Newsletter of the Community of Duckweed Research and Applications, edited by the ISCDRA

DUCKWEED FORUM

ISCDRA
International Steering Committee on Duckweed Research and Applications

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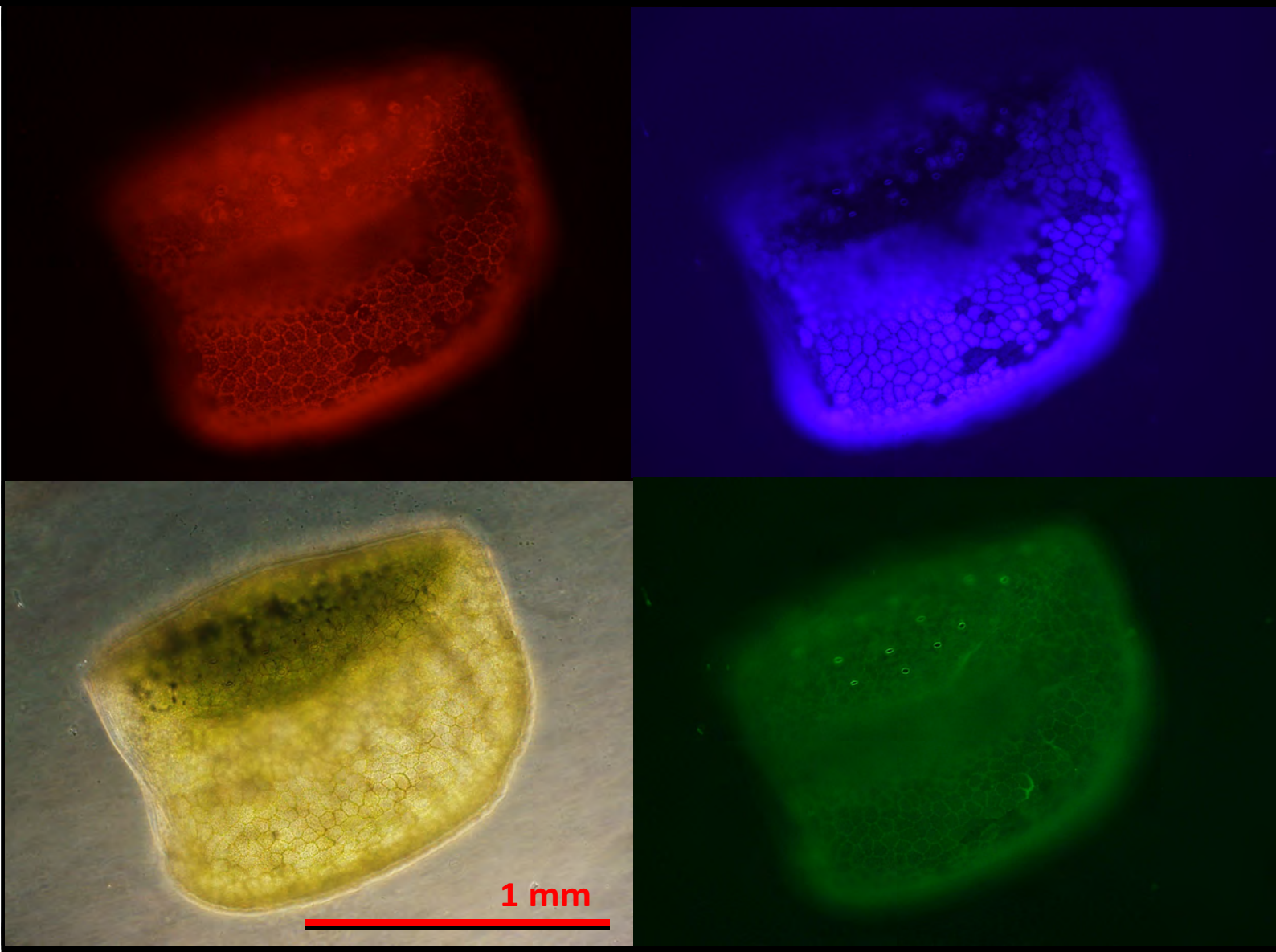


Clockwise from upper left: *Wolffia cylindracea* 9056 photographed with red, blue, and green filters for fluorescence emission under different excitation wavelengths; and under transmission light (lower left).

