules (in summer) are normally narrow-shaped, with a length-width ratio of approximately 2:1. Sometimes more inflated capsules with a length-width ratio of ca 3:2 occur. The nature of such capsules is somewhat unclear. The round-shaped capsules can be immature stages, e.g. when early-spring plants are collected. Perhaps however these are aberrant hybrid forms. In this respect it is interesting to know whether *Z. stirtonii* type collection plants contain SS-sporophytes or presumed SV-hybrid sporophytes. For this to know, the type collection needs to be checked for male plants.

Zygodon viridissimus var. *stirtonii* in the Netherlands

The distribution of *Zygodon viridissimus* var. *stirtonii* and var. *viridissimus* in the Netherlands is given in Fig. 1 and Fig. 2, respectively.
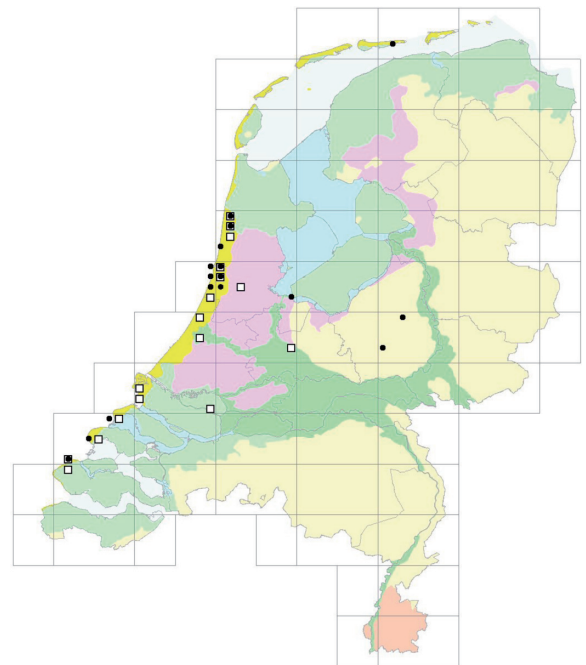

Figure 1. Distribution of *Zygodon viridissimus* var. *stirtonii* in the Netherlands. Open squares: grid cells with the taxon before 1980; filled dots: grid cells with the taxon from 1980 onwards.
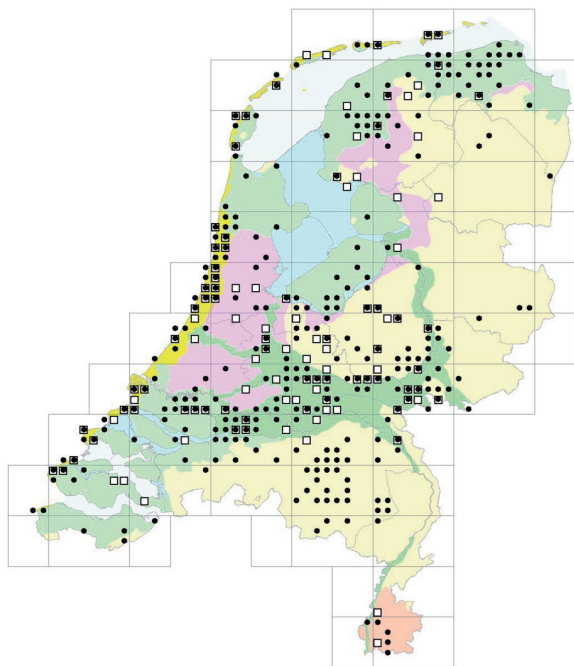
Figure 2. Distribution of *Zygodon viridissimus* var. *viridissimus* in the Netherlands. Data (not all revised) from the BLWG, <www.verspreidingsatlas.nl>. For meaning of symbols see Fig. 1.

Var. *stirtonii* has always been much less common than var. *viridissimus*. In recent decades the number of finds of var. *viridissimus* has substantially increased, from about 110 square grid records before 1980 to about 320 after 1980. Very surprisingly, in the same period no increase of var. *stirtonii* was observed at all, with 17 records before 1980 and 16 from 1980 onwards. Of course, distribution maps not always reflect real distribution of species in time and space. In the course of time intensity of recording has been highly variable. Also, the intensity of recording has not been evenly distributed over the country. With some exaggeration one may state that distribution of bryophytes sometimes reflects distribution of bryologists. However, in the case of var. *stirtonii* and var. *viridissimus* the differences cannot be interpreted as a recording effect, because the dynamics of recording are the same for both taxa which are highly similar in appearance and habitat. Plants of *Zygodon* cannot be identified with certainty in the field and it is common practise to collect some material for microscopic identification.

Maybe ecological preferences account for differences in distribution. Var. *stirtonii* might 'prefer' more calcareous habitats than var. *viridissimus*, as in the Netherlands it is predominantly found in the calcareous dunes south of Bergen and is almost absent from the non-calcareous dunes of the Wadden Sea islands in the north. Var. *viridissimus* is found throughout the entire dune region. However, other data suggest var. *stirtonii* having a broad ecological ampli-

tude. It has been found on a wide variety of genera of trees (most frequently *Populus*, also *Ulmus, Quercus, Sambucus, Fagus, Fraxinus, Tilia* and *Salix*; moreover on dead wood of poles and fences) as well as on man-made stone structures (concrete walls, bunkers, brick walls, gravestones). Old, calcareous concrete occurs everywhere nowadays in the Netherlands. Why did var. *stirtonii* not colonize these habitats, as var. *viridissimus* did? Plants of both taxa also very often grow intermixed, with no macroscopically visible boundaries between populations, indicating that ecological demands overlap. Variety *stirtonii* may also 'prefer' coastal habitats, as indeed most locations in the Netherlands are along the coast. However on a European scale the taxon does not have a strictly Atlantic distribution, e.g. it protrudes some hundreds of kilometres to the east in Germany (Meinunger and Schröder 2007). In this respect it is interesting to look at another sub-Atlantic *Zygodon, Z. conoideus*. This species, like *Z. viridissimus* var. *viridissimus* also increased spectacularly in the Netherlands, from only seven square grid records before 1980 to about 350 after 1980 (Fig. 3). So why did var. *stirtonii* not increase in the same way?

Var. *viridissimus* might have had a 'head start', because it was already more common before 1980 than var. *stirtonii*. New habitats had a greater chance of being colonized by diaspores of var. *viridissimus* than of var. *stirtonii*. This process may have accelerated the expansion of var. *viridissimus*, but it does not explain the complete lack of expansion of var. *stirtonii*.
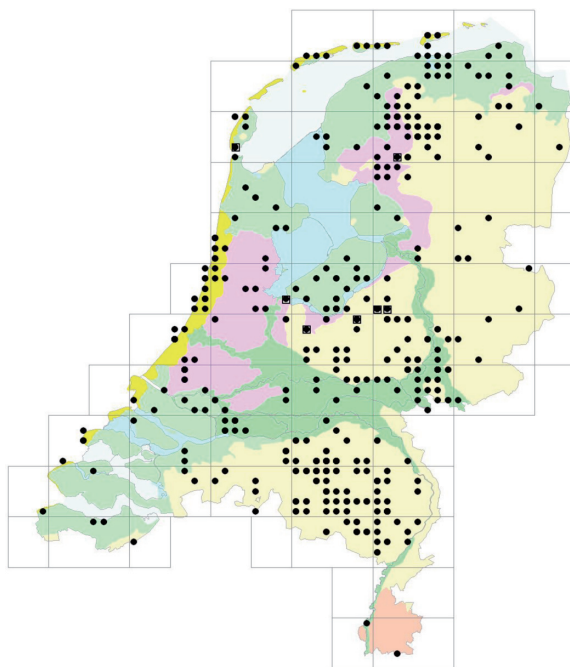

Figure 3. Distribution of *Zygodon conoideus* in the Netherlands. Data (not all revised) from the BLWG, <www.verspreidingsatlas.nl>. For meaning of symbols see Fig. 1.

I think the formation of sporophytes might play a role, because of the different behaviour of the two varieties in this respect in the Netherlands and perhaps also abroad. In Fig. 4 and 5 the distribution of sporulating populations in the Netherlands of var. *stirtonii* and var. *viridissimus* is given. For completeness, sporulating populations of *Z. conoideus* can be found in Fig. 6.

Sporophytes on plants of both varieties of *Z. viridissimus* have been found in coastal areas, but this 'preference' is shown by var. *stirtonii* in stronger degree than by var. *viridissimus*. Of course information of sporophyte formation is only fragmentary. Nevertheless, formation of sporophytes seems to have decreased greatly for both varieties. After 1870 *stirtonii*-plants with sporophytes have only been collected in the Netherlands in 1977 (three plants with broken setas) and in 1994 (with unripe capsules). In comparison, var. *viridissimus* seems to have had a somewhat more continuous history of sporophyte formation. After the 19th century there are records of plants with sporophytes from 1925 and 1977 and several from 1990 until now. Especially the region between Noordwijk and Haarlem is a recent stronghold for sporophyte production.

Maybe var. *stirtonii* could not expand its range rapidly because only 'heavy' gemmae were available, while var. *viridissimus* could by means of easily dispersed, lighter spores. It would be interesting to know about the history on sporophyte formation in Britain and Ireland. With prevailing westerly winds this region is probably an important source of (dia)spores. In Smith (2004) sporophytes on var. *stirtonii* are reported 'rare', on var. *viridissimus* 'occasional' and on *Z. conoideus* 'frequent'. The rapid expansion of *Z. viridissimus* var. *viridissimus* and *Z. conoideus* in the Netherlands may well have been fed by British sporulating populations.

Dutch species of *Zygodon* perhaps 'need' lighter spores for expansion over longer distances. Contradicting with this hypothesis, there are also examples of several epiphytes (e.g. *Metzgeria fruticulosa* (Dicks.) A.Evans, *Orthotrichum lyellii*, *O. obtusifolium* Brid., *Syntrichia papillosa* (Wilson) Jur. and *Ulota phyllantha* Brid.) that have recently expanded their range spectacularly in the Netherlands although relying on distribution via 'heavy' propagules (with sporophytes being rare or even unknown). However, most of these species grow higher on trees and branches where diaspores experience higher wind velocities. Plants of *Zygodon* often grow low on tree trunks where gemmae may not easily get airborne.

Formation of sporophytes with *Zygodon viridissimus* s.l.

Expression of sexual organs and mate availability are important conditions that govern the formation of sporophytes in dioicous bryophytes. Ecological factors probably play an important role with sex-expression. In the Netherlands sex-expressing plants of *Thamnobryum alopecurum*
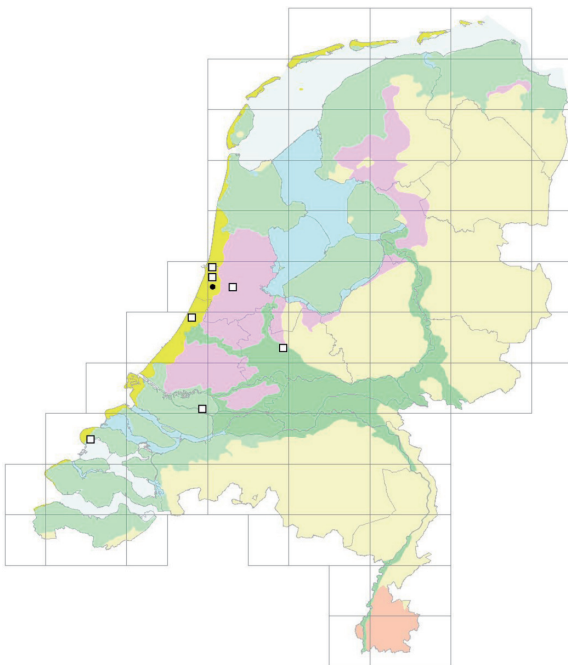

Figure 4. Distribution of Zygodon *viridissimus* var. *stirtonii* with sporophytes (often probably of a hybrid nature) in the Netherlands. For meaning of symbols see Fig. 1.
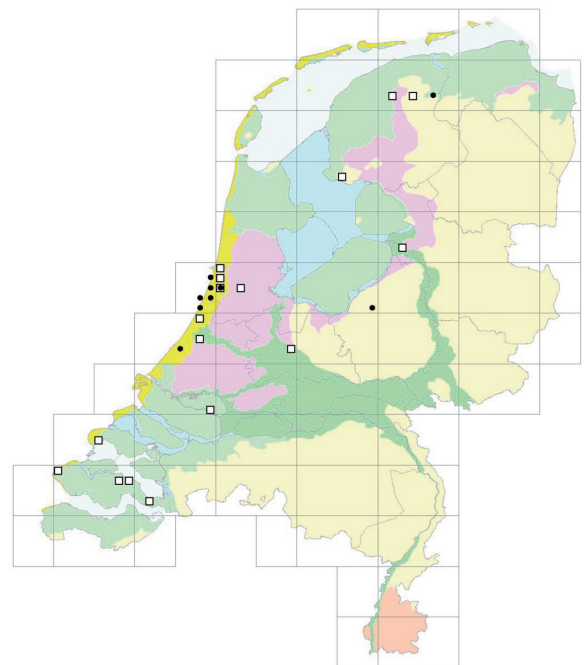

Figure 5. Distribution of *Zygodon viridissimus* var. *viridissimus* with sporophytes in the Netherlands. For meaning of symbols see Fig. 1.
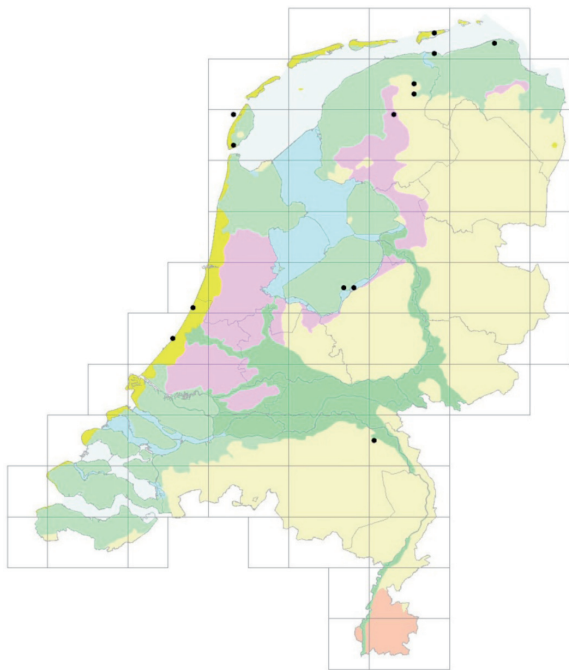
Figure 6. Distribution of *Zygodon conoideus* with sporophytes in the Netherlands. For meaning of symbols see Fig. 1.

(Hedw.) Gangulee (and correlated with these, plants with sporophytes) were most frequently found on sites with constantly high air humidity (During 1978). Sexual organs are best formed in healthy, full-grown plants, with the mass of individual shoots above a critical minimum (Stark et al. 2001). Plants in 19th century collections of *Z. viridissimus* s.l. are often much more vigorous (longer, with up to five annual growth segments) than in recent collections. Although no exact data are yet available on the frequency of sex-expressing plants in recent collections, in my experience sex-expression has decreased with time. Increased levels of air pollution or decreased overall air humidity may be responsible.

Mate availability is greatly governed by chance. Many dioicous bryophytes probably have sporophytes on particular locations, simply for historical reasons. A species can have sporophytes now, because it had sporophytes before at that site. As an example *Aulacomnium androgynum* (Hedw.) Schwägr. may be quoted. This common dioicous moss rarely has sporophytes, but they have been found repeatedly near Bergen (Touw and Rubers 1989). Around sporulating plants the concentration of moss spores per volume of air will decrease rapidly with distance. Therefore in the direct vicinity of sporulating plants chances are highest that distances between newly established male and female colonies are small enough (e.g. when both grow on the same tree) to enable fertilization and formation of sporophytes. Further away, new populations can easily remain single male or female (for example on different

trees, with uninhabitable forest floor between them). In this respect formation of propagules with dioicous species can be looked upon as an adaptation that enables future contact between isolated sexes and enhances sporophyte formation in the long term (During 1990, 2007).

Zygodon viridissimus s.l. produces sporophytes mainly in coastal areas. Here, an ecological factor probably also plays a role in mate availability. I noticed that many sporulating patches contain fair amounts of sand between plants. If in these coastal, open habitats wind velocities are so high that sand grains can be transported, much lighter gemmae of *Zygodon* probably also can. In cushions of *Zygodon* regeneration of shoots on organic matter between old plants is commonly seen. In this way clonal populations of separate sexes probably get more easily intermixed and formation of sporophytes becomes easier. After the 19th century the drift of sand in the dunes has decreased substantially, being limited to the outermost dune-ridges near the sea. This may have contributed to the decline in sporophyte formation in populations of *Zygodon*.

The non-expansion of var. *stirtonii* may be explained by the extinction of sporulating populations. However, it could also be that dispersal via spores is more difficult for var. *stirtonii* than var. *viridissimus* in any case. So far in the Netherlands populations of *Z. stirtonii* with ripe capsules of presumed SS-sporophytes have never been detected. This can be a recording effect, but it is also possible that ♀ and ♂ *stirtonii*-plants are reproductively incompatible and that fully developed sporophytes are only produced in case of hybridisation with var. *viridissimus*. A hypothesis can be that '*stirtonii*-genes' only end up in spores that can be easily dispersed in the scenario of ♀ *viridissimus*-plants being fertilized by ♂ *stirtonii*-plants, since there is limited evidence that only presumed vs-hybrid sporophytes produce viable spores. Also, theoretically only 50% of the spores would then contain '*stirtonii*-genes'. For var. *viridissimus* the production of viable spores in capsules of VV-sporophytes is probably less problematic. For successful fertilization no other taxon is required, and theoretically 100% of the spores pass on the '*viridissimus*-genes'.

Conclusion

In actual practise in bryophyte taxonomy species are still described chiefly on morphological (and more recently on molecular) characters. Information on the ability to cross-fertilize and the viability of the progeny is mostly wanting. I think that with dioicous taxa this information is sometimes indirectly available, via the study of herbarium collections with sporophyte bearing plants. The results of my study on sporulating herbarium collections of *Zygodon stirtonii* in the Netherlands indicate that *Z. stirtonii* and *Z. viridissimus* are much more related to each other than recently thought and are better treated as varieties.

Many questions are still unsolved. If fresh material of sporulating plants of var. *stirtonii* becomes available my theory of cross-fertilization and theory of viable hybrid *Zygodon*-spores can be verified by culture experiments. Theoretically sporophytes of presumed SV-hybrid nature should produce about 50% individuals of var. *stirtonii* and 50% of var. *viridissimus* (or none in case of one-directional viability of hybrid spores). Presumed SS-sporophytes should produce 100% individuals of var. *stirtonii* (or none in case of reproductive incompatibility). A paternity analysis using microsatellites as done e.g. in van der Velde et al. (2001) could also be informative. All plants that were sorted out in this study (male, female or female with sporophytes, of var. *stirtonii* and var. *viridissimus*) are packed in separate convolutes and are (for the greatest part) kept at the National Herbarium in Leiden. Although these plants are (very) old, perhaps they can be used for future DNA- or electrophoretic analysis.

Sporophytes of *Zygodon stirtonii* have also been found in Britain. I hope a comparable analysis will also be carried out on these British collections. What kind of males can be found between sporulating plants of *Z. stirtonii* (also in the type collection) and does *Z. stirtonii* also co-sporulate with *Z. viridissimus* s.s. over there? Have ripe capsules of SS-sporophytes ('genuine' *stirtonii*) ever been found? And, where and when have *Z. stirtonii* and *Z. viridissimus* s.s. been found with sporophytes in Britain?

I think in general for a better understanding of taxonomical problems with dioicous taxa, it is important to try to examine the male component that is actually present in collections with sporulating plants. For this to be successful, collections should be of a reasonable size to ensure that the fertilizing males are also gathered.

I also think that the publication of maps of sporulating populations of taxa can be of use for a better understanding of the distribution of bryophytes. Such maps can in particular provide important information for the conservation of bryophytes. Sporulating populations are the sources from which threatened species can still spread comparatively fast by means of far travelling spores to regions where they have become endangered or extinct.

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