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DIPLOMARBEIT

Titel der Diplomarbeit

Sexuelle Fortpflanzung und taxonomische Fragestellungen bei der Süßwasseralge *Spirogyra* (Zygnemataceae, Streptopyhta)

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag. rer.nat.)

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Einleitung

Allgemeines zum Artbegriff

Die Kategorisierung der Welt im Allgemeinen und von Organismen im Besonderen hat eine lange Tradition. Die Anfänge naturwissenschaftlicher Klassifikation und damit auch der Beginn der Debatte um die Definition von Arten reichen zurück bis in die Antike. Vor allem die Lehren des griechischen Philosophen Platon, der die Struktur aller Dinge zu beschreiben suchte, sowie deren Weiterführung durch seinen Schüler Aristoteles, hatten großen Einfluss auf nachfolgende Philosophen und Naturwissenschaftler. Unter ihnen war auch der schwedische Naturforscher Carl Linnaeus, dessen Mitte des 18. Jahrhunderts erschienenes Werk „Systema Naturae“ den Grundstein für die auch heute noch verwendete Einteilung von Organismen in verschiedene Hierarchieebenen legte. Die Art hat einen besonderen Stellenwert in dieser Hierarchie, da sie als Fortpflanzungsgemeinschaft die Basis bildet für sowohl höhere, als auch niedrigere taxonomische Ränge. Während Linnaeus stark von seiner Religiosität beeinflusst war und an die von Gott gegebene Unveränderlichkeit aller Dinge glaubte, revolutionierte Charles Darwin ein knappes Jahrhundert später mit seiner Evolutionstheorie die Naturwissenschaft und lenkte somit auch die Debatte um den Artbegriff in eine neue Richtung. Ernst Mayr etablierte schließlich 1942 erstmals das biologische Artkonzept und fügte der Diskussion somit einen weiteren wichtigen Aspekt hinzu. Die Debatte besteht bis zum heutigen Tag, geführt unter verschiedenen Gesichtspunkten. Manche Anhänger der Kladistik schlagen sogar vor, alle Hierarchieebenen, inklusive der Art, abzuschaffen und durch den Begriff „clade“ (Stamm) zu ersetzen. Für eine detaillierte historische Darstellung des Themas, sowie für weiterführende Literatur siehe Stuessy (2009).

Ernst Mayr war der Erste, der den Begriff „Konzept“ im Zusammenhang mit der Definition von Arten benutzte (Hey, 2006). Das stellte den Beginn einer Reihe von unterschiedlichen Artkonzepten dar, die in der zweiten Hälfte des 20. Jahrhunderts entwickelt wurden und von denen 22 größtenteils anerkannt sind (Mayden, 1997). Alle diese Konzepte sind monistisch, d.h. sie beanspruchen alleinige Gültigkeit für sich (siehe auch Hull, 1997). Manche davon sind zwar von großer theoretischer Bedeutung, allerdings oft nicht anwendbar. Ein gutes Beispiel ist das biologische Artkonzept, in dem Ernst Mayr 1969 Arten als „Gruppen natürlicher Populationen, die sich fortpflanzen und reproduktiv isoliert von anderen solchen Gruppen sind“ definiert hat (zitiert in Stuessy, 2009). Im Falle von höheren Pflanzen beispielsweise, wo es sehr oft zu Hybridisierungsvorgängen kommt, kann diese Auslegung der Artabgrenzung in der Praxis nicht zum Tragen kommen. Von dieser Definition ausgeschlossen werden auch asexuelle Organismen, wie z.B. auch einige Algengruppen,

bei denen bis dato keine Form der sexuellen Fortpflanzung bekannt ist (John and Maggs, 1997). Die Unvereinbarkeit von universeller theoretischer Gültigkeit und Anwendbarkeit in der Praxis ist praktisch allen Artkonzepten anhaftend (Hull, 1997) - ein Problem, das sich in der Literatur als sogenanntes „species problem“ manifestiert hat (Hey, 2001). Damit ist die Diskrepanz zwischen der Definition des Artbegriffs und der tatsächlichen Identifikation von Arten in der Natur gemeint.

Ungeachtet der Diskussion rund um Begrifflichkeiten ist die Unterscheidung von Arten ein wichtiges Fundament für viele wissenschaftliche Arbeiten, nicht zuletzt für jene, die sich mit Biodiversität und Naturschutz auseinandersetzen. Aus diesem Grund ist es von (auch politischer) Bedeutung, einen Konsens darüber zu finden, welche Einheiten in der Natur denn nun geschützt werden sollen. In der Praxis kommt dabei meistens das morphologische Artkonzept zum Einsatz. Das morphologische Artkonzept kennt mehrere Definitionen (siehe Stuessy, 2009 für Beispiele), es sollte jedoch grundsätzlich möglich sein, Arten anhand relativ einfach zu erkennender morphologischer Merkmale zu unterscheiden. Dabei fließt auch ein großer Anteil an subjektivem Empfinden durch den Beobachter mit ein, was auch einer der wichtigsten Kritikpunkte an diesem Konzept ist. In diesem Zusammenhang diskutieren Hey et al. (2003) auch die Anwendung von ESUs (Evolutionary Significant Units) als Alternative für den Artenschutz. Das ESU Konzept basiert auf „Populationen (oder Gruppen von interagierenden Populationen) die von anderen Populationen genetisch isoliert sind und wesentlich zur ökologischen und genetischen Diversität innerhalb eines bestimmten Taxons beitragen“. Das ist allerdings eine sehr vage Formulierung, die viele Interpretationen hinsichtlich der Abgrenzung von solchen ESUs offen lässt.

Die Gattung *Spirogyra*: Lebenszyklus und Artabgrenzung

Spirogyra ist eine weltweit verbreitete, häufig in verschiedensten Süßwasserhabitaten vorkommende Grünalgengattung, die zur Ordnung der Zygnematales gehört. Mikroskopisch ist sie an der Bildung von nicht verzweigten Fäden und an ihren spiralig geformten, wandständigen Chloroplasten leicht erkennbar. Im Freiland findet man sie oft zusammen mit verwandten Gattungen als makroskopisch erkennbare, grüne, schleimige Watten, die entweder an der Wasseroberfläche schwimmen, untergetaucht oder festgewachsen sind. Die Gattung als solche ist kaum mit anderen zu verwechseln, was sie zu einem beliebten Objekt für diverse Schüler- oder Studentenkurse macht.

Der Lebenszyklus von *Spirogyra* (Abb.1) umfasst sowohl vegetative, als auch sexuelle Fortpflanzung. Vegetative Fortpflanzung erfolgt meist durch Fragmentierung des Zellfadens,

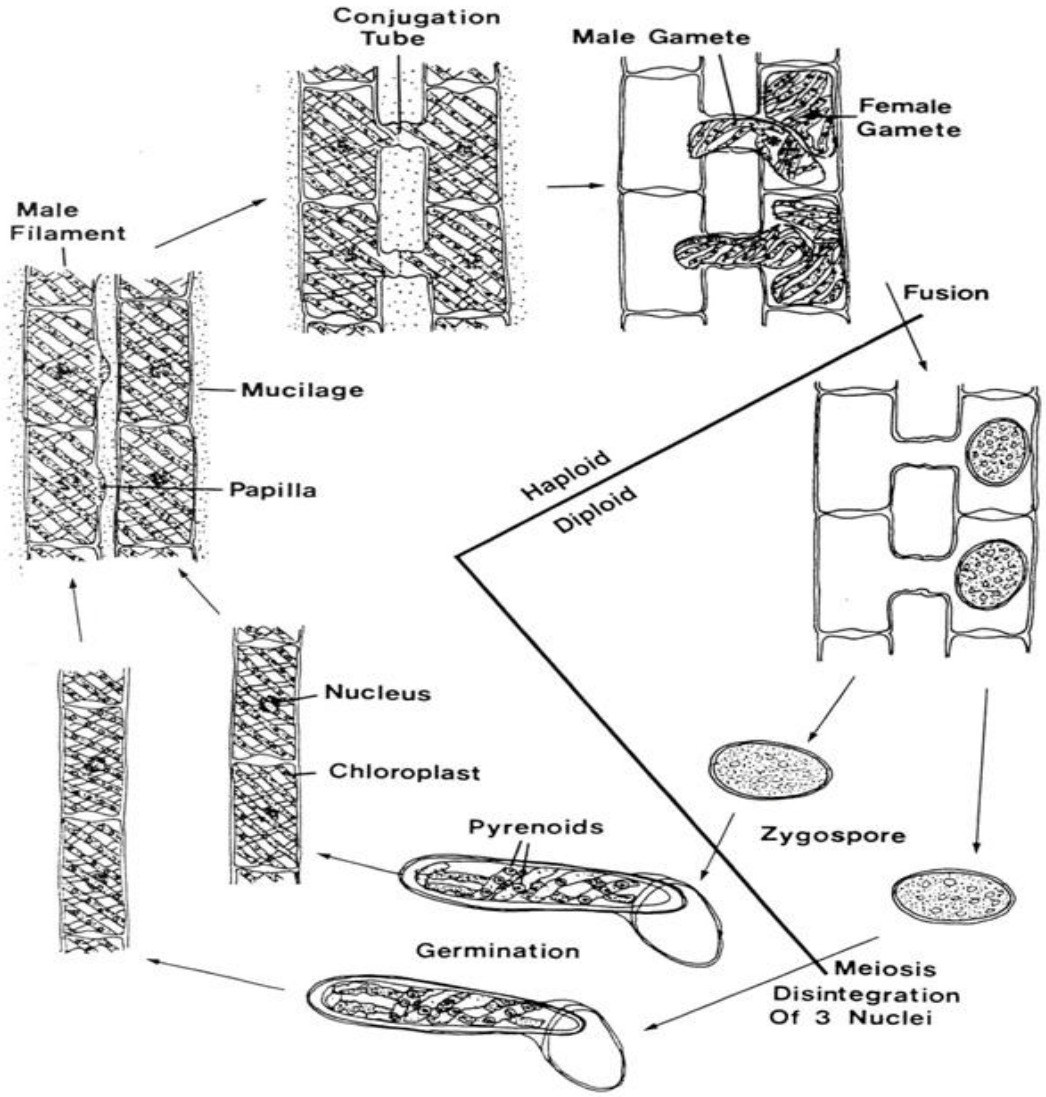

Abb. 1:. Lebenszyklus von *Spirogyra*, sexuelle Fortpflanzung hier mittels leiterförmiger Konjugation (verändert nach Lee, 2008).

mit anschließendem Wachstum durch Zellteilung. In einigen Fällen kommt es auch zur ungeschlechtlichen Bildung von Dauerstadien. Das können entweder Akineten (entstehen durch eine Verdickung der Zellwand), Aplanosporen (Bildung einer neuen Zellwand um den Protoplasten innerhalb der alten Zelle) oder Parthenosporen (sexualisierte Keimzellen, bei denen es allerdings nicht zur Kopulation kam) sein. Die sexuelle Vermehrung erfolgt mittels Konjugation, ein besonderer Vorgang, der alle Zygnematales auszeichnet. Bei der Konjugation kommt es zuerst zur Differenzierung von vegetativen Zellen zu Gametangien, welche die Keimzellen (Gameten) enthalten. Danach erfolgt die Verschmelzung zweier unbegeißelter Gameten entweder im Konjugationskanal, der sich zwischen zwei Zellen formt, oder im „weiblichen“ Gametangium. Dabei kann kein äußerlicher Unterschied zwischen „männlichen“ und „weiblichen“ Keimzellen festgestellt werden. Es existieren

verschiedene Formen der Konjugation. Beispiele sind die leiterförmige (Abb. 1, die häufigste Art bei *Spirogyra*) und die seitliche Konjugation (Abb. 2, seltener). Das Produkt der geschlechtlichen Fortpflanzung ist eine diploide Zygote, die als Dauerstadium die Fähigkeit besitzt, ungünstige Lebensbedingungen zu überstehen. Vor der Keimung findet eine Reduktionsteilung (Meiose) statt, bei der es allerdings nur im Falle eines der vier Tochterkerne zur Bildung eines haploiden Keimlings kommt (Kadlubowska, 1984).

Für die Unterscheidung von Arten innerhalb der Gattung *Spirogyra* mittels morphologischer Merkmale ist das Vorhandensein sexueller Stadien des Lebenszyklus unumgänglich. Dabei wird besonderes Augenmerk auf die Größe und Form der reifen Zygoten bzw. auf die Struktur der Zygotenhülle gelegt (Abb. 3).

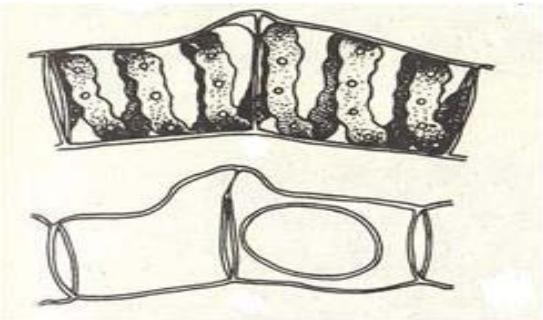

Abb. 2: Seitliche Konjugation bei *Spirogyra* (verändert nach Kadlubowska, 1984).

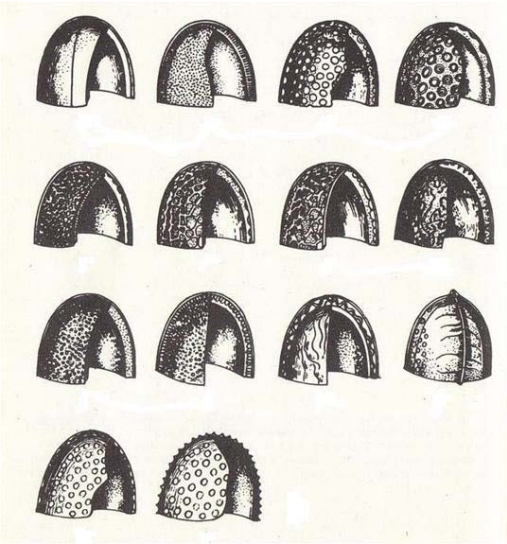

Abb. 3: Verschiedene Ausprägungen der Zygotenhülle bei Spirogyra (verändert nach Kadlubowska, 1984).

Oft ist die Identifikation von Arten jedoch nicht möglich, da *Spirogyra* trotz ihres häufigen Vorkommens im Freiland nur selten konjugierend vorgefunden wird (McCourt et al., 1986). Daraus ergibt sich eine Notwendigkeit, Konjugation in Laborversuchen künstlich zu initiieren. Es wurden bereits vereinzelt Studien zu diesem Thema durchgeführt, die zumindest teilweise erfolgreich waren. Trotzdem kann man nicht mit Sicherheit sagen, welcher Faktor, bzw. welche Kombination aus einzelnen Faktoren; die sexuelle Phase bei *Spirogyra* in Gang setzt. Czurda (1933) machte als erster umfassende Versuche über das Auslösen von Kopulation bei diversen Mikroorganismen und kam zu dem Schluss, dass es nach einer Phase intensiver vegetativer Fortpflanzung und mit Erreichen eines kritischen pH Wertes zur sogenannten „Kopulationsdisposition“ kommt. Diesen Status vermochte er allerdings nicht genau zu definieren. Andere Studien nahmen vor allem Nährstofflimitation und Licht als die Hauptfaktoren für die geschlechtliche Vermehrung an (Allen, 1958; Grote, 1977; Yamashita and Sasaki, 1979; Simons et al., 1984).

Abgesehen von den oben beschriebenen Schwierigkeiten ergeben sich auch noch andere Probleme bezüglich der Artabgrenzung bei *Spirogyra*. Die aktuellste Monographie von Kadlubowska (1984) enthält Beschreibungen von insgesamt 386 Arten basierend auf morphologischen Unterscheidungsmerkmalen, welche jedoch allesamt auf Einzelfunden verschiedener Autoren bzw. auf nachfolgende Ergänzungen durch andere beruhen. Es existieren einige Nachweise, dass innerhalb der Gattung unter bestimmten Bedingungen aus einem einzigen Klon spontan mehrere polyploide Formen gebildet werden können, die sich untereinander auch eingeschränkt sexuell fortpflanzen (Allen, 1958; Hoshaw et al., 1985). Diese Formen unterscheiden sich oft stark in ihrer morphologischen Ausprägung, was dazu führen kann, dass sie mittels eines traditionellen Bestimmungsschlüssels verschiedenen Arten zugeordnet werden. Aus dieser Perspektive ist es mit Sicherheit in vielen Fällen aufschlussreich, *Spirogyra*-Kulturen über einen längeren Zeitraum zu beobachten und auf eventuelle Veränderungen in der Physiognomie hin zu überprüfen. Diese Möglichkeit morphologischer Variabilität wurde von der konventionellen Taxonomie jedoch in keinster Weise berücksichtigt (in diesem Zusammenhang siehe auch McCourt and Hoshaw, 1990).

Die vorliegende Studie beschäftigt sich mit den Voraussetzungen, die für eine Einleitung der sexuellen Phase bei *Spirogyra* erforderlich sein könnten. Zu diesem Zweck wurden mehrere Stämme im Labor kultiviert und verschiedene potentielle Schlüsselfaktoren getestet, wie etwa die Limitation bestimmter Nährstoffe, unterschiedliche Lichtbedingungen, Temperaturschwankungen oder pH Werte. Außerdem wurden statistische Tests durchgeführt, um die abiotischen Bedingungen von Standorten, an denen *Spirogyra* konjugierend gefunden wurde, mit jenen zu vergleichen, an denen die Alge nur im vegetativen Zustand anzutreffen war.

Im zweiten Teil der Arbeit wurden einige *Spirogyra* Stämme mithilfe konventioneller Monographien bis auf die Art bestimmt. Die dabei auftretenden Probleme werden im Hinblick auf die Anwendung des morphologischen, sowie auch des biologischen und phylogenetischen Artkonzeptes diskutiert.

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Manuscript 1

Induction of sexual reproduction in the genus *Spirogyra* (Zygnemataceae, Streptophyta) and related problems

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Abstract

Species identification of the ubiquitous green algae genus *Spirogyra* based on the traditional morphological species concept requires sexual reproduction stages including ripe hypnozygotes. Since these stages are only infrequently observed in nature, an artificial onset of the sexual phase in the laboratory would be essential. We therefore tried to induce conjugation in 95 strains of *Spirogyra* originating from diverse sampling localities mainly in Central Europe. Altogether, 681 experimental setups were conducted with a focus on variation of nutrient supply and light conditions. Results were then compared to environmental data obtained in situ. No clear pattern could be revealed concerning the conditions for sexual reproduction in the genus, although the importance of a certain nutrient ratio according to specific ecological demands seems likely.

Key words: green algae, *Spirogyra*, conjugation, zygote formation

1. Introduction

The freshwater green alga *Spirogyra* (Zygnemataceae, Streptophyta) has been subject to many investigations in the past concerning its life cycle, morphology and ecology (Allen 1958, Hoshaw et al. 1985, Berry and Lembi 2000). Due to its spiral, parietal chloroplasts, the genus is easily recognized. Therefore, genus distribution is well recorded (Hoshaw and McCourt, 1988), which, however, does not apply for species distribution. According to traditional identification keys, reproductive stages, which are rarely found in nature, are required for species identification. McCourt et al. (1986) reported from large scale field collections that they observed these stages in only 10% out of 632 *Spirogyra* strains.

Within the family of Zygnemataceae, sexual reproduction occurs by means of conjugation. This unique process involves the differentiation of vegetative cells to gametangia and the fusion of amoeboid gametes, which takes place either in the conjugation tube, which is formed between the cells, or in the “female” gametangium. The gametal fusion results in the formation of a dormant stage, the so-called hypnozygote, which is able to survive unfavourable environmental conditions. In some species, asexual reproduction stages like aplanospores, parthenospores or akinetes do occur. In the most recent worldwide Zygnematalean flora of Kadlubowska (1984), 381 *Spirogyra* species are described, based on morphological characters like cell width, number of chloroplasts, type of end wall, type of reproduction, formation of conjugation channels, shape of gametangia, and as central characteristics shape, pigmentation, dimensions, and sculpturing of spores.

Taking into account the infrequent in situ observations of zygospores, there is an obvious need to induce sexual reproduction in the laboratory under defined conditions. This is of importance for species identification, for scrutinizing the traditional species concept, and also for implementing the taxon into biomonitoring surveys. Czurda (1933) was the first one who performed a broad study to trigger conjugation in *Spirogyra varians* Kützing. He assumed that the existence of a general principle underlies the initiation of the reproductive stage in the cell cycle of all microorganisms. Czurda (1933) stated that sexual disposition occurs after a period of intense vegetative growth. He could not clearly define the physiological conditions of that state (he called it “disposition for copulation”), but related it to a critical pH range, which the cells have to pass through. He also found that these pH values varied in every species, presumably according to the different ecological demands of the organisms. The direct influence of other external factors, such as nutrient depletion, was excluded by him, as well as the existence of an inner periodicity of the conjugation process. In another study, Allen (1958) investigated the complete life cycle of several strains of a homothallic clone of *Spirogyra* and thereby developed a standard technique to induce conjugation and zygote formation at will. She did not believe the pH optimum to be the only external factor. In her

conjugation experiments, Allen (1958) tested a broad variety of culture conditions and established a quantitative relationship between the amount of light energy and the occurrence of sexual stages above a certain minimum of light. Grote (1977) induced conjugation and spore formation in *Spirogyra majuscula* Kützing in a nutrient solution without any available nitrogen source. Also, she could not find certain vegetative and generative pH optima. Subsequent studies emphasized the role of nitrogen depletion and light as key factors to initiate the sexual phase in *Spirogyra* (Yamashita and Sasaki, 1979; Simons et al., 1984). Simons et al. (1984) were able to induce formation of mature zygospores in 31 species, which represented 40% of the total number of *Spirogyra* species described in the Netherlands at that time. The important role of nitrogen depletion for the onset of sexual reproduction was confirmed by the work of Dell'Uomo and Masi (1985), who observed a community of Zygnemataceae in nature over a period of one year. The impact of light was stressed by Kadlubowska (1984), who did not observe conjugation in *Spirogyra fluviatilis* Hilse until the algae reached the water surface.

Based on the above-mentioned research, the aim of the present study was to examine multiple *Spirogyra* strains with regard to potential key factors for the artificial induction of conjugation and zygote formation. Various culture conditions were tested, with a focus on nutrient depletion and light. Successfully obtained spores were then used for species identification. Furthermore, environmental data from field collections of mating as well as non-mating *Spirogyra* strains were analysed and compared to the results of the laboratory experiments.

2. Materials and methods

2.1. *Spirogyra* collection

The material originated from various field localities in Austria as well as in Germany, Croatia and Egypt. The strains were isolated and stored either in 100 ml or 250 ml Erlenmeyer flasks with culture medium C at 18°C, a light:dark cycle of 16:8h and overall low light intensity of 15 µmol photons m^{-2} s^{-1}. The composition of all culture media is given in the appendix. Several different techniques of precultivating the algal material were applied in order to obtain luxuriant vegetative growth before starting the mating induction experiments (for details, see appendix). White light was provided by cool white fluorescent tubes (either Philips TLD 36W/33 or Osram FQ 39W/840 LUMLUX Cool White) and in certain cases the precultures were stored in a SANYO MLR-350 versatile environmental chamber.

2.2. Environmental data

Analyses were done to examine the conditions for sexual reproduction in nature. For this purpose, environmental data from 137 *Spirogyra* sampling sites in Central Europe were used (fig. 1). For detailed information on the sampling methods and the water analyses procedures see Hainz et al. (2009). In order to compare sampling sites where conjugation occurred and sampling sites where no conjugation was observed, statistical analyses were conducted with the SigmaPlot SPW11 and with the SPSS Statistics 17.0 software packages. When data were normally distributed, T-tests were chosen to prove significant differences; otherwise Mann-Whitney-U-tests were performed.

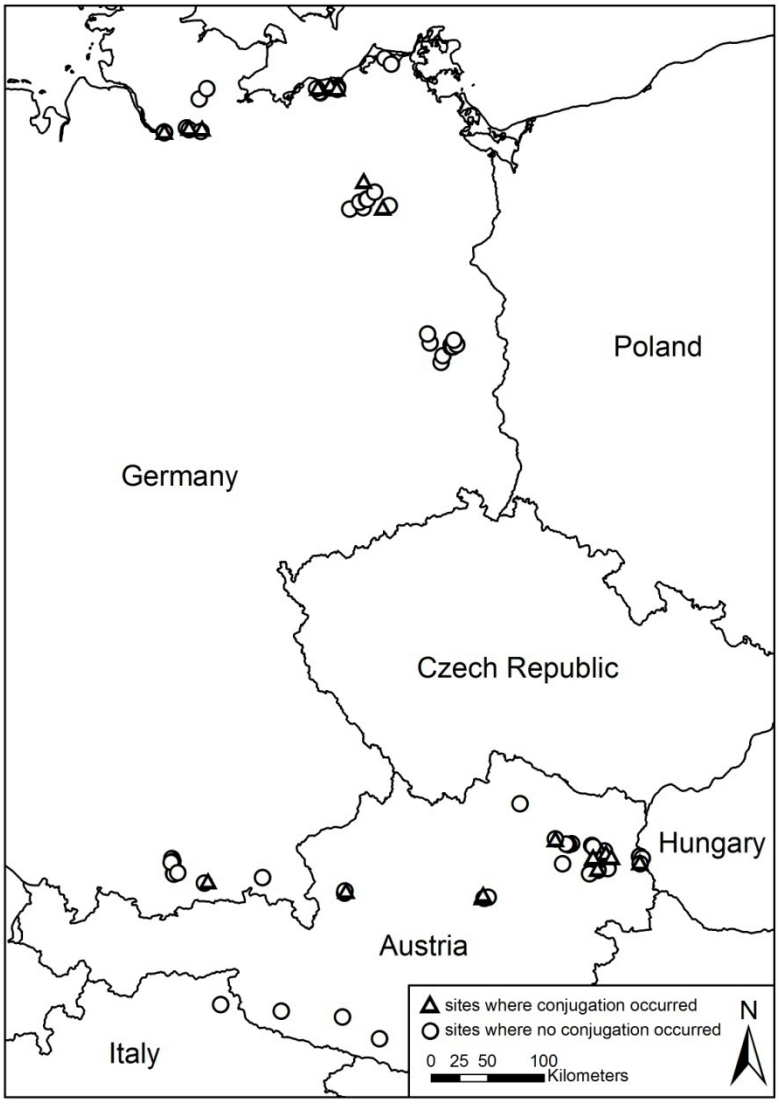

Fig. 1. Sampling sites in Central Europe.

2.3. Mating induction experiments

A total of 95 of the isolated strains of *Spirogyra spp.* were tested in multiple experimental setups. Different abiotic parameters were combined and varied to induce sexual reproduction. For this purpose, the precultivated material was transferred into small sterile plastic petri dishes of 55 mm diameter. Nutrient composition of the medium, light intensity, light quality, light cycle, pH, and temperature were considered as potential key parameters. Three types of nutrient media were used: (1) organic media, which contained soil extract or bog water; (2) inorganic media of clearly defined composition; (3) autoclaved water originating from sampling localities, diluted to 50% with distilled water. The substrate was either liquid or contained 1.5-3% agar. White light was provided by the aforementioned cool white fluorescent tubes. The SANYO MLR-350 versatile environmental chamber was used in some experiments. The light:dark cycle was 16:8h, 13:11h, or 24:0h. Additional varicoloured light was provided either by Philips 60W spot lights or by red, green, and blue foil, which was filtering the white light emitted from Voltolux 40W standard incandescent lamps. Additional UV light was always provided by 300W Osram Ultra-Vitalux sunlamps, which emitted irradiances similar to natural sunlight. A detailed description of the experimental design is given in the appendix, as well as the emission spectra for each lamp. The light intensity was measured with the Skye Quantum Sensor SKP 215/S and arbitrarily divided into three categories: (1) $\leq$ 33 µmol $m^{-2}s^{-1}$ (low); (2) > 33 to $\leq$ 70 µmol $m^{-2}s^{-1}$ (medium); and (3) > 70 µmol m^{-2} s^{-1} (high), with its maximum at about 160 µmol photons m^{-2} s^{-1}. In case of higher light intensities, the chloroplasts were contracting rapidly, bleaching, or changing their colour to brownish-black. pH was determined with a Metrohm AquatrodePlus combined pH glass electrode using the Metrohm PC Control Titrando sample processor and software. The petri dishes were regularly checked by means of a stereo microscope, in order to find out whether conjugation or zygote formation had occurred.

2.4. Species identification

Zygospores obtained from the field, as well as from the laboratory experiments, were examined under a Reichert-Jung Polyvar microscope, or under a Zeiss Axio Imager A1 microscope. Microphotography was done using the Olympus cell^F or the analySIS 3.0 software and digital camera. In certain cases, zygospores were treated with 5% KOH in 80% ethanol for the purpose of dissolving the cell content. The remaining spore walls were centrifuged and rinsed in ethanol and distilled water. Subsequently, the zygospores were put on a slide and embedded in glycerine. (cf. Kadlubowska 1984). This method should enhance the sculpture of the spore walls. Some zygospores were fixed in ethanol or Strasburger solution and treated mechanically in an attempt to separate the spore wall layers. In that

case, the zygospores were put on a slide and pressed slightly while moving the cover glass (cf. Simons et al., 1982). Species identification was carried out using the monographs of Transeau (1951), Randhawa (1959), and Kadlubowska (1984).

3. Results

The analyses of the environmental data displayed no significant differences between sites where conjugation was observed and sites where no conjugation occurred (table 1, fig. 2-4). At sites where only vegetative filaments were found, NO_3-N ranged from 0 (i.e. it was below the detection limit) to around 20 mg l^{-1}, NH_4-N = 0.002 – 1.3 mg l^{-1}, SRP was between 0 (below the detection limit) and 1.4 mg l^{-1}, the weight ratio of N_{tot}:P_{tot} = 1 – 445, pH = 6.2 - 9.1, alkalinity = 0.6 - 7.9 mval l^{-1}, temperature = 9.6 - 30.4°C, and conductivity = 76 – 1508 µS cm^1. At sites where conjugation was observed, NO_3-N was between 0.002 and around 15 mg l^{-1}, NH_4-N = 0.001 – 0.08 mg l^{-1}, and SRP ranged from below the detection limit to 0.05 mg l^{-1}. Here, we found a weight ratio of N_{tot}:P_{tot} between 2.6 and 4243. pH was between 7.2 and 8.9, alkalinity = 0.9 - 6.6 mval l^{-1}, temperature = 7.9 - 28.6°C, and conductivity = 180 - 958 µS cm^1.

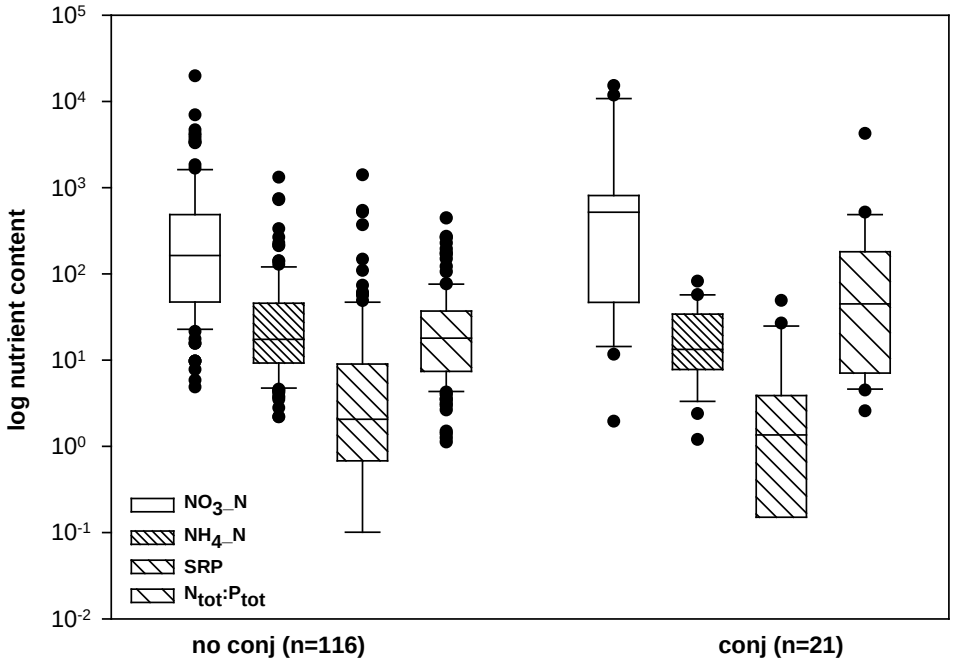

Fig. 2. Nutrient content of the sampling sites.

Table 1: Statistical tests and probability values of all environmental parameters tested. Sites where no conjugation occurred: n=116 with temperature being the only exception, where n=112. Sites where conjugation occurred: n=21.

parameter	median (vegetative)	IQR (vegetative)	median (conjugation)	IQR (conjugation)	statistical test	p
NO_3-N [µg l^{-1}]	163.5	440.3	520.0	764.5	Mann-Whitney-U	0.341
NH_4-N [µg l^{-1}]	17.4	36.5	13.3	26.4	Mann-Whitney-U	0.285
SRP [µg l^{-1}]	2.1	8.3	1.4	3.7	Mann-Whitney-U	0.221
N_{tot}:P_{tot}	18.0	29.6	44.9	173.1	Mann-Whitney-U	0.109
alkalinity [mval l^{-1}]	3.2	2.7	3.1	1.4	Mann-Whitney-U	0.770
conductivity [µS cm^{-1}]	457.0	320.3	378.0	357.5	Mann-Whitney-U	0.548
parameter	**mean (vegetative)**	**SD (vegetative)**	**mean (conjugation**	**SD (conjugation)**	**test**	**p**
pH	7.8	0.5	8.0	0.4	t-Test	0.068
temperature [°C]	18.1	4.7	18.2	4.7	t-Test	0.940

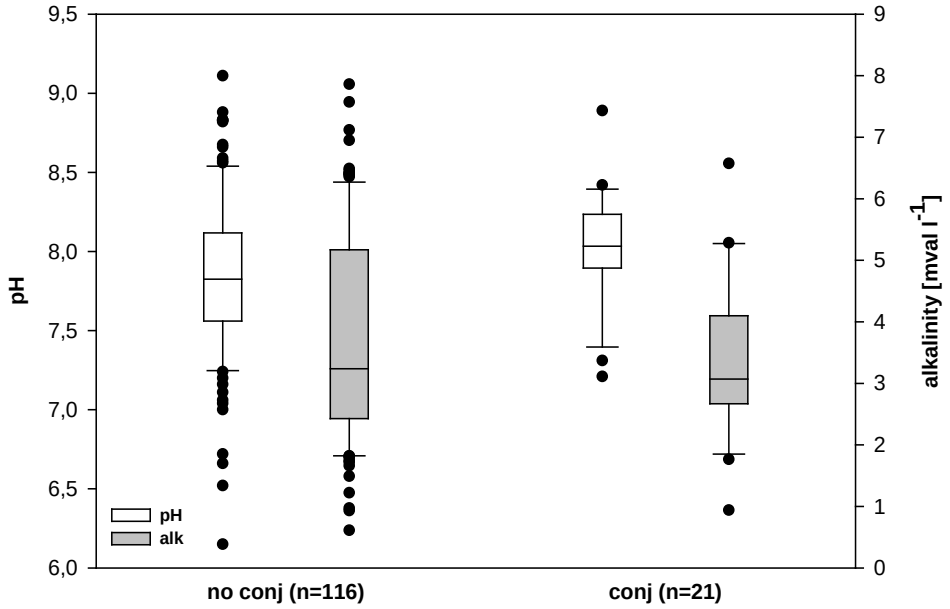

Fig. 3. pH and alkalinity of the sampling sites.

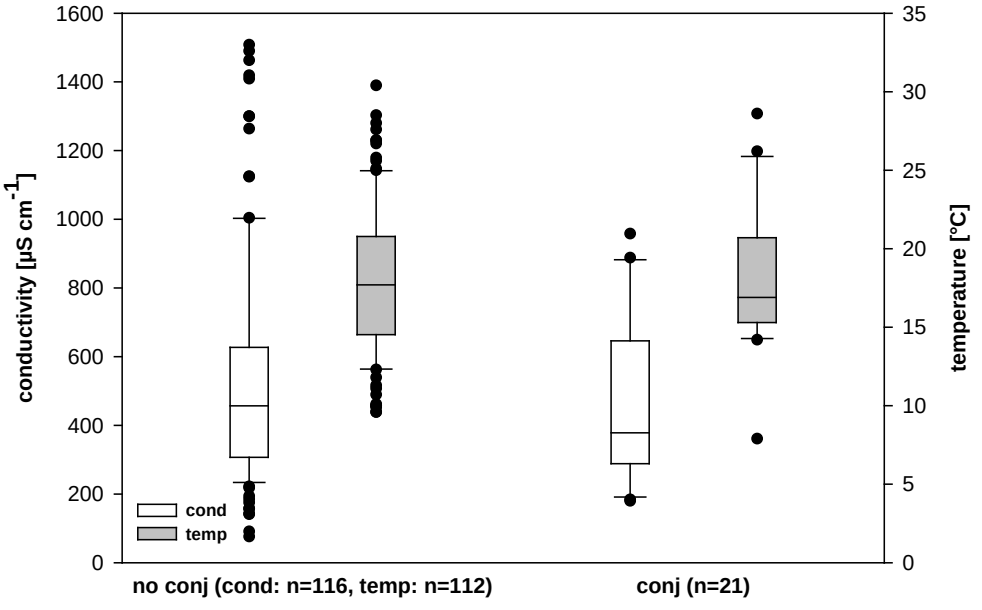

Fig. 4. Conductivity and temperature of the sampling sites.

In the laboratory, 681 different experimental setups were performed (tables 2, 3). Artificial induction of the mating process was obtained in 8 *Spirogyra* strains. Sexual reproduction took place in all three categories of light intensity, regardless of the day length. Onset of conjugation was observed under white, red, and green light, whereas zygote formation occurred only under white and red light (tables 4-6). Blue or UV light experiments always yielded negative results. Sexual reproduction under red and green light always occurred in nitrogen depleted media. Under white light, it was mainly observed in substrates without nitrogen or in phosphorus depleted substrates.

Table 2: Experimental setups given in absolute numbers. LI=light intensity (1=low, 2=medium, 3=high), LC=light:dark cycle in hours. In the case of experimental setups containing UV light, additional UV light was switched on for either 1h (setup in the first column), 3 times for 2h at a time (second column), 4h (third column), or 4 times for 1h at a time (fourth column) during the 16h light phase. 2 standard media containing phosphorus and nitrogen were used (+N+P liquid and agar). The other media were depleted either from nitrogen (-N+P liquid and agar), or from phosphorus (+N-P liquid), or from both of these nutrients (-N-P agar).

White							
	Media						
		LI=1/LC=16:8	LI=2/LC=16:8	LI=3/LC=16:8	LI=1/LC=13:11	LI=2/LC=24:0	**Sum**
	(+)N (+)P liquid	70	42	0	12	0	124
	(+)N (-)P liquid	7	7	0	0	0	14
	(-)N (+)P liquid	42	49	0	4	0	95
	(+)N (+)P agar	6	0	0	0	30	36
	(-)N (+)P agar	18	15	0	0	0	33
	(-)N (-)P agar	43	52	30	0	0	125
	Sum	186	165	30	16	30	**427**
UV							
	Media						
		LI=3/LC=16:8	LI=3/LC=16:8	LI=3/LC=16:8	LI=3/LC=16:8		**Sum**
	(+)N (+)P liquid	0	22	0	8		30
	(+)N (-)P liquid	0	3	0	4		7
	(-)N (+)P liquid	0	10	0	4		14
	(+)N (+)P agar	6	0	6	0		12
	(-)N (+)P agar	3	0	3	0		6
	(-)N (-)P agar	3	0	3	0		6
	Sum	12	35	12	16		**75**

A list of species, which could artificially be induced to spore formation is given in table 7. Sexual reproduction in nature was not observed in these. It was not possible to identify all strains, because of the lack of mature hypnozygotes in some cases. The same applies for the conjugating strains of *Spirogyra* found in nature (table 8).

Table 3: Experimental setups given in absolute numbers. LI=light intensity (1=low, 2=medium, 3=high), LC=light:dark cycle in hours. Red, blue, or green light was either additionally switched on for 8h during the 16h light phase, or it was provided continuously. 2 standard media containing phosphorus and nitrogen were used (+N+P liquid and agar). The other media were depleted either from nitrogen (-N+P liquid and agar), or from phosphorus (+N-P liquid), or from both of these nutrients (-N-P agar).

Red					
	Media				
		LI=2/LC=16:8+8	LI=3/LC=16:8+8	LI=1/LC=16:8	**Sum**
	(+)N (+)P liquid	0	22	8	30
	(+)N (-)P liquid	0	3	4	7
	(-)N (+)P liquid	0	25	4	29
	(+)N (+)P agar	6	0	0	6
	(-)N (+)P agar	3	15	0	18
	(-)N (-)P agar	3	0	0	3
	Sum	12	65	16	**93**
Green					
	Media				
		LI=3/LC=16:8+8	LI=1/LC=16:8		**Sum**
	(+)N (+)P liquid	6	8		14
	(+)N (-)P liquid	3	4		7
	(-)N (+)P liquid	6	4		10
	(+)N (+)P agar	6	0		6
	(-)N (+)P agar	3	0		3
	(-)N (-)P agar	3	0		3
	Sum	27	16		**43**
Blue					
	Media				
		LI=3/LC=16:8+8	LI=1/LC=16:8		**Sum**
	(+)N (+)P liquid	6	8		14
	(+)N (-)P liquid	3	4		7
	(-)N (+)P liquid	6	4		10
	(+)N (+)P agar	6	0		6
	(-)N (+)P agar	3	0		3
	(-)N (-)P agar	3	0		3
	Sum	27	16		**43**

4. Discussion

The occurrence of *Spirogyra* in a broad range of habitats is well documented and reflected by the results of our environmental data analyses. However, unlike the study of Dell'Uomo and Masi (1985), no long time monitoring of the particular sampling sites was carried out. Therefore, no conclusions can be drawn concerning the changes in abiotic environmental conditions which might have caused an onset of sexual reproduction. Regarding this, daily fluctuations of parameters like temperature in algal mats might also be of importance.

Table 4: Results of mating induction experiments under white light. LI=light intensity (1=low, 2=medium, 3=high), LC=light:dark cycle in hours. Conj=0: no conjugation, Conj=1: conjugation, Conj=2: zygote formation. All data are given in percentages.

Conj=0						
	Media					
		LI=1/LC=16:8	LI=2/LC=16:8	LI=3/LC=16:8	LI=1/LC=13:11	LI=2/LC=24:0
	(+)N (+)P liquid	99	100	x	100	x
	(+)N (-)P liquid	86	100	x	x	x
	(-)N (+)P liquid	93	94	x	100	x
	(+)N (+)P agar	100	x	x	x	87
	(-)N (+)P agar	89	87	x	x	x
	(-)N (-)P agar	100	100	100	x	x
Conj=1						
	Media					
		LI=1/LC=16:8	LI=2/LC=16:8	LI=3/LC=16:8	LI=1/LC=13:11	LI=2/LC=24:0
	(+)N (+)P liquid	1	0	x	0	x
	(+)N (-)P liquid	0	0	x	x	x
	(-)N (+)P liquid	5	0	x	0	x
	(+)N (+)P agar	0	x	x	x	0
	(-)N (+)P agar	0	0	x	x	x
	(-)N (-)P agar	0	0	0	x	x
Conj=2						
	Media					
		LI=1/LC=16:8	LI=2/LC=16:8	LI=3/LC=16:8	LI=1/LC=13:11	LI=2/LC=24:0
	(+)N (+)P liquid	0	0	x	0	x
	(+)N (-)P liquid	14	0	x	x	x
	(-)N (+)P liquid	2	6	x	0	x
	(+)N (+)P agar	0	x	x	x	13
	(-)N (+)P agar	11	13	x	x	x
	(-)N (-)P agar	0	0	0	x	x

The present study did not resolve the difficulties regarding the induction of the sexual phase within the genus *Spirogyra*. Even though conjugation and zygote formation in the laboratory indicate a relation to nitrogen depletion, no clear pattern could be found. Furthermore, sexual reproduction seems to happen in a wide range of light conditions, which probably reflects the different ecological demands of the strains used in the experiments. It seems likely that a certain shift in nutrient ratio is an important factor in triggering the conjugation process. The true role of light intensity and nitrogen deficiency in the sexual process is still uncertain. The lack of certain nitrogen compounds seems to be involved in the differentiation of gametangia (Simons et al., 1984). Yamashita and Sasaki (1979) found a high intracellular C/N ratio in generative cells. They stated that the accumulation of photosynthetic products, mainly starch, is necessary for the formation of ripe hypnozygotes. This finding was confirmed by Simons et al. (1984), who also assumed that nitrogen depletion results in the formation of secondary carotenoids and consequently in sporopollenin synthesis.

Table 5: Results of mating induction experiments under red light. LI=light intensity (1=low, 2=medium, 3=high), LC=light:dark cycle in hours. Red light was either additionally switched on for 8h during the 16h light phase, or it was provided continuously. Conj=0: no conjugation, Conj=1: conjugation, Conj=2: zygote formation. All data are given in percentages.

Conj=0				
	Media			
		LI=2/LC=16:8+8	LI=3/LC=16:8+8	LI=1/LC=16:8
	(+)N (+)P liquid	x	100	100
	(+)N (-)P liquid	x	100	75
	(-)N (+)P liquid	x	92	75
	(+)N (+)P agar	100	x	x
	(-)N (+)P agar	100	100	x
	(-)N (-)P agar	100	x	x
Conj=1				
	Media			
		LI=2/LC=16:8+8	LI=3/LC=16:8+8	LI=1/LC=16:8
	(+)N (+)P liquid	x	0	0
	(+)N (-)P liquid	x	0	25
	(-)N (+)P liquid	x	4	25
	(+)N (+)P agar	0	x	x
	(-)N (+)P agar	0	0	x
	(-)N (-)P agar	0	x	x
Conj=2				
	Media			
		LI=2/LC=16:8+8	LI=3/LC=16:8+8	LI=1/LC=16:8
	(+)N (+)P liquid	x	0	0
	(+)N (-)P liquid	x	0	0
	(-)N (+)P liquid	x	4	0
	(+)N (+)P agar	0	x	x
	(-)N (+)P agar	0	0	x
	(-)N (-)P agar	0	x	x

Table 6: Results of mating induction experiments under green light. LI=light intensity (1=low, 2=medium, 3=high), LC=light:dark cycle in hours. Green light was either additionally switched on for 8h during the 16h light phase, or it was provided continuously. Conj=0: no conjugation, Conj=1: conjugation. No zygote formation was observed in these setups. All data are given in percentages.

Conj=0			
	Media		
		LI=2/LC=16:8+8	LI=1/LC=16:8
	(+)N (+)P liquid	100	100
	(+)N (-)P liquid	100	75
	(-)N (+)P liquid	83	75
	(+)N (+)P agar	100	x
	(-)N (+)P agar	100	x
	(-)N (-)P agar	100	x
Conj=1			
	Media		
		LI=2/LC=16:8+8	LI=1/LC=16:8
	(+)N (+)P liquid	0	0
	(+)N (-)P liquid	0	25
	(-)N (+)P liquid	17	25
	(+)N (+)P agar	0	x
	(-)N (+)P agar	0	x
	(-)N (-)P agar	0	x

Table 7: List of species, in which artificially induced formation of zygotes was obtained. The species marked with an asterisk conjugated spontaneously in culture. Abiotic conditions at the sampling date are given. At that time, neither conjugation nor zygote formation was observed in nature.

species	*S. subsalsa* Kützing 1845	*S. decimina* (Müller) Kützing 1845	*S. mirabilis** (Hassall) Kützing 1849	*S. fluviatilis* Hilse 1862	*S. polymorpha* Kirchner 1878	*S. varians* (Hassall) Kützing 1849
sampling date	25/04/2006	15/05/2006	15/05/06	10/08/2006	22/04/2007	23/04/2007
sampling site	Danube back water Vienna	pond in Vienna	rivulet in Vienna	Lake Ossiach Carinthia Austria	rivulet in the area of Rostock	kettle hole in the area of Rostock
O_2 [%]	166,5	77,4	140,6	-	330,0	68,9
O_2 [mg l^{-1}]	14,6	6,9	13,45	-	37,5	8,4
t [°C]	20,5	21,5	19,2	-	11,1	9,9
cond [µS m^{-2} s^{-1}]	321,0	1409,0	1264	218,0	530,0	296,0
mean pH	8,9	8,0	8,3	8,8	7,5	6,5
alk [mval l^{-1}]	1,8	6,2	6,4	2,5	2,7	1,5
NO_3-N [µg l^{-1}]	152,0	205,0	475,0	105,0	6962,2	4019,4
NO_2-N [µg l^{-1}]	0,2	0,7	38,5	5,4	92,2	42,6
NH_4-N [µg l^{-1}]	14,3	26,6	18,7	18,7	23,7	139,8
N_{org} [µg l^{-1}]	185,8	572,3	767,4	285,6	360,5	1705,0
N_{tot} [µg l^{-1}]	338,0	778,0	1281,0	396,0	7414,8	5767,0
SRP [µg l-1]	0,0	0,8	3,3	0,6	1,3	6,4
P_{tot} [µg l^{-1}]	7,5	31,1	25,5	14,2	16,6	575,1
Na [mg l^{-1}]	17,4	55,3	61,2	3,9	25,0	20,1
K [mg l^{-1}]	2,0	25,3	28,8	1,7	6,9	22,5
Ca [mg l^{-1}]	23,2	82,1	79,3	27,8	102,5	44,8
Mg [mg l^{-1}]	14,9	109,4	110,4	6,9	15,9	6,0
Cl [mg l^{-1}]	31,7	173,4	136,8	4,5	58,7	28,8
SO4 [mg l^{-1}]	33,9	279,0	268,2	11,3	120,8	61,1

Another essential aspect one has to consider is the evidence of polyploidy within the genus. Allen (1958) isolated three strains of different cell width from a single clone of *Spirogyra*, which indicates polyploidy. All three strains conjugated in a similar manner. Additional, successful interbreeding between the three cell width groups was obtained. Hoshaw et al. (1985) also observed ploidal changes in a clonal culture of *Spirogyra.* In their study, they examined vegetative and sexual reproductive behaviour of four cell width groups, and they too succeeded in crossing morphologically different strains. According to their observations, they concluded that all four strains belong to a species complex of *Spirogyra communis* (Hassall) Kützing. These results were confirmed in a subsequent study, in which the natural occurrence of this species complex was evident (Wang et al., 1986).

Apart from polyploidy, some other aspects should be taken into account when dealing with the sexual process. Heterothallism is one of them, although to date there is no evidence for heterothallism in *Spirogyra*. However, there are rare observations of this phenomenon within the family of Zygnemataceae, e.g. in some *Zygnema* species (Hoshaw, 1968). Furthermore, it is possible that sexual reproduction does not occur in all *Spirogyra* species, or polyploid variants belonging to the same species complex.

Table 8: List of species, which were found reproducing sexually in nature. Abiotic conditions at the sampling date are given.

species	*S. longata* (Vaucher) Kützing 1843	*S. fluviatilis* Hilse 1862	*S. majuscula* Kützing 1849	*S. longata* (Vaucher) Kützing 1843	*S. longata* (Vaucher) Kützing 1843	*S. fluviatilis* Hilse 1862	*S. distenta* Transeau 1934	*S. porticalis* (Müller) Cleve 1868	*S. velata* Nordstedt 1873	*S. teodoresci* Transeau 1934	*S. neglecta* (Hassall) Kützing 1849	*S. fuellebornii* Schmidle 1902	*S. velata* Nordstedt 1873	*S. semiornata* Jao 1935	*S. hassalii* (Jenner) Petit 1880	*S. columbiana* Czurda 1932
sampling date	25/04/06	29/05/06	06/06/06	16/06/06	21/06/06	21/06/06	24/08/06	19/04/07	20/04/07	23/04/07	23/04/07	23/04/07	25/04/07	25/04/07	25/04/07	26/04/07
sampling site	Danube back water Vienna	pond in Vienna	pond in Vienna	Theißerin river Eastern Austria	pond in the area of Vienna	pond near Slovakian border Eastern Austria	pond in Lunz lower Austria	pond in the area of Berlin	pond in the area of Rostock	fire water pond in the area of Rostock	botanical garden pond Rostock	botanical garden pond Rostock	Wandse river retention basin Hamburg	Wandse river retention basin Hamburg	marsh in the area of Hamburg	botanical garden pond Hamburg
O_2 [%]	166,5	83,8	115,2	127,1	113,6	256,0	168,6	59,2	111,8	210,0	137,8	137,8	106,3	106,3	-	-
O_2 [mg l^{-1}]	14,6	8,3	10,9	11,2	9,4	19,5	15,5	7,4	11,9	21,8	18,1	18,1	18,4	18,4	-	-
t [°C]	20,5	18,2	17,2	20,9	24,6	28,6	16,4	14,2	7,9	15,3	15,3	15,3	16,7	16,7	16,9	14,6
cond [µS m^{-2} s^{-1}]	321,0	401,0	222,0	635,0	958,0	860,0	337,0	652,0	308,0	536,0	640,0	640,0	365,0	365,0	269,0	443,0
mean pH	8,9	8,0	8,0	8,0	8,2	8,1	8,3	8,0	7,7	8,0	7,9	7,9	8,1	8,1	7,2	7,3
alk [mval l^{-1}]	1,8	3,3	2,9	5,3	4,4	2,8	4,1	3,8	2,9	2,9	5,2	5,2	2,2	2,2	0,9	4,1
NO_3-N [µg l^{-1}]	152,0	704,9	55,7	2129,2	15221,7	11822,0	736,2	6555,6	11,7	42,9	2,0	2,0	610,4	610,4	103,4	29,3
NO_2-N [µg l^{-1}]	0,2	8,9	3,2	35,9	88,8	78,9	3,6	65,8	0,1	1,1	0,2	0,2	19,8	19,8	3,1	0,6
NH_4-N [µg l^{-1}]	14,3	55,7	8,4	57,5	52,9	1,2	14,8	9,4	7,0	13,3	10,8	10,8	25,5	25,5	10,8	2,4
N_{org} [µg l^{-1}]	185,8	207,5	715,4	272,0	276,1	739,5	173,5	185,2	538,0	558,2	322,6	322,6	616,5	616,5	2827,5	206,4
N_{tot} [µg l^{-1}]	338,0	921,3	774,3	2437,2	15586,6	12640,3	913,4	6806,6	549,9	602,2	324,7	324,7	1246,7	1246,7	2934,0	236,2
SRP [µg l-1]	0,0	2,7	49,1	2,9	4,7	1,4	0,3	0,0	1,3	26,9	16,7	16,7	0,0	0,0	5,1	2,6
P_{tot} [µg l^{-1}]	7,5	6,0	108,7	10,2	30,1	35,1	8,7	1,6	97,8	117,1	72,4	72,4	58,2	58,2	1138,2	33,5
Na [mg l^{-1}]	17,4	12,0	0,6	10,6	18,6	16,2	8,2	13,6	16,1	48,9	24,5	24,5	23,0	23,0	17,1	48,4
K [mg l^{-1}]	2,0	1,7	0,7	3,5	5,5	1,3	0,0	2,0	3,5	15,0	2,3	2,3	5,7	5,7	2,5	4,9
Ca [mg l^{-1}]	23,2	53,7	45,9	61,6	91,4	82,4	44,7	106,2	64,9	62,1	126,9	126,9	60,9	60,9	47,8	62,4
Mg [mg l^{-1}]	14,9	18,1	6,0	29,5	55,4	33,3	20,3	14,1	7,7	16,7	20,2	20,2	5,8	5,8	4,2	9,1
Cl [mg l^{-1}]	31,7	23,5	0,4	24,7	70,5	65,7	11,9	38,7	29,2	79,1	42,5	42,5	42,9	42,9	27,8	43,3
SO4 [mg l^{-1}]	33,9	47,7	1,6	87,2	159,7	117,7	11,7	115,4	35,5	75,2	123,3	123,3	123,1	123,1	103,3	26,3

For these reasons, the traditional species concept, which is based exclusively on morphological criteria, has to be scrutinized. The large morphological plasticity within the genus makes the description of species extremely difficult. In this respect, molecular analyses could provide some solutions. Coleman (2000) compared Internal Transcribed Spacer (ITS) sequences of rDNA among unicellular Volvocaleans and found qualitative sequence change related to known mating affinities. The sequenced secondary structure of ITS2 might be an adequate tool for the identification of mating types and hence genetic relatedness among organisms. If this also holds true for *Spirogyra*, species identification would no longer be dependent on rare findings of sexual stages and true phylogenetic relationships might be deciphered. Another recent study in population genetics suggests the use of genetic markers in combination with statistic tools to estimate the rate of recombination in a population of partially clonal organisms (Halkett et al., 2005). Strictly asexual lineages are generally believed to have a short evolutionary age. Consequently, a minimum of sexual recombination would occur in every organism. Regarding this, the employment of adequate genetic markers might also be useful when studying the genealogy and evolution of *Spirogyra* species. The application of these methods might also lead to a considerable reduction of species number. There are still a lot of questions remaining concerning the species delineation of *Spirogyra*. In the future, some of these gaps will hopefully be filled by applying new methods, which should include molecular studies.

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Manuscript 2

The application of the morphological species concept in the genus *Spirogyra* (Zygnemataceae, Streptophyta) and resulting taxonomic implications

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Abstract

According to traditional monographs, species delimitation within the Zygnematalean genus *Spirogyra* is based upon morphology, considering asexual and sexual stages of its life cycle. In this study, we identified altogether 16 Spirogyra strains from 14 sampling sites in Central Europe down to species level and then compared our observations to species descriptions in literature. Results demonstrate the many uncertainties underlying the exclusive application of the morphological species concept, which indicates that many *Spirogyra* species might have been circumscribed to narrow. On this account, a combination of several approaches, including molecular as well as morphological and physiological data, could provide useful taxonomic information and might help to resolve identification problems on the intrageneric level.

Keywords: green algae, *Spirogyra*, taxonomy, species concepts

1. Introduction

1.1. Species concepts in eukaryotic algae

As a result of the frequent occurrence of asexual lineages and hybridisation events, the biological species concept is often not applicable when dealing with eukaryotic algae. Nonetheless, several studies revealed congruence between morphological traits and reproduction in certain cases, especially in rhodophytes and diatoms. Usually, the identification of algae species relies on the discrimination of morphological characters. While quite a few versions of the morphological species concept do exist (for examples see Stuessy, 2009), all of them imply that species should be readily identified by some distinct morphological features. This kind of convenient applicability is certainly the great advantage of the morphological species concept. On the other hand, it is also subjective in a sense that it depends strongly on the observer. Furthermore, it often does not consider the phenetic plasticity displayed by many groups of organisms. Phenetic plasticity can arise due to certain environmental conditions, although it might also be partly genetically controlled (John and Maggs, 1997). Last but not least, morphological characters might not necessarily reflect true relationships.

To overcome such problems, phylogenetic analyses might provide a more objective tool for researchers who aim to determine the historical relatedness between groups of organisms. The phylogenetic (or cladistic) species concept comprises several approaches (Stuessy, 2009). Two of them are discussed by Manhart and McCourt (1992). One defines species as “the smallest cluster of organisms, which possess at least one diagnostic character”. The nature of this character is not illustrated, but it is not necessarily an apomorphy. The other one focuses on monophyletic lineages that share one or more derived characters. In the last two decades, molecular data, e.g. analysis of mitochondrion, chloroplast and nucleus DNA, have widely been used among phycologists to support their hypotheses. However, molecular analyses also have their limitations, and not every set of data is compatible with every molecular method (Manhart and McCourt, 1992; Sites and Marshall, 2003).

1.2. The Zygnemataceae and the genus *Spirogyra*

The Zygnemataceae constitute a family of filamentous green algae within the order Zygnematales, which belongs to the conjugating green algae (Zygnemophyceae) along with the Desmidiales. The unicellular Mesotaeniaceae, or saccoderm desmids, form the second family within the order. The order is circumscribed by some distinct ultrastructural features of the cell wall, which does not shows any ornamentation and which is not segmented.

Recently, several attempts have been made to clarify phylogenetic relationships of the conjugating green algae using molecular markers. Both studies based on mitochondrial or chloroplast genes (McCourt et al., 1995; McCourt et al., 2000; Hall et al., 2008) and studies based on small subunit ribosomal DNA (SSU rDNA) sequences (Gontcharov et al., 2002) suggested that the traditional family of Zygnemataceae is polyphyletic, although the authors offer different solutions how to resolve relationships below the family level. This might be largely due to fact that taxon sampling as well as sampling size varied greatly between all of these studies.

Within the family of Zygnemataceae, twelve genera are traditionally recognized that occupy all sorts of freshwater habitats. The genus *Spirogyra*, easily distinguishable by its parietal, coiled chloroplasts, comprises nearly 400 species (Kadlubowska, 1984). Species identification relies on certain morphological characters of the vegetative filaments, like cell width, number of chloroplasts, and transverse cell walls. However, reproductive stages are essential, with the type of conjugation and the shape of gametangia as important characteristics, although the focus lies on the ultrastructure of the zygospore wall. Simons et al. (1982) examined spores of twenty *Spirogyra* species and found that the spore wall consists of at least four layers. The hyaline exo- and endospore layers comprise cellulose. The brown- or yellow-coloured mesospore contains sporopollenin and has two layers, the inner layer often being sculptured. Even though they observed variations in some species, they assumed this to represent the basic pattern in all Zygnemataceae. According to Kadlubowska (1984), the exospore as well as the mesospore might consist of two layers and both can either be smooth or ornamented.

In the present study, several strains of *Spirogyra* originating from various field collections were assigned to species by means of the morphological features mentioned above. The application of the morphological species concept with a focus on the genus *Spirogyra* is discussed.

2. Material and Methods

2.1. *Spirogyra* collection

A total of 314 sites in central Europe were investigated, 137 of which contained *Spirogyra*. At only 21 of these sites sexually reproducing *Spirogyra* strains were found (fig. 1). For detailed information on the sampling sites and the determination of environmental parameters see Hainz et al. (2009).

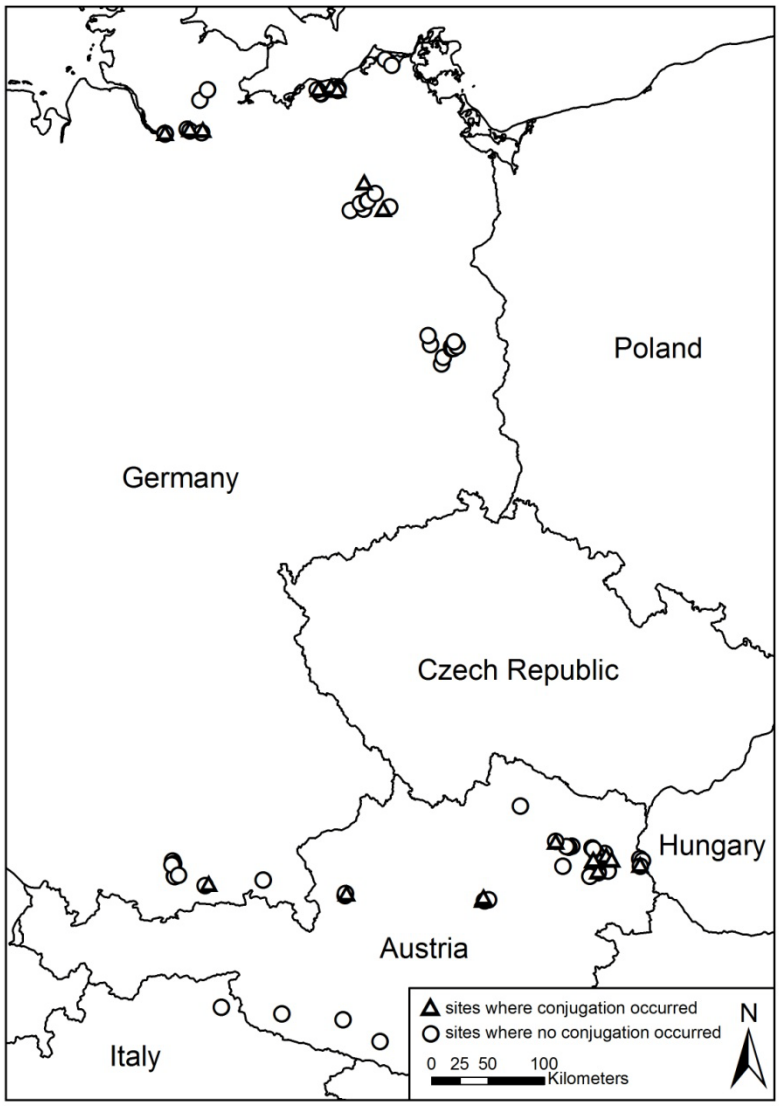

Fig. 5: Sampling sites in Central Europe.

Table 9: Abiotic conditions of the sampling sites.

site	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Danube back water Vienna	city park pond Vienna	city park pond Vienna	Theißerin river Eastern Austria	pond in the area of Vienna	pond near Slovakian border Eastern Austria	pond in Lunz lower Austria	pond in the area of Berlin	pond in the area of Rostock	fire water pond in the area of Rostock	botanical garden pond Rostock	Wandse river retention basin Hamburg	marsh in the area of Hamburg	botanical garden pond Hamburg
sampling date	25/04/06	29/05/06	06/06/06	16/06/06	21/06/06	21/06/06	24/08/06	19/04/07	20/04/07	23/04/07	23/04/07	25/04/07	25/04/07	26/04/07
O_2 [%]	166,5	83,8	115,2	127,1	113,6	256,0	168,6	59,2	111,8	210,0	137,8	106,3	-	-
O_2 [mg l^{-1}]	14,6	8,3	10,9	11,2	9,4	19,5	15,5	7,4	11,9	21,8	18,1	18,4	-	-
t [°C]	20,5	18,2	17,2	20,9	24,6	28,6	16,4	14,2	7,9	15,3	15,3	16,7	16,9	14,6
cond [µS m^{-2} s^{-1}]	321,0	401,0	222,0	635,0	958,0	860,0	337,0	652,0	308,0	536,0	640,0	365,0	269,0	443,0
mean pH	8,9	8,0	8,0	8,0	8,2	8,1	8,3	8,0	7,7	8,0	7,9	8,1	7,2	7,3
alk [mval l^{-1}]	1,8	3,3	2,9	5,3	4,4	2,8	4,1	3,8	2,9	2,9	5,2	2,2	0,9	4,1
NO_3-N [µg l^{-1}]	152,0	704,9	55,7	2129,2	15221,7	11822,0	736,2	6555,6	11,7	42,9	2,0	610,4	103,4	29,3
NO_2-N [µg l^{-1}]	0,2	8,9	3,2	35,9	88,8	78,9	3,6	65,8	0,1	1,1	0,2	19,8	3,1	0,6
NH_4-N [µg l^{-1}]	14,3	55,7	8,4	57,5	52,9	1,2	14,8	9,4	7,0	13,3	10,8	25,5	10,8	2,4
N_{org} [µg l^{-1}]	185,8	207,5	715,4	272,0	276,1	739,5	173,5	185,2	538,0	558,2	322,6	616,5	2827,5	206,4
N_{tot} [µg l^{-1}]	338,0	921,3	774,3	2437,2	15586,6	12640,3	913,4	6806,6	549,9	602,2	324,7	1246,7	2934,0	236,2
SRP [µg l-1]	0,0	2,7	49,1	2,9	4,7	1,4	0,3	0,0	1,3	26,9	16,7	0,0	5,1	2,6
P_{tot} [µg l^{-1}]	7,5	6,0	108,7	10,2	30,1	35,1	8,7	1,6	97,8	117,1	72,4	58,2	1138,2	33,5
Na [mg l^{-1}]	17,4	12,0	0,6	10,6	18,6	16,2	8,2	13,6	16,1	48,9	24,5	23,0	17,1	48,4
K [mg l^{-1}]	2,0	1,7	0,7	3,5	5,5	1,3	0,0	2,0	3,5	15,0	2,3	5,7	2,5	4,9
Ca [mg l^{-1}]	23,2	53,7	45,9	61,6	91,4	82,4	44,7	106,2	64,9	62,1	126,9	60,9	47,8	62,4
Mg [mg l^{-1}]	14,9	18,1	6,0	29,5	55,4	33,3	20,3	14,1	7,7	16,7	20,2	5,8	4,2	9,1
Cl [mg l^{-1}]	31,7	23,5	0,4	24,7	70,5	65,7	11,9	38,7	29,2	79,1	42,5	42,9	27,8	43,3
SO4 [mg l^{-1}]	33,9	47,7	1,6	87,2	159,7	117,7	11,7	115,4	35,5	75,2	123,3	123,1	103,3	26,3

2.2. Species identification

Field material containing zygospores was examined under a Reichert-Jung Polyvar microscope, or under a Zeiss Axio Imager A1 microscope. Microphotography was done using the Olympus cell^F or the analySIS 3.0 software and digital camera. In certain cases, zygospores were treated with 5% KOH in 80% ethanol for the purpose of dissolving the cell content. The remaining spore walls were centrifuged and rinsed in ethanol and distilled water. Subsequently, the zygospores were put on a slide and embedded in glycerine (c.f. Kadlubowska, 1984). This method should enhance the sculpturing of the spore walls. Some zygospores were fixed in ethanol or Strasburger solution and treated mechanically in an attempt to separate the spore wall layers. In that case, the zygospores were put on a slide and pressed slightly while moving the cover glass (c.f. Simons et al., 1982). Species identification was carried out using the monographs of Transeau (1951), Randhawa (1959), and Kadlubowska (1984).

3. Results

In most cases, several strains of *Spirogyra* were observed at each sampling site, often in their vegetative state, which differed at least in one morphological character, e.g. cell width, number of chloroplasts or formation of rhizoids. Mature hypnozygotes of altogether 16 strains were found at only 14 of the 21 sites where sexual reproduction was observed. Therefore, these strains could be identified down to species level. The remaining sites contained early reproduction stages, which were inappropriate for species identification. Table 1 summarizes the abiotic characteristics of the sampling sites. Table 2 and 3 list the species and give an overview of some important traits, which lead to the particular species description. At both the sites 11 and 12, two conjugating strains were detected and identified as two distinct species. Two strains had replicate end walls and four strains showed sculptured spore walls. Lateral conjugation was observed in only two strains. All in all, 12 species could be distinguished. *Spirogyra longata* (Vaucher) Kützing was found three times and hence was the most common species in the samples.

Table 10: An overview of important morphological species characteristics. Observations of the authors are compared to species descriptions in two monographs.

	Transeau (1951)	Kadlubowska (1984)	observations of the authors
***S. longata* (Vaucher) Kützing 1843**			
			site1 /site4 /site5
cell width [µm]	26-38	26-32	23-28/ 24-28 /21-25
no. of chlorplasts	1	1	1 in all 3 strains
end wall	plane	plane	plane in all 3 strains
conjugation type	scalariform, lateral	scalariform, lateral	scalariform/ lateral (scalariform) /scalariform
gametangia (f)	-	cylindric	cylindric /enlarged /enlarged
form of zygote	ovoid, globose	ellipsoid (pointed poles), cylindric (rounded poles)	cylindric /ellipsoid, ovoid /ellipsoid
size of zygote [µm]	28-38 x 50-83	25-35 x 46-100	26 x 76-79 /25-29 x 47-67 /23-28 x 56-60
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth in all 3 strains
***S. fluviatilis* Hilse 1862**			
			site2 /site6
cell width [µm]	30-45	30-45	33-38 /33-56
no. of chlorplasts	3-4	3-4	4 /3
end wall	plane	plane	plane in both strains
conjugation type	scalariform	scalariform	scalariform in both strains
gametangia (f)	inflated to 70 µm	inflated to 100%	inflated in both strains
form of zygote	ovoid	ellipsoid	ellipsoid /ellipsoid, ovoid
size of zygote [µm]	47-85 x 68-110	47-85 x 68-110	46-48 x 98-101 /51-57 x 80-99
exospor /mesospor	smooth /corrugate	smooth /corrugate	smooth /corrugate in both strains
***S. majuscula* Kützing 1849**			
			site 3
cell width [µm]	50-80	54-100	61-73
no. of chlorplasts	(3-) 5-8	5-10	6
end wall	plane	plane	plane
conjugation type	scalariform, lateral	scalariform	scalariform
gametangia (f)	cylindric, enlarged	inflated (not conjuating side only)	enlarged (not conjugating side only)
form of zygote	lenticular	lenticular	lenticular, globose
size of zygote [µm]	57-62 x 45-60	53-90 x 53-98 x 35-60	50-66 x 65-70
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. distenta* Transeau 1934**			
			site 7
cell width [µm]	48-52	48-52	51-54
no. of chlorplasts	2	2	2
end wall	plane	plane	plane
conjugation type	scalariform	scalariform	scalariform
gametangia (f)	cylindric, enlarged	cylindric, enlarged	enlarged
form of zygote	ovoid	cylindric (poles rounded), globose	ovoid, globose
size of zygote [µm]	49-55 x 50-100	49-55 x 50-100	42-51 x 45-80
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. porticalis* (Müller) Cleve 1868**			
			site 8
cell width [µm]	40-50	40-55	39-44
no. of chlorplasts	1	1	1
end wall	plane	plane	plane
conjugation type	scalariform	scalariform	scalariform
gametangia (f)	cylindric, enlarged	cylindric, enlarged	enlarged
form of zygote	ovoid, globose-ovoid	ellipsoid	ellipsoid
size of zygote [µm]	38-50 x 50-83	35-54 x 42-83	44-47 x 67-81
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. velata* Nordstedt 1873**			
			site 9 /site 12
cell width [µm]	29-41	29-40	33-34 /36-44
no. of chlorplasts	1 (-2)	1 (-2)	1 in both strains
end wall	plane	plane	plane in both strains
conjugation type	scalariform	scalariform	scalariform in both strains
gametangia (f)	cylindric, enlarged	cylindric, enlarged	cylindric, enlarged /cylindric
form of zygote	ovoid, cylindric-ovoid, (ellipsoid)	ellipsoid, (globose), (cylindric)	ellipsoid, ovoid, (cylindric) /cylindric, ovoid
size of zygote [µm]	37-57 x 60-100	35-48 x 46-150	34-36 x 58-61 /34-39 x 53-70
exospor /mesospor	scrobiculate (inner layer) /smooth	scrobiculate (inner layer) /smooth	scrobiculate (inner layer) /smooth in both strains

Table 11: An overview of important morphological species characteristics, continued

	Transeau (1951)	Kadlubowska (1984)	observations of the authors
***S. teodoresci* Transeau 1934**			
			site 10
cell width [µm]	24-30	24-30	23-27
no. of chlorplasts	1	1	1
end wall	plane	plane	plane
conjugation type	scalariform, lateral	scalariform	scalariform
gametangia (f)	inflated (conjuagting side only)	inflated (conjuagting side only)	enlarged (conjugating side only)
form of zygote	ellipsoid	ellipsoid	ellipsoid
size of zygote [µm]	26-33 x 45-55	22-38 x 36-55	25-28 x 31-36
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. neglecta* (Hassall) Kützing 1849**			
			site 11
cell width [µm]	55-67	57-67	58-68
no. of chlorplasts	3	2-4	2-3
end wall	plane	plane	plane
conjugation type	scalariform, lateral	scalariform	scalariform
gametangia (f)	enlarged, inflated	cylindric	enlarged
form of zygote	ovoid	ellipsoid (poles rounded)	ellipsoid
size of zygote [µm]	54-64 x 75-100	54-69 x 70-110	56-68 x 68-80
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. fuellebornii* Schmidle 1902**			
			site 11
cell width [µm]	40-42	40-46	39-47
no. of chlorplasts	3	2-4	2-3
end wall	plane	plane	plane
conjugation type	scalariform, lateral	scalariform	scalariform
gametangia (f)	cylindric	cylindric	enlarged
form of zygote	ellipsoid (poles pointed)	ellipsoid	ellipsoid
size of zygote [µm]	32-44 x 50-80	32-50 x 50-110	39-46 x 45-62
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. semiornata* Jao 1935**			
			site 12
cell width [µm]	27-32	25-33	25-46
no. of chlorplasts	1	1	1-2
end wall	replicate	replicate	replicate
conjugation type	scalariform, lateral	scalariform, lateral	scalariform
gametangia (f)	enlarged	cylindric, enlarged	enlarged
form of zygote	ovoid	ellipsoid	ellipsoid, (cylindric)
size of zygote [µm]	35-46 x 61-106	30-46 x 61-120	34-41 x 67-83
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. hassallii* (Jenner) Petit 1880**			
			site 13
cell width [µm]	26-33	28-35	24-30
no. of chlorplasts	2	2-4	2-3
end wall	replicate	replicate	replicate
conjugation type	lateral, scalariform	lateral, (scalariform)	lateral
gametangia (f)	inflated to 50 µm	inflated (60%)	enlarged
form of zygote	ellipsoid	ellipsoid	cylindric
size of zygote [µm]	39-48 x 58-146	37-54 x 47-72	32-37 x 97-125
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth
***S. columbiana* Czurda 1932**			**site 14**
cell width [µm]	48-54	46-54	50-58
no. of chlorplasts	(1-) 3	3	2-3
end wall	plane	plane	plane
conjugation type	scalariform	scalariform	scalariform
gametangia (f)	cylindric	cylindric	cylindric
form of zygote	ellipsoid (poles pointed)	ellipsoid	ellipsoid
size of zygote [µm]	50 x 70	42-55 x 70-124	45-56 x 82-113
exospor /mesospor	smooth /smooth	smooth /smooth	smooth /smooth

4. Discussion

4.1. Species identification within the genus *Spirogyra* and related difficulties

Several problems arise when dealing with species identification based solely on morphology within the genus *Spirogyra*. First of all, observations of zygotes resulting from sexual reproduction in situ are rare (McCourt et al., 1986), a fact that is also evident in our study. Though there were some attempts to induce conjugation in *Spirogyra* cultures (Czurda, 1933; Allen, 1958; Grote, 1977; Yamashita and Sasaki, 1979; Simons et al., 1984), their success was only limited. However, even if all morphological traits are available, species identification turns out to be difficult. The present study demonstrated that certain important characters used to delimit species in the monographs are often not clearly assignable. These characters are mainly the cell width of vegetative filaments, the degree of enlargement of female gametangia, and the form and size of zygospores, due to their great plasticity. We often found slight deviations from the species characterizations in literature, mainly regarding cell width and zygote size (table 2). Actually, *Spirogyra hassalii* (Jenner) Petit differed remarkable in zygote size as well as zygote form compared to previous descriptions. Other characters, like the type of end wall, the type of conjugation, the spore wall ornamentation, and the number of chloroplasts, were always found to be consistent with the information in at least one of the monographs used. In general, species identification proved to be difficult due to the fact that some features were either not clearly visible or deformed after preparation. The assignment of an appropriate species was always done when the observed characters came closest to its description in literature.

Our study confirms the many uncertainties inherent to species identification in the genus *Spirogyra*. Species delineations often relied only on field observations of single authors and were then extended by others, based upon their own findings. Hence, they contain a large subjective element, which is certainly one great flaw of the morphological species concept.

We suspect that species descriptions within the genus *Spirogyra* might be too narrow in certain cases A lot of authors, when describing a new species, did not take into account that considerable morphological variance might occur within a putative species under certain circumstances (McCourt and Hoshaw, 1990). This led to a notable increase in species number. Transeau (1951) recognized 275 *Spirogyra* species in his monograph, Randhawa (1959) listed 289 and Kadlubowska (1984) described 386. However, there are some species at least whose morphological variance is acknowledged. For example, *Spirogyra majuscula* Kützing was circumscribed by Transeau (1951) as a “complex group of elementary forms, differing in dimensions and numbers of chromatophores”.

4.2. Polyploidy and species complexes

There is some evidence that morphological plasticity within the genus *Spirogyra* is related to autopolyploidy, i.e. ploidal changes occur within a group of filaments that do not require initial hybridisation. A few studies proposed the existence of so-called species complexes in clonal cultures of *Spirogyra* (Allen, 1958; Hoshaw et al., 1985; Hoshaw et al., 1987). They all have in common that a single clone of *Spirogyra* gave rise to some morphologically distinct strains. If the morphological species concept was applied here, all ploidal stages within a single strain would belong to different species, since they differed in characters like cell width, chloroplast number, and zygote size. The fact that all strains originated from a single common ancestor is completely neglected. Furthermore, biological species would not correspond to the observed morphospecies, since some strains were capable of interbreeding while others were not. Thus, a single monophyletic lineage might comprise several distinct morphotypes, which exhibit limited sexual compatibility (McCourt and Hoshaw, 1990). The existence of a species complex of *Spirogyra communis* (Hassall) Kützing in nature was confirmed by Wang et al. (1986).

4.3. Phylogenetic analyses

Drummond et al. (2005) used *rbc*L (RUBISCO large subunit) sequence data to investigate the phylogeny of *Sirogonium* and *Spirogyra* at the species level. In their study, both species complexes of *Spirogyra pratensis* Transeau (Allen, 1958) and *Spirogyra communis* (Hassall) Kützing (Hoshaw et al., 1985; Wang et al., 1986) turned out to be monophyletic. Furthermore, strains with replicate end walls formed a single clade, as did strains with only loosely spiral chloroplasts. The latter clade contained all *Sirogonium* strains as well as *Spirogyra maxima* (Hassall) Wittrock. According to their findings, Drummond et al. (2005) presumed that these particular morphological characters constitute synapomorphies and might therefore prove to be adequate for species identification. In this context, zygospore ultrastructure also could be regarded as a suitable character for resolving intrageneric relationships (Drummond et al., 2005; Poulickova et al., 2007), since it is generally believed to be relatively stable.

4.4. Concluding remarks

Evolutionary relatedness is probably neither reflected in morphological discontinuities, nor is it in breeding groups. Nonetheless, it always depends on the objective of the researcher and on the organism investigated how taxonomic units are likely to be delimited. Hainz et al. (2009) grouped vegetative *Spirogyra* strains into morphotypes, which turned out to be related to certain environmental conditions. This approach could facilitate the implementation *Spirogyra* into biomonitoring surveys.

The answer to the problem of species delineation is certainly not easy. Moreover, it will presumably take more than one perspective to find a solution. In this regard, a combination of suitable morphological, physiological and molecular data, as recently suggested by Pröschold and Leliaert (2007), can be considered as useful for taxonomic revision.

In this regard, the family of Zygnemataceae in general and the genus *Spirogyra* in particular will definitely continue to provide a challenging task for future studies.

Acknowledgements

This study is part of the FWF project P18465-B03. We gratefully acknowledge Roland Hainz for doing a great part of the sampling and water analyses and for providing a map of the sampling sites, Martin Gruber for his help during sampling trips and laboratory work, and Hubert Kraill (University of Vienna) for the ion analyses. Uta Raeder, Viktoria Tscherne (Limnologische Station Iffeldorf - TU München), Ulf Karsten, Henning Baudler, Jana Wölfel (Insitut für Biowissenschaften - University of Rostock), Dieter Hanelt, Ludwig Kies (Fachbereich Biologie - University of Hamburg) kindly supported the collections in Germany.

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Appendix A) Experimental setups

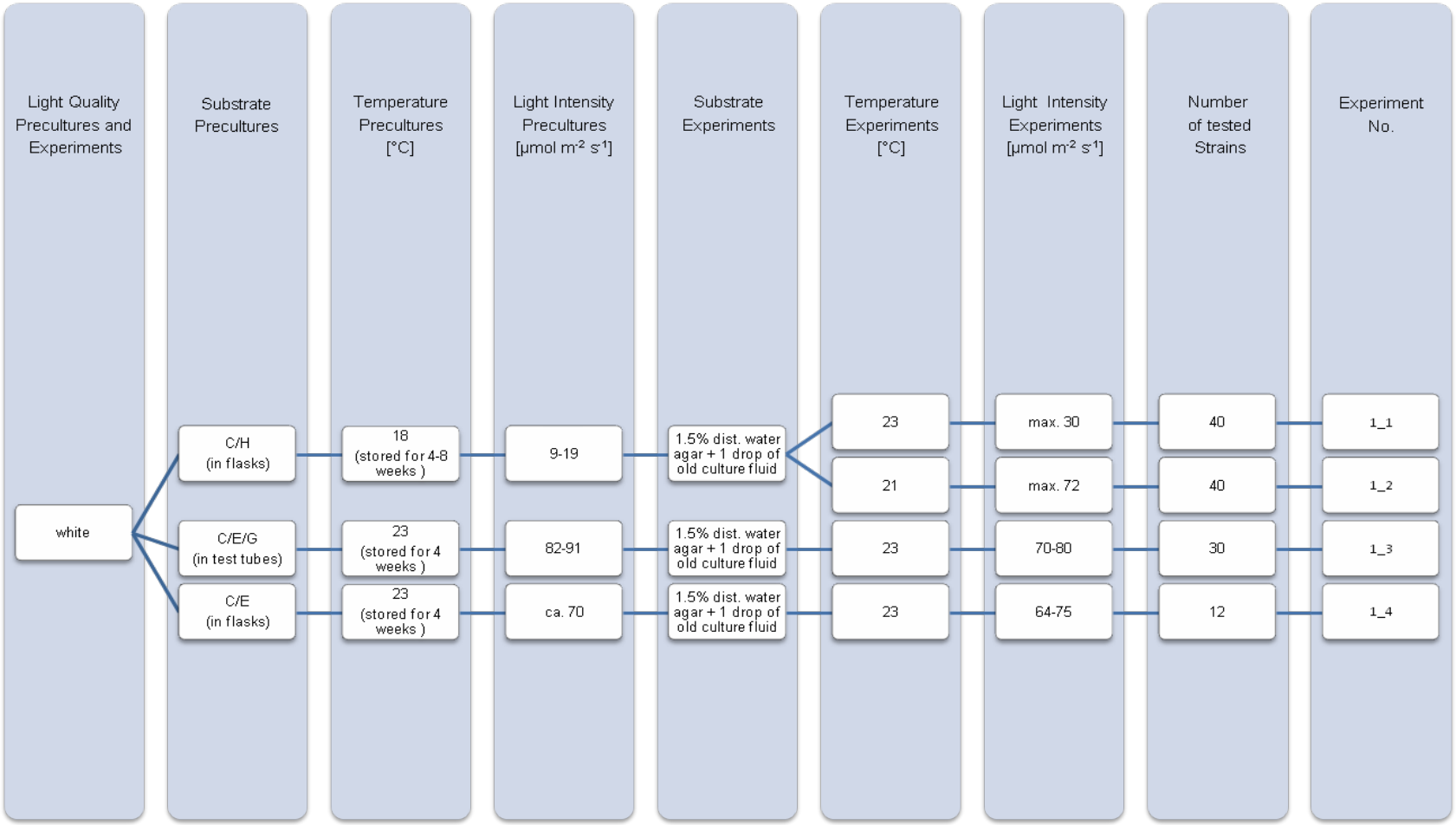

Fig. A 1. Allen Standard Method (Allen, 1958), modified and precultures included. The test duration was two weeks for each experiment. The light cycle was 16:8h in all precultures and experiments. No conjugation or zygote formation was observed.

In Fig. A2-A6, the light cycle always was 16:8h. Additional varicoloured or UV light always was provided during the 16h light phase.

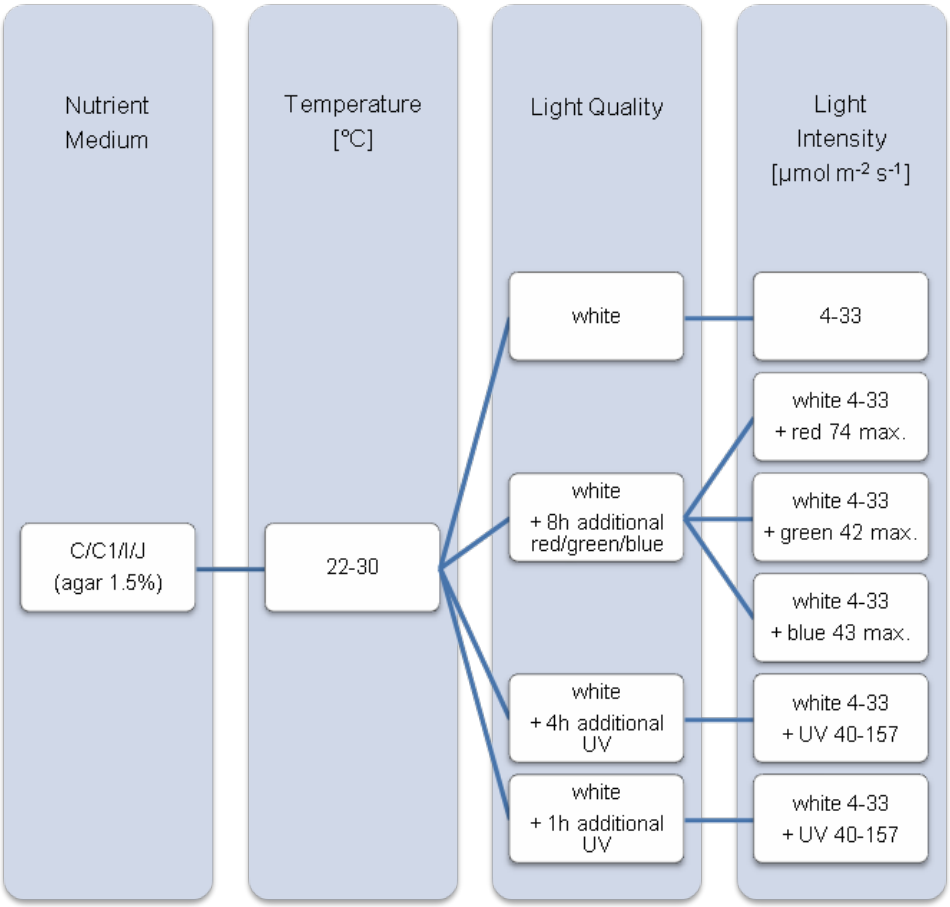

Fig. A 2. Light quality and different nutrient media. The test duration was 22 weeks. Varicoloured light was provided by white 40W standard incandescent lamps, which were filtered by red, green, or blue foil. The number of tested strains was 3. No conjugation or zygote formation was observed.

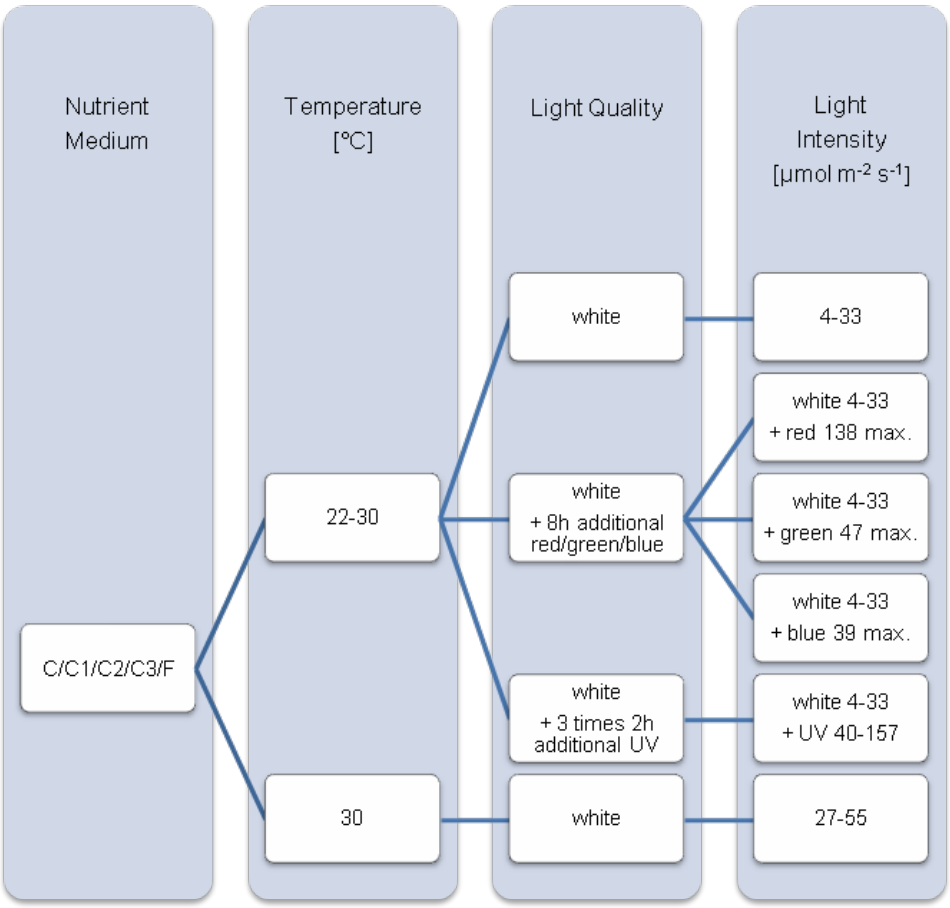

Fig. A 3. Light quality and different nutrient media. The test duration was 18 weeks. Varicoloured light was provided by 60W red, green, or blue spot light. The number of tested strains was 3. Conjugation and zygote formation was observed in 1 strain.

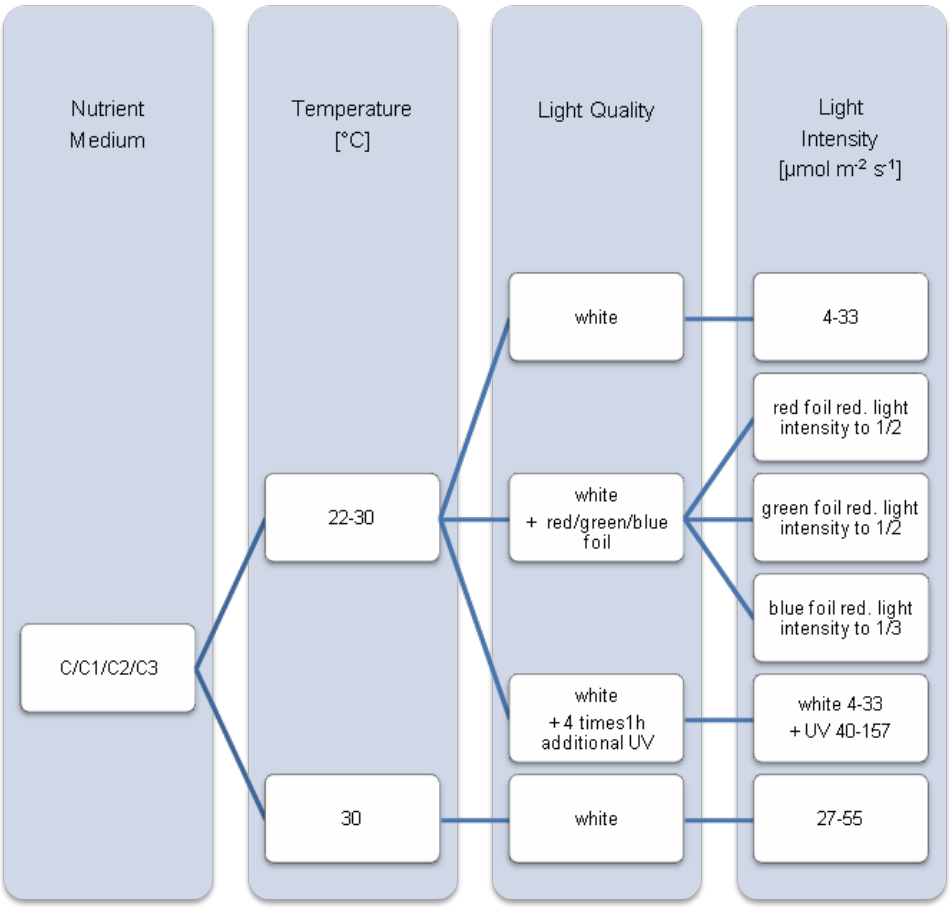

Fig. A 4. Light quality and different nutrient media. The test duration was 16 weeks. White light was continuously filtered by red, green, or blue foil during the 16h light phase. The number of tested strains was 4. Conjugation and zygote formation was observed in 1 strain.

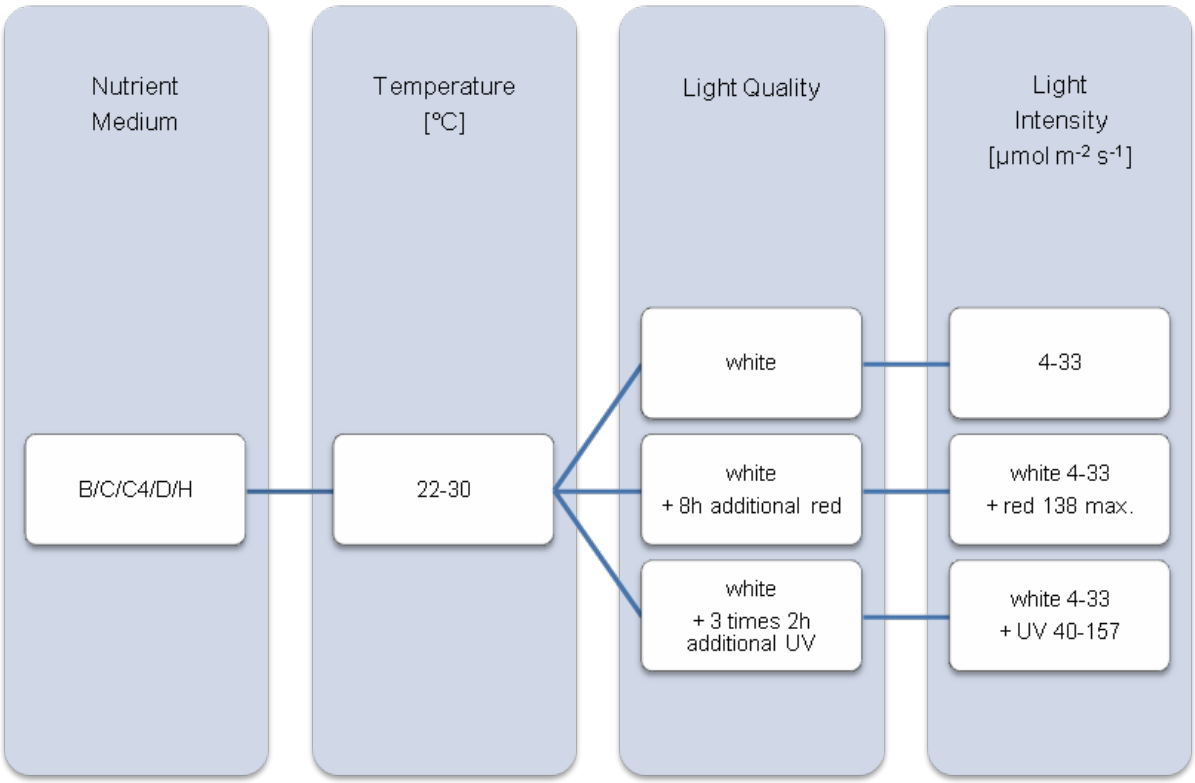

Fig. A 5. Light quality and different nutrient media. The test duration was 15 weeks. Red light was provided by 60W red spot light. The number of tested strains was 4. Conjugation was observed in 1 strain, but no zygote formation occurred.

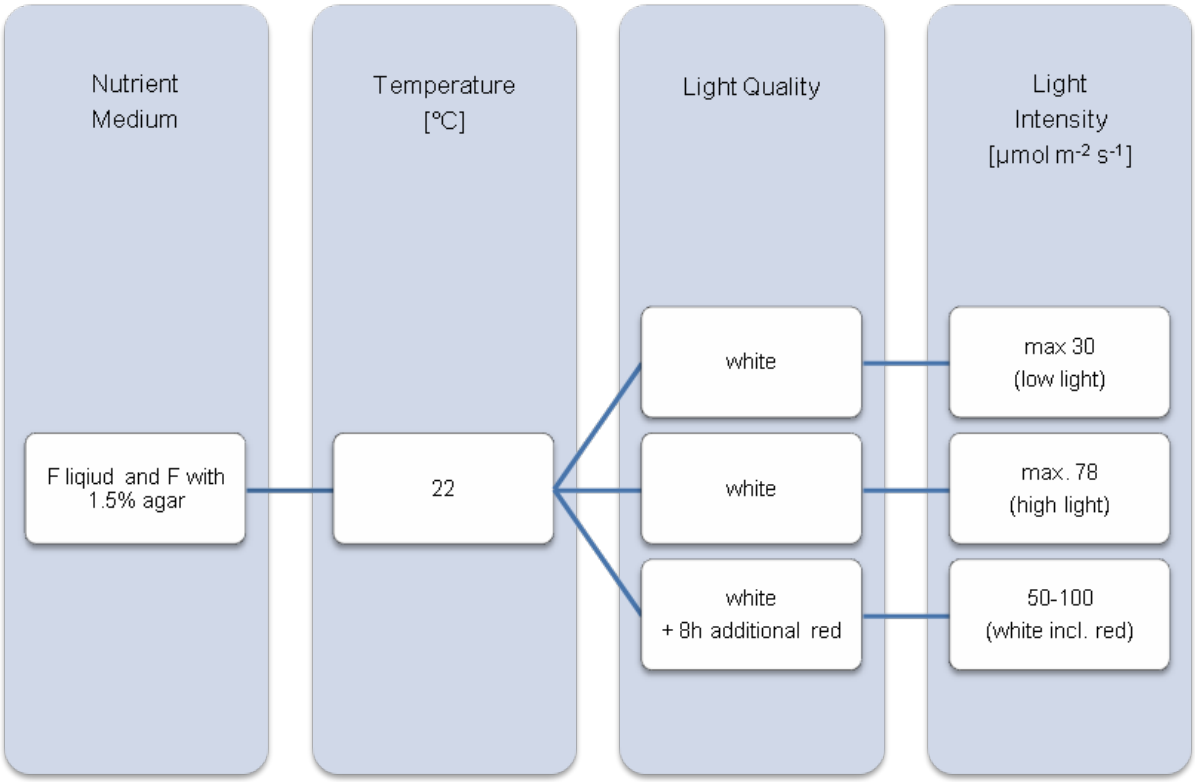

Fig. A 6. Light quality and light intensity. The test duration was 3 weeks. Red light was provided by white a 40W standard incandescent lamp, which was filtered by red foil. The number of tested strains was 15. Conjugation and zygote formation was observed in 2 strains.

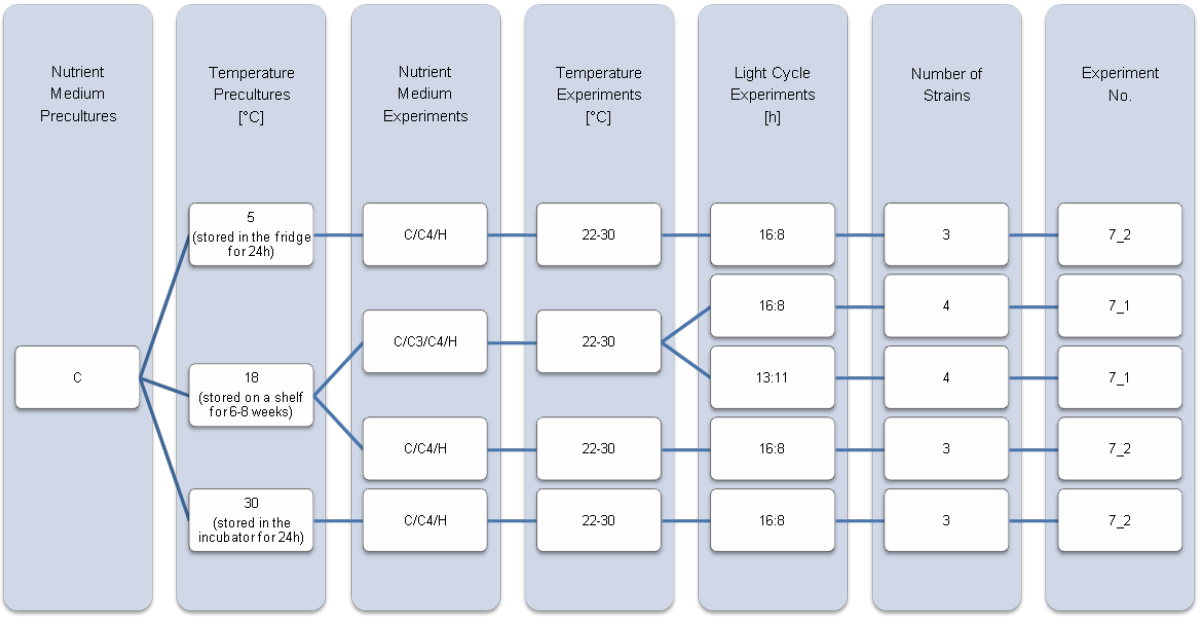

Fig. A 7. Influence of temperature fluctuation and day length, precultures included. The test duration was 6 weeks. The light intensity was 4-33 µmol m^{-2} s^{-1} (white light). No conjugation or zygote formation was observed.

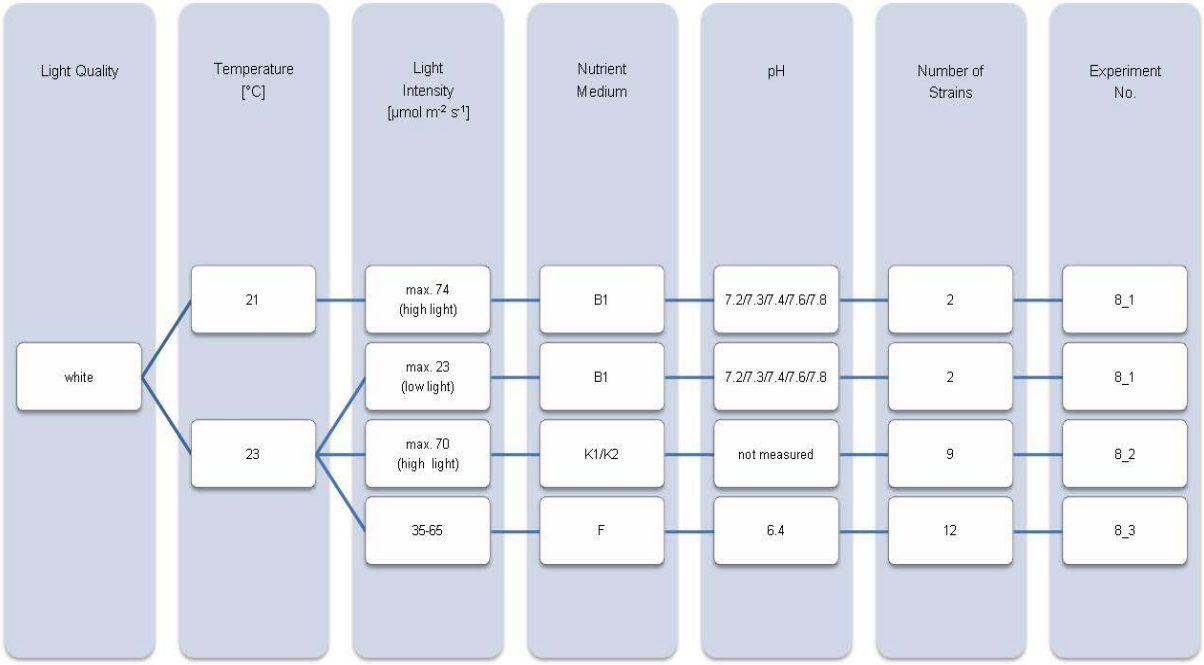

Fig. A 8. Light intensity, pH, and mixing of strains. The test duration was 2 weeks for each experiment. The light cycle was 16:8h in each experiment. The pH for nutrient medium B1 was adjusted using a Na_2HPO_4/citric acid buffer. In experiment 8_3, 2 different strains at a time were mixed and put into a single petri dish. Conjugation was observed in 1 strain during experiment 8_1, and conjugation as well as zygote formation occurred in 1 strain during experiment 8_3.

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Appendix B) Nutrient Media

A) Basal culture medium (Yamashita and Sasaki, 1979)

	mg l^{-1} dH_2O	
KNO_3	200	
$MgSO_4*7H_2O$	10	
KH_2PO_4	20	
$FeSO_4*7H_2O$	5	
agar		3%

The pH was adjusted to 7.0 or 7.5 using a Na_2HPO_4/citric acid buffer.

B) Czurda Medium (Allen, 1958)

	mg l^{-1} dH_2O	
KNO_3	100	
K_2HPO_4	10	
$MgSO_4$	10	
EDTA solution		1 ml
$CaSO_4$	5	

Starr iron sequestrine (EDTA) solution (Allen, 1958)

	g per 500 ml dH_2O
EDTA	2.61
$FeSO_4*7H_2O$	2.49
1 N KOH	27

B1) Phosphorus depleted Czurda Medium (Allen, 1958)

Same composition as medium B, but devoid of K_2HPO_4.

C) Desmids (Kusel-Fetzmann and Schagerl, 1993)

	mg l^{-1}	
KNO_3	100	
$(NH_4)_2HPO_4$	20	
$MgSO_4*7H_2O$	20	
CaSO4*2H2O	15	
FeIIINaEDTA	33	
vitamin stock		1 ml
sterile bog water		200 ml
dH_2O		800 ml

Vitamin stock solution

	mg l^{-1} dH_2O
vitamin B12	0.005
vitamin B1	0.5

C1) Nitrogen depleted Desmids

Basically the same composition as medium C, but sterile bog water and KNO_3 were omitted and $(NH_4)_2HPO_4$ was substituted for K_2HPO_4.

C2) Phosphorus depleted Desmids

Basically the same composition as medium C, but sterile bog water was omitted and $(NH_4)_2HPO_4$ was substituted for NH_4Cl.

C3) Desmids x 2

The concentration of all components of medium C was doubled.

C4) Nitrogen depleted Desmids x 2

The concentration of all components of medium C1 was doubled.

D) Godward (Allen, 1958)

	mg l^{-1} dH_2O
KNO_3	250
$MgSO_4$	80
Na_2SO_4	58
$Ca(NO_3)_2$	20
K_2SiO_3	2.7
K_2HPO_4	28
$CaCO_3$	10

E) Pringsheim's culture medium with soil extract added (Ueno and Sasaki, 1978)

mg l^{-1} dH_2O		
KNO_3	200	
$(NH_4)_2HPO_4$	20	
$MgSO_4*7H_2O$	10	
$CaCl_2$	0.5	
$FeCl_2*6H_2O$	0.5	
Jüttner trace metal mix		1 ml
soil extract		20%

Jüttner trace metal mix (Kusel-Fetzmann and Schagerl, 1993)

	mg l^{-1} dH_2O
H_3BO_3	30.1
$MnCl*4H_2O$	20
$ZnSO_4*7H_2O$	2.9
$NaMoO_4*2H_2O$	4.8
$CuSO_4*5H_2O$	0.5
$CoCl_2*6H_2O$	0.5

Soil extract

Boil 1 kg of soil in 1 litre of distilled water for 60 minutes and leave it in the dark for 2 days. Filter the extract and dilute to 2/3. Add to culture medium at a final concentration of 20%.

The pH of the culture medium was adjusted to 7.2 using 1N NaOH.

F) Reichert/Grote (Simons et al., 1984)

	mg l^{-1} dH_2O	
Na_2EDTA	25	
K_2HPO_4	20	
$MgSO_4*7H_2O$	10	
$FeSO_4$	10	
$CaSO_4*2H_2O$	50	
$NaHCO_3$	200	
HEPES	1000	
Jüttner trace metal mix		1 ml
Desmids vitamin stock		1 ml

G) Utex soil water medium (adaptation of Pringsheim, 1946)

Put 2-3 teaspoons garden soil in 200 ml distilled water. (No supplements are added.) Steam for 3h on each of 2 consecutive days.

H) Woods Hole (Simons et al., 1984)

	mg l^{-1} dH_2O	
$CaCl_2*2H_2O$	36.76	
$MgSO_4*7H_2O$	36.97	
$NaHCO_3$	12.6	
K_2HPO_4	8.71	
$NaNO_3$	85.01	
$Na_2SiO_3*9H_2O$	28.42	
Na_2EDTA	4.36	
$FeCl_3*6H_2O$	3.15	
HEPES	500	
Jüttner trace metal mix		1 ml
Desmids vitamin stock		1 ml

I) Distilled water with supplements

1 g l^{-1} $NaHCO_3$ and 10 ml l^{-1} $CaSO_4$ (from Desmids stock solution, which contains 1.5 g $CaSO_4*2H_2O$ per 100 ml), were used as supplements. The osmolality was 25 mOsm kg^{-1} of solution, which was determined via freezing point depression with the micro - osmometer "The Advanced™ 3MO plus" (Advanced Instruments Inc., Norwood, Massachusetts, U.S.A.).

J) Tap water without supplements

K) Autoclaved water from 2 sampling localities, which was diluted to 50% with distilled water.

L) Allen standard substrate (Allen, 1958)

1.5% distilled water agar+1 drop of old culture fluid

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Yamashita, T., Sasaki, K., 1979. Conditions for the induction of the mating process and changes in contents of carbohydrates and nitrogen compounds during the mating process of *Spirogyra*. J. Fac. Sci. Hokkaido Univ. Ser. 5, 279-287.

Appendix C) Emission Spectra

All light spectra were established with the USB 400 Fiber Optic Spectrometer using the Spectra Suite Spectrometer Operating Software.

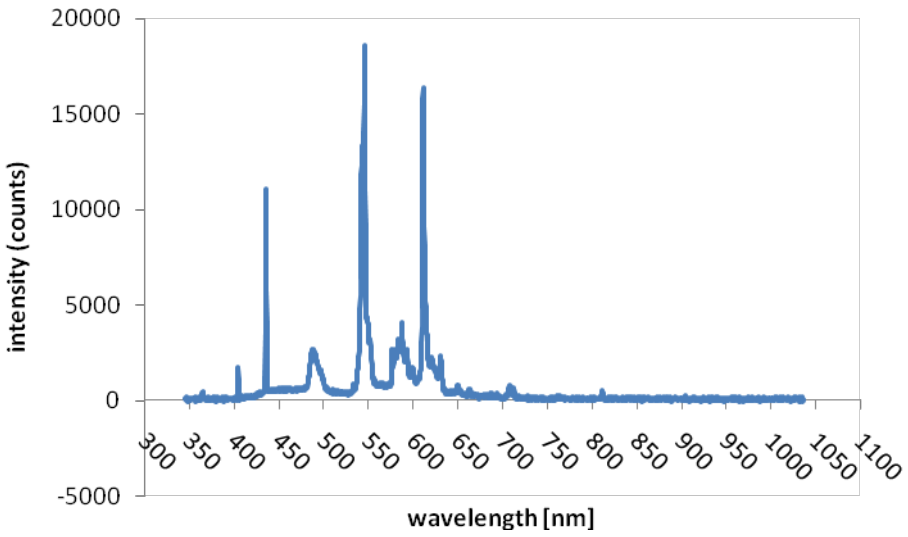


Fig. C 1. Philips TLD 36W/33 cool white fluorescent tubes, used in precultures.

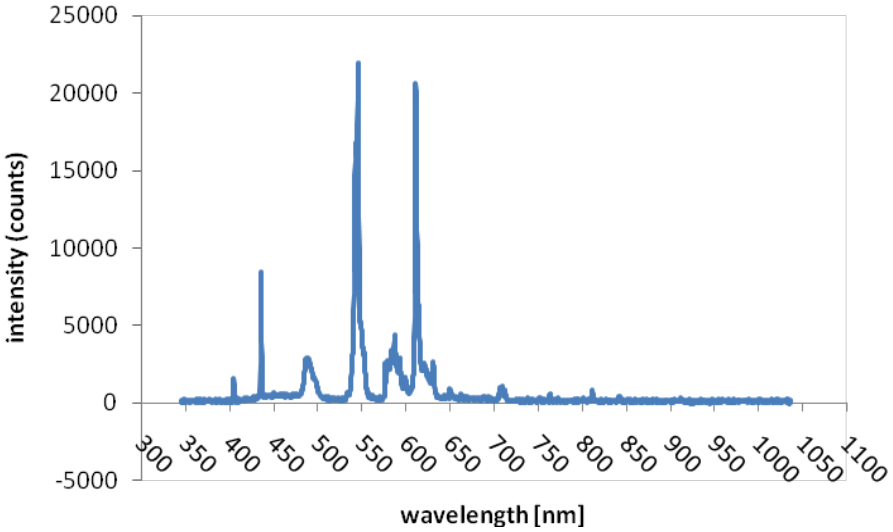


Fig. C 2. Osram FQ 39W/840 HO LUMLUX cool white fluorescent tubes, used in mating experiments.

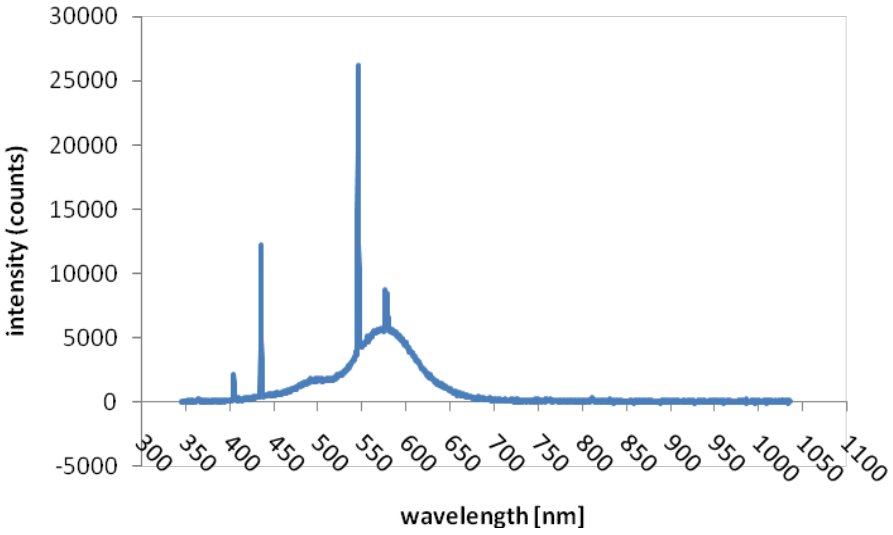


Fig. C 3. Cool white fluorescent tubes in the SANYO MLR-350 Versatile Environmental Chamber.

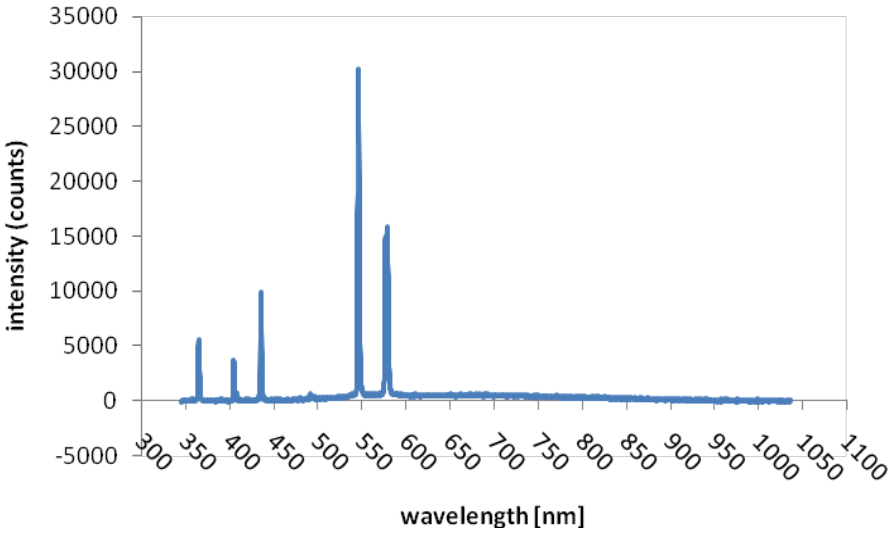


Fig. C 4. 300W Osram Ultra-Vitalux sunlamp.

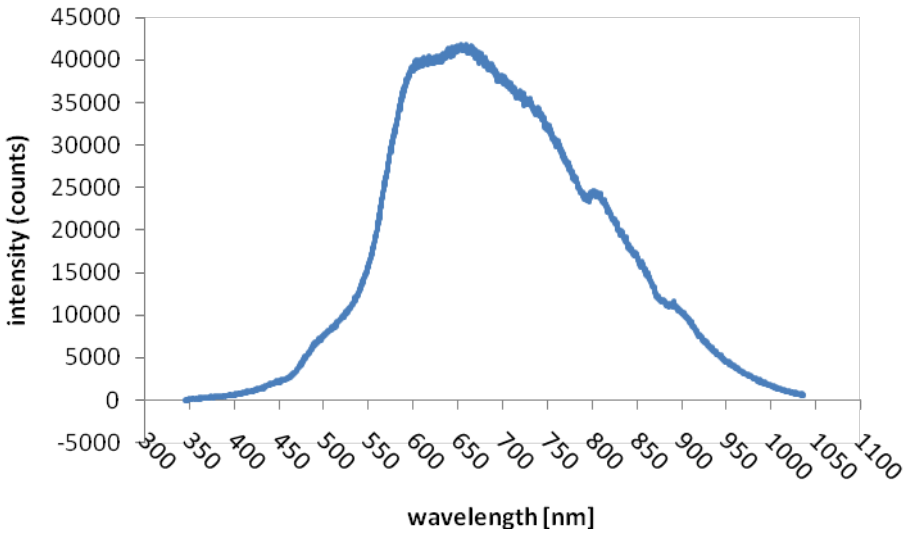


Fig. C 5. 40W Voltolux white standard incandescent lamp, filtered by red foil.

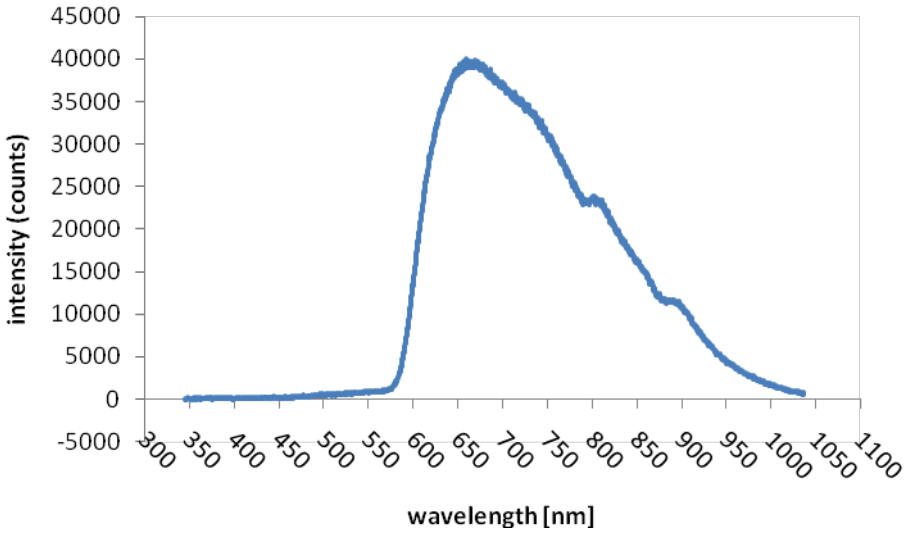


Fig. C 6. 60W Philips red spot light.

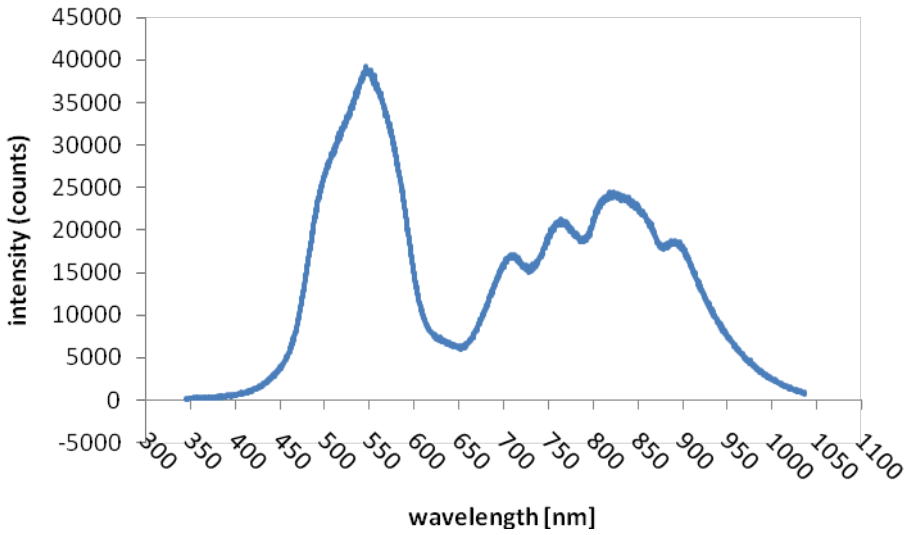


Fig. C 7. 40W Voltolux white standard incandescent lamp, filtered by green foil.

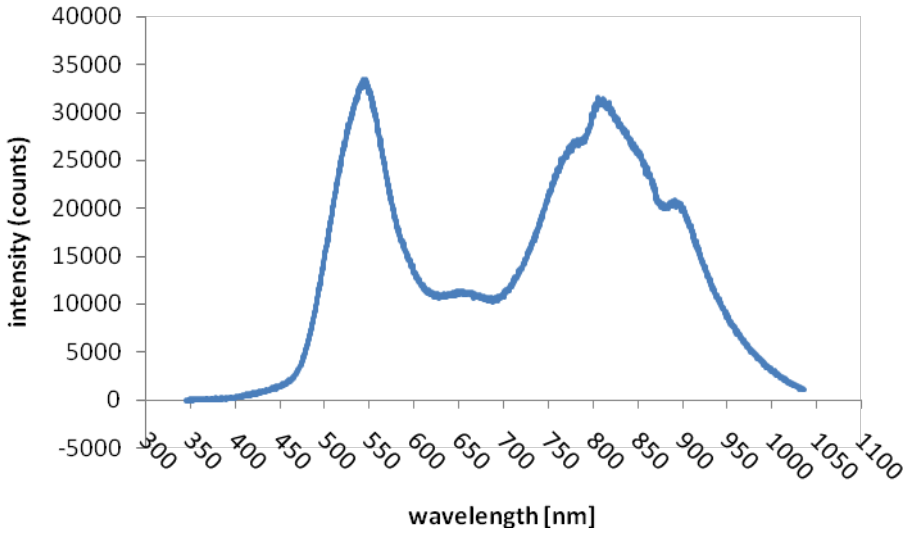


Fig. C 8. 60W Philips green spot light.

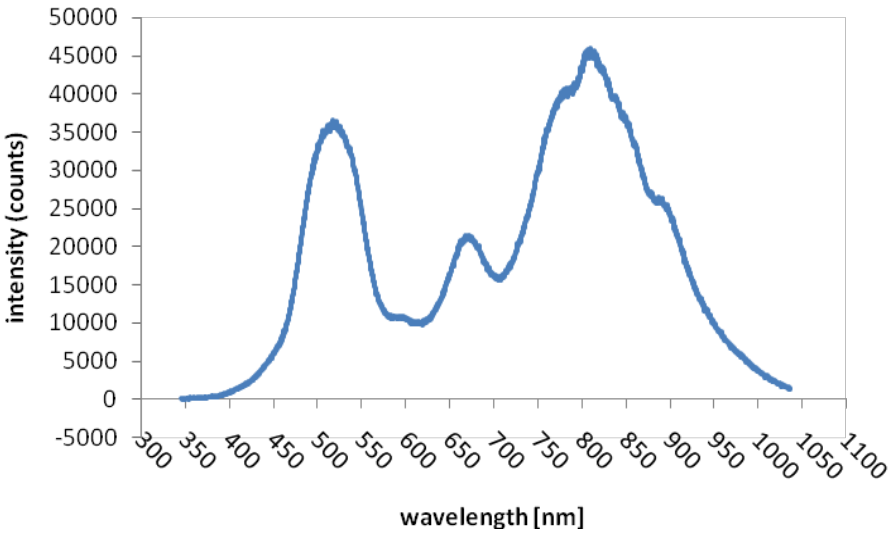


Fig. C 9. 40W Voltolux white standard incandescent lamp, filtered by blue foil.

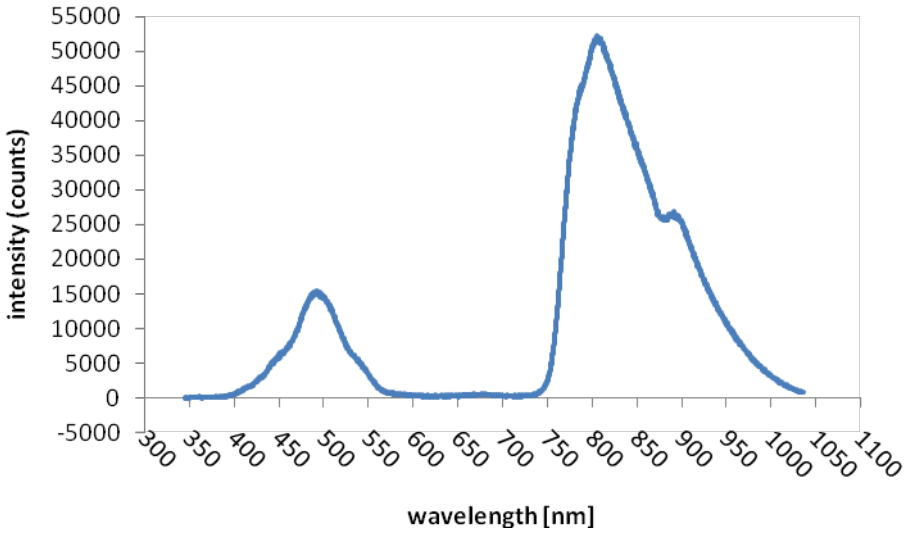


Fig. C 10. 60W Philips blue spot light.

Zusammenfassung

Die Artabgrenzung bei der Süßwasseralge *Spirogyra* (Zygnemataceae, Streptophyta) erfolgt traditionell aufgrund morphologischer Unterscheidungsmerkmale, wobei das Vorhandensein sexueller Stadien des Lebenszyklus vonnöten ist. Da sexuelle Reproduktion im Freiland nur selten beobachtet wird, ist es von Bedeutung diesen Prozess im Labor unter kontrollierten Bedingungen zu initiieren. Daher wurden 95 *Spirogyra* Stämme, die von diversen Standorten hauptsächlich in Mitteleuropa stammten, wechselnden Umweltfaktoren ausgesetzt, um ihr Potenzial für sexuelle Fortpflanzung zu testen. In insgesamt 681 Versuchsanordnungen. wurden dabei vor allem Nährstoffverfügbarkeit und Lichtbedingungen, aber auch andere Parameter wie Temperatur oder pH Wert variiert und die Ergebnisse mit im Freiland erhobenen Daten verglichen. Die Versuchsergebnisse zeigten kein klares Muster bezüglich der für die Auslösung der sexuellen Phase bei *Spirogyra* verantwortlichen Faktoren. Es ist jedoch wahrscheinlich, das eine Verschiebung bestimmter Nährstoffverhältnisse, abhängig von autökologischen Ansprüchen, eine wichtige Rolle spielt.

Außerdem wurden 16 Spirogyra Stämme von 14 Standorten mittels konventioneller Monographien bis auf die Art bestimmt. Aus den Ergebnissen sind einige Probleme im Zusammenhang mit der exklusiven Anwendung des morphologischen Artkonzeptes ersichtlich. Die kombinierte Anwendung von morphologischen, physiologischen und molekularen Methoden könnte zu einer beträchtlichen Reduktion der Artenzahl bei *Spirogyra* (derzeit sind 386 Arten beschrieben) führen.

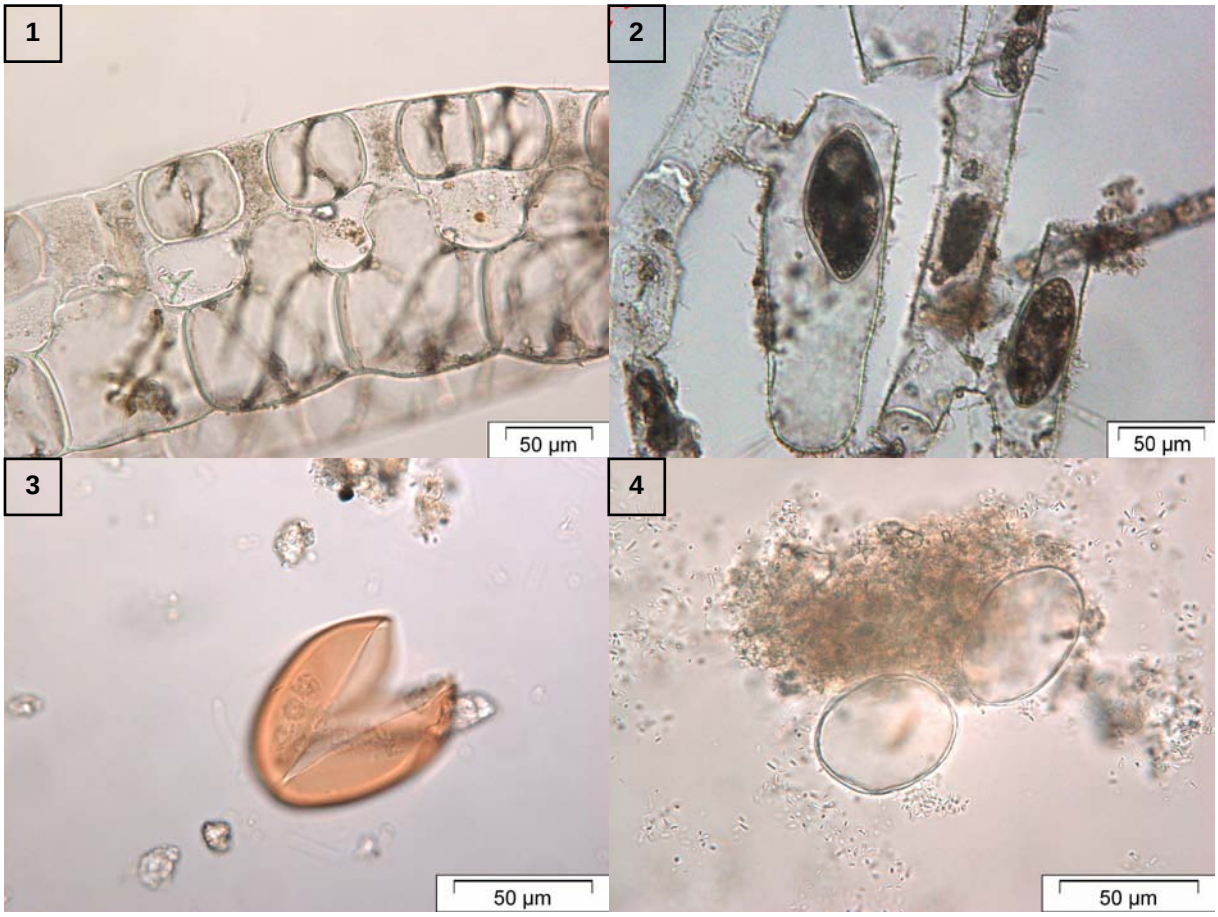
Summary

According to traditional monographs, species delimitation within the ubiquitous freshwater algae genus *Spirogyra* (Zygnemataceae, Streptophyta) is based upon morphology considering asexual and sexual stages of its life cycle. Since sexual reproduction is only infrequently observed in nature, an artificial onset in the laboratory would be essential for species identification. We therefore tried to induce conjugation in 95 strains of *Spirogyra* originating from diverse sampling localities mainly in Central Europe. Altogether, 681 experimental setups were conducted with a focus on variation of nutrient supply and light conditions. Results were then compared to environmental data obtained in situ. No clear pattern could be revealed concerning the conditions for sexual reproduction in the genus, although the importance of a certain nutrient ratio according to specific ecological demands seems likely.

We also identified altogether 16 Spirogyra strains from 14 sampling sites in Central Europe down to species level and then compared our observations to species descriptions in literature. Results demonstrate the many uncertainties underlying the exclusive application of the morphological species concept, which indicates that many *Spirogyra* species might have been circumscribed to narrow. On this account, a combination of several approaches, including molecular as well as morphological and physiological data, could provide useful taxonomic information and might help to resolve identification problems on the intrageneric level.

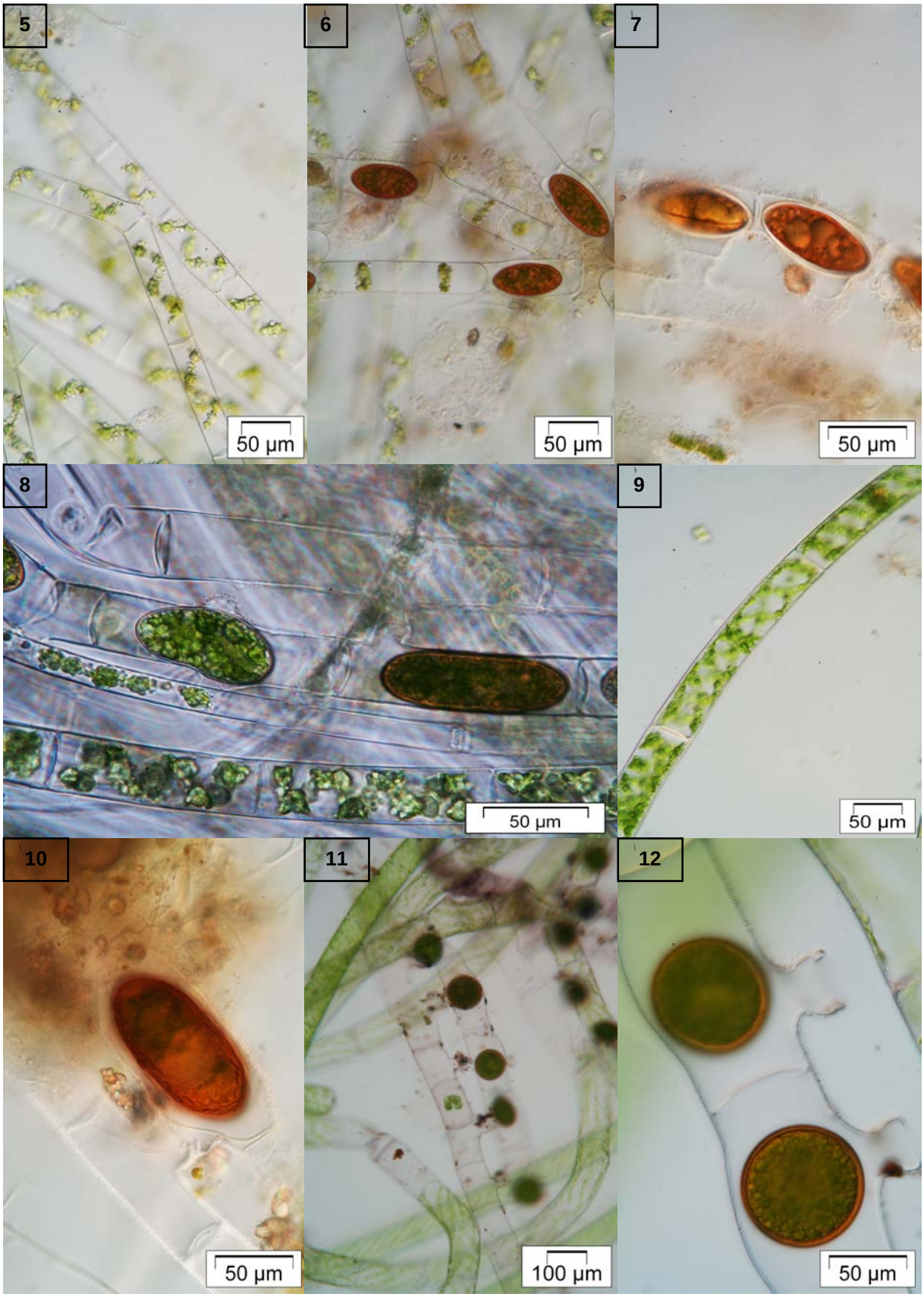
Fototafeln

Alle mikroskopischen Fotos stammen von Charlotte Chen, Roland Hainz und der Verfasserin.



1, 2: Hybridisierung zweier Filamente mit unterschiedlicher Fadendicke.

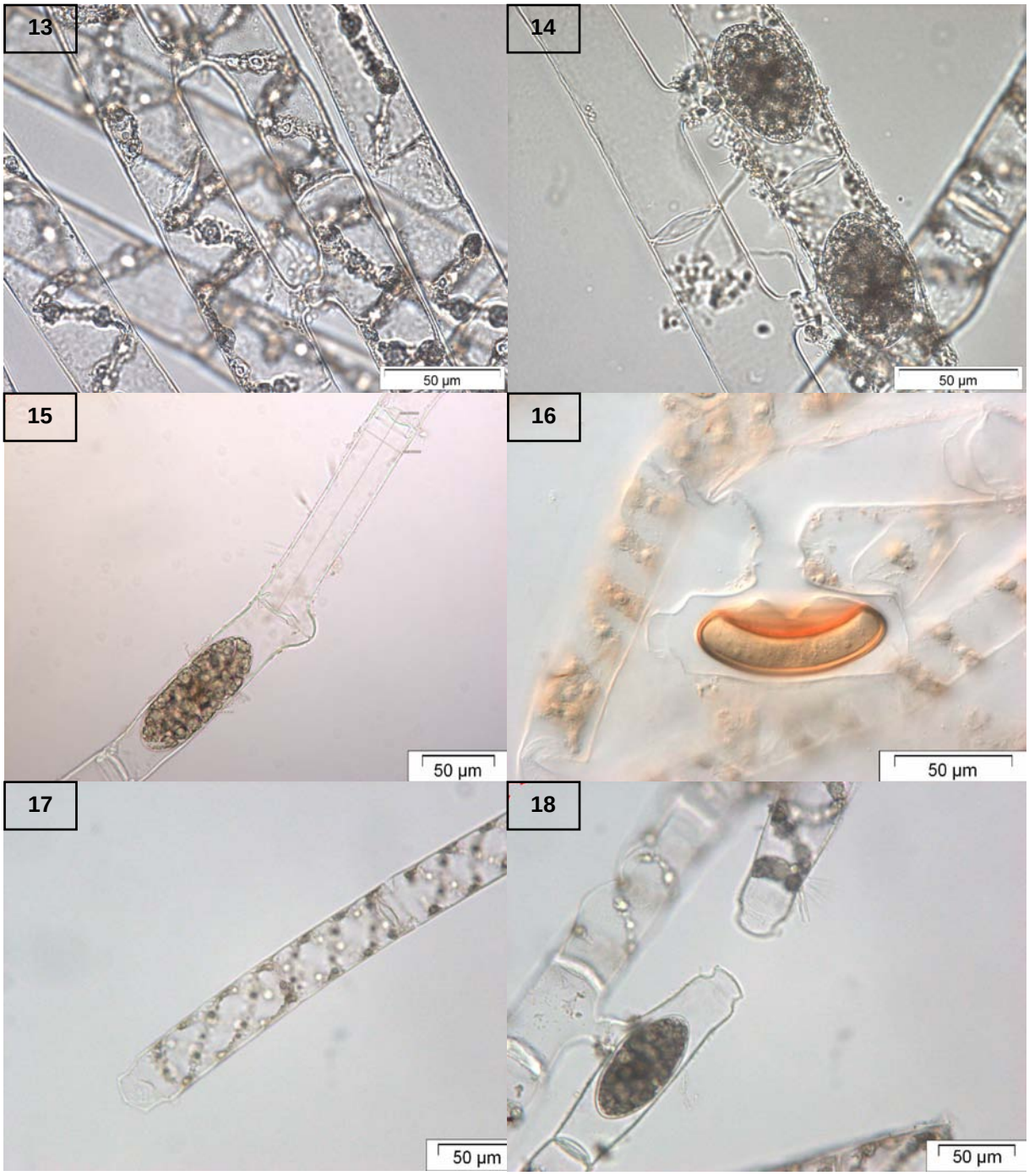
3, 4: *Spirogyra spp*., Exo- und Mesospor der Zygotenwände glatt.



5-8: *Spirogyra longata* von 2 unterschiedlichen Fundorten, leiterförmige und seitliche Konjugation.

9, 10: *Spirogyra fluviatilis*, eine Art mit zerfurchter Sporenwand.

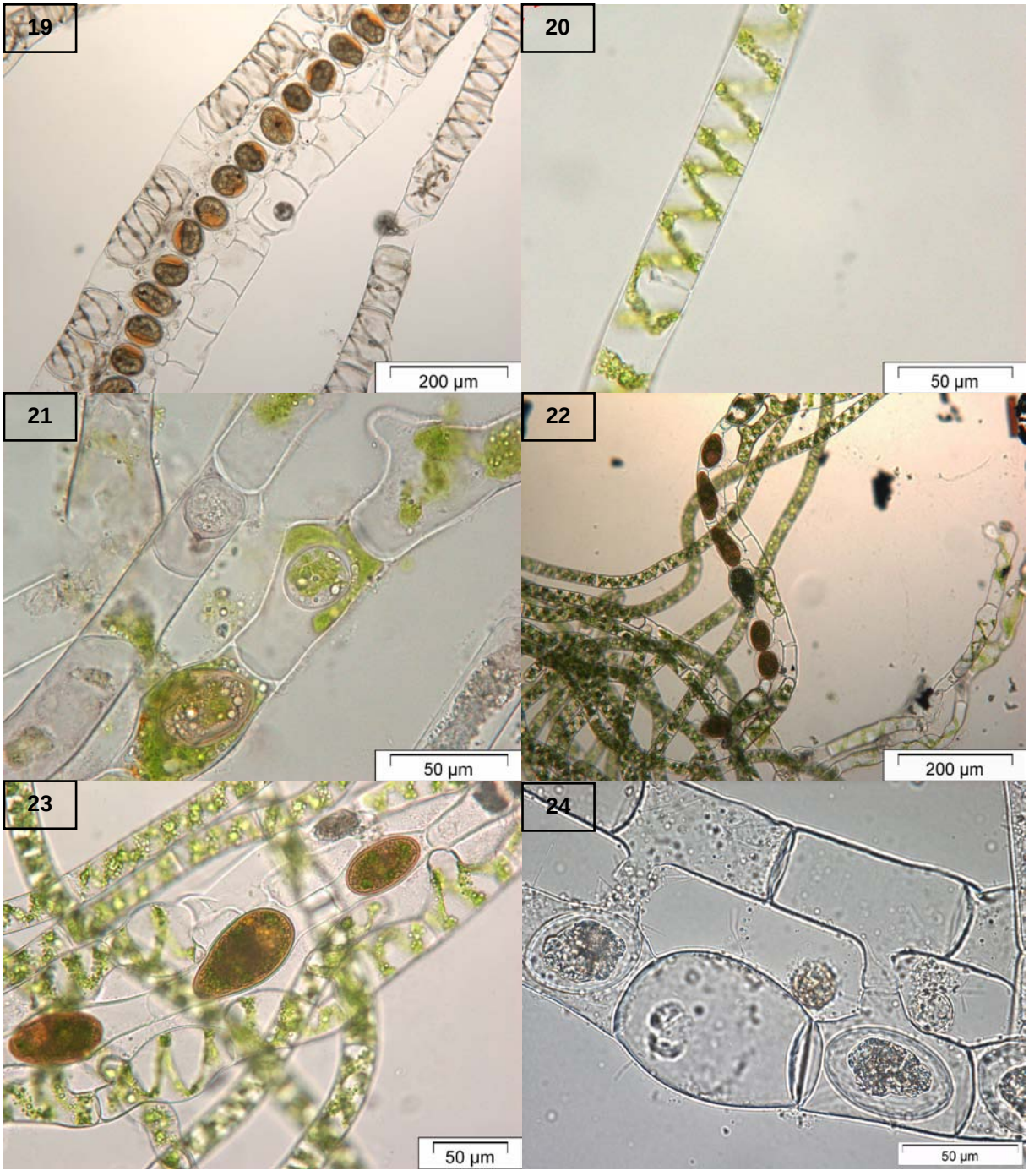
11, 12: *Spirogyra majuscula*, leiterförmige Konjugation.



13, 14: *Spirogyra velata*, Zellwand mit Vertiefungen.

15: *Spirogyra hassallii*, seitliche Konjugation.

16-18: *Spirogyra semiornata*, gefaltete Querwände der Zellen.



19: *Spirogyra neglecta*, leiterförmige Konjugation.

20, 21: *Spirogyra mirabilis*, Bildung von Parthenosporen.

22, 23: *Spirogyra polymorpha*, Zygoten desselben Filaments unterscheiden sich merklich in Form und Größe.

24: *Spirogyra varians*, sterile Zelle im konjugierenden Filament stark aufgetrieben.

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