Cover page

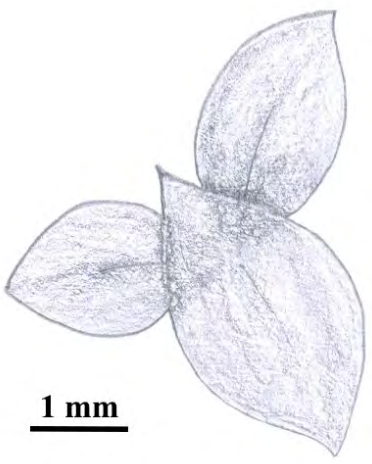
The cover for this issue of the Duckweed Forum features the final remaining species ***Wolffia cylindracea*** (clone 9056 from Zimbabwe, Africa). This is the last of the 37 known species in the Lemnaceae family that have been featured on the cover of our newsletter. To highlight the various chemical components in the cells of a duckweed plant, we used fluorescence microscopy that utilize different colors of light to excite the plant and capture the fluorescence light that is then emitted through the use of appropriate filters. Clockwise from top left: excitation at 530-550 nm and emission at 575 nm and above to visualize chlorophyll fluorescence; excitation at the ultraviolet range of 360-370 nm and emission of blue light at 420-460 nm to visualize phenolic compounds typically associated with the cell wall of plants; excitation in the blue region of 460-495 nm and emission in the green region of visible light at 510-550 nm to visualize other compounds in plants. Note the particularly strong green fluorescence observed on the edges of guard cells that facilitate gas exchange. *W. cylindracea* has been found in African countries such as South Africa, Angola, Tanzania and Zimbabwe. While its distinction from *W. globosa* was questioned by Landolt and others, more recent genotyping results using molecular markers clearly established it as a separate group with the closest species being *W. columbiana* and *W. arrhiza* (Bog et al., 2013; Borisjuk et al. 2014). Photographs taken by Dr. Eric Lam at the Rutgers Duckweed Stock Cooperative (Rutgers University, NJ).

In this issue

The 3rd International Steering Committee on Duckweed Research and Applications Members

All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: *Lemna tenera* Kurz



Lemna tenera Kurz is a little known species of Lemnaceae, in the words of Landolt. The fronds have a curved tip which makes it easier for identification purpose. This species has a unique habitat, reported to grow in forest swamps under the shade of trees. This species has been spotted in South East Asia and in Australia. Like *Lemna trisulca*, this species of *Lemna* also grows submerged except for the time when it flowers. However, in the laboratory growth conditions, unlike *L. trisulca*, *L. tenera* can hardly maintain its submerged nature and is found floating in most of the nutrient media that are commonly used for culturing aquatic plants. Drawing by Dr. K. Sowjanya Sree, Central University of Kerala.

Letter from the Editor

Dear Duckweed Community,

The approaching autumn is evident in the turning colors of foliage here in the Northeast of the United States and reminds me that the end for this year of 2018 is rapidly approaching. I hope this year has been very good for all of you. It certainly has been a busy one in the Duckweed arena, as evident by some of the contents in this issue of the DF newsletter.

We start this issue with the second circular for our community's upcoming International Conference to be hosted at Rehovot, Israel. The official website is now in place and I encourage all of you to consider attending and contributing to this biennial event that helps to foster advances in our field through sharing of information and collaboration. This is followed with a Commentary by Todd Michael (JCVI, San Diego, USA) and I that describe the recent application of cutting edge genomics tools together with advanced flourescence microscopy to generate a new "Gold Standard" genome for the Greater Duckweed *Spirodela polyrhiza*. This new map for the basal duckweed genome should enable many applications and research by providing a high-fidelity reference map to help characterize and utilize this remarkable plant. Our committee member Tsipi Shoham then provided an updated list of current duckweed-centric developers to keep us informed of the status of commercialization efforts. While some start-ups have gone, new ones are being formed to explore other avenues. Thanks to all the individuals who provided update information to Tsipi since it is important to keep this listing as current as possible.

In this issue of the DF, we are beginning our new section of Historical Accounts with two installments: a short biography of Matthias Jacob Schleiden, who is one of the pioneers of duckweed research, by Manfred Eichhorn and a personal perspective of flowering research with duckweed by Charles F. Cleland. I hope everyone will enjoy these historical pieces as much as I have.

The Student Spotlight section in this issue is a personal account by Phuong Thi Nhu Hoang, a student of Prof. Ingo Schubert (IPK, Gatersleben, Germany) who is a main contributor for the most recent *S. polyrhiza* genome map that is featured in the Commentary section. Following this piece are two invitations: the first is an invitation for an upcoming Duckweed Workshop in the 27th Plant and Animal Genome (PAG) Conference and the second is to invite community contribution to future Cover Photos for the DF. I hope to see many participants in these efforts by our community members.

Finally, our DF issue ends with the Database section by our committee member Prof. Klaus Appenroth. Many of the articles in a special section of Frontiers in Chemistry are now online, with still more to come in the future. They collectively illustrate that the field of duckweed research is most certainly thriving once again, and new insights and technologies that can benefit our understanding of plants and Biology can result from working with this unique family.

As always, I want to thank all the contributors to this issue of DF and wish everyone the very best for the remaining months in 2018.

Eric Lam, Chair of the ISCDRA

5th ICDRA: 2nd Circular

5th International Conference Duckweed Research & Applications

9-12 September, 2019

Weizmann Institute of Science, Rehovot, Israel

PLENARY & LECTURE TOPICS - Advances in duckweed genomics & molecular biology, ecosystems & ecotoxicology, microbe interactions, natural products, biomass production, biofuels and other commercial applications.

SPOTLIGHT ON POSTERS - All delegates (including speakers) are urged to prepare and bring a poster. Those doing so will benefit from a reduction in registration fee. Posters will remain up for the entire conference period. A **Poster Lecture Session** will be held and a **Best Poster Prize** will be awarded. Details for poster preparation on the Conference web site (to be available soon).

VENUE - Conference Centre at the Weizmann Institute of Science (WIS). WIS is located in a lushly landscaped campus in the university town of Rehovot, 25 min. from Tel Aviv and 50 min. from Jerusalem. Accommodations for convention delegates are available on campus and at a leading international hotel located next to campus.

REGISTRATION FEE

Academia (research, teaching)	$400
Postdocs & students	$250
Non-Academia (commercial)	$600
Accompanying persons	$250

Registration fee rebate of $100 cash (in Israeli currency) for conference delegates submitting an abstract and displaying a poster.

Fee includes: admission to all sessions, conference kit, coffee breaks, lunch and dinner on conference days, half day trip, transport to and from airport.
Registration closes on 9th July, 2019 ($50 penalty for late registration).

ABSTRACTS - See Registration Link. **Abstract submission deadline: 3rd July, 2019** (Late abstracts might not make it into the Conference abstract book).

ACCOMODATIONS

	per night
WIS Faculty guest house	$ 80
HUJ Guest house	$110
Leonardo Boutique Hotel	$200

All accommodations are within a 10 min stroll to conference hall.

Reservation details soon to be available on the Conference web site.

TRAVEL - There are a multitude of direct and connecting international flights to Israel's Ben-Gurion International airport (TLV). See https://www.touristisrael.com/full-list-flights-tel-aviv-israel/12331/

QUESTIONS? - Please email **Inbal** at the Conference Secretariat: inbal.azoulay@weizmann.ac.il

REGISTRATION LINK for ICDRA 2019 Duckweed Conference

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The *Spirodela* genome gets a face lift

Todd Michael[1] and Eric Lam[2]

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The *Spirodela* genome got a face lift recently (Hoang et al., 2018). The 158 megabase (Mb) *Spirodela* genome is the highest quality duckweed assembly published to date, which should greatly facilitate research, discovery and industry in this important aquatic plant family. Most current sequencing technologies generate sequencing reads much shorter than the chromosomes, so assembling a genome is like putting together a huge jigsaw puzzle with millions of pieces. Hopefully, the A, T, C and Gs are arranged in the proper order and organized into the correct chromosomes. However most genomes to date, including those produced for *Spirodela polyrhiza* over the past several years, still have missing sequences and improperly assembled regions. Now, research teams led by Ingo Schubert and Eric Lam leveraged two independent technologies to validate the structure of the *Spirodela* genome and filled in much of the missing sequences, which resulted in a greatly improved *Spirodela* reference genome for the duckweed community (Hoang et al., 2018).

S. polyrhiza, the Greater Duckweed, was chosen for initial genome sequencing because it has the smallest genome in the Duckweed family at ~158 Mb (Wang et al., 2011). A later survey of 38 clones of this species resulted in an average genome size of 160±3 Mbp/1C±SD (Bog et al., 2015) demonstrating very low variation. The *Spirodela* accession 7498 (Sp7498) was sequenced under the Department of Energy Joint Genome Institute (DOE JGI) Community Sequencing Project (CSP) using a combination of 454, fosmids and BAC-end (Bacterial Artificial Chromosomes) sequencing. 454 sequencing produces reads between 200-700 basepairs (bp) long with some level of systematic per base error, which forms the basis of the initial assembly that is fragmented and did not result in 20 chromosome models. BACs are generally much longer ranging from 50-150 kilobases (kb) and only the 800-1,000 bp of high quality (low error) is sequenced from the beginning and end of each insert (ends). BAC-ends are then used as an orthogonal technology to create a physical map of the genome, which takes the fragmented pieces of the 454-based assembly and organizes as well as orients them. The resulting assembly covered 90% of the genome in 32 pseudo-molecules, which is close to the 20 chromosomes, and 10.7% Ns, where bases could not be assembled or ordered (Wang et al., 2014). The assembly enabled the discovery that *Spirodela* has the fewest protein coding genes of any known flowering plant.

In a later attempt to improve the *Spirodela* genome Michael et al., (2017) sequenced and assembled a second accession of *S. polyrhiza* 9509 (Sp9509) using a combination of high quality short (100 bp) Illumina reads and novel single-molecule BioNano optical maps. The resulting assembly covered 87% of the predicted genome size in 20 chromosomes with only 8% Ns, which enabled the accurate identification of 10% more of the repeat content (retrotransposons) in the genome as compared to the Sp7498 assembly (Michael et al., 2017). The higher resolution of the repeat sequences not only enabled a proper count of full length long terminal repeat (LTR) retrotransposons but also the estimation of the solo:intact ratio, which is the ratio of degraded or purged LTRs (solos) versus full length LTR elements (intact). This work revealed that *Spirodela* has the highest solo:intact ratio of any plant tested to date, which indicates that it is actively purging jumping LTRs to maintain its small genome size. This finding is consistent with the reduced protein coding gene content as well as the

new finding in this study that it also has a greatly reduced ribosomal DNA (rDNA) array consistent with a minimized genome and fast growth. In addition, the Sp9509 genome enabled the discovery that *Spirodela* has the lowest DNA methylation level of known flowering plants, specifically in protein coding regions that have been recently duplicated, control growth and photosynthesis, and have very low sequence variation as compared to another accession of *S. polyrhiza* (Sp7498). In general, *Spirodela* has a very low level of variation, as has been recently corroborated and extended by a large-scale population sequencing study (Xu et al., 2018).

In the recent update to the *Spirodela* genome, Hoang et al., (2018) leveraged two distinct technologies to validate and further improve the chromosome scale assembly of the *Spirodela* genome, demonstrating that using multiple orthogonal technologies is critical to generate a high-quality reference genome. First, as in previous work that utilized multicolor fluorescence *in situ* hybridization (mcFISH) to resolve the 32 Sp7498 pseudo-molecules into 20 chromosomes (Cao et al., 2016), this method was used to correct and update the 20 Sp9509 chromosomes using additional FISH probes to increase resolution. Second, we leveraged Oxford Nanopore Technology (ONT) single molecule, long read sequence to assemble the most complete *Spirodela* genome to date. In conjunction with the mcFISH data, the new ONT assembly identified seven chimeric pseudomolecules and revealed assembly errors in the previous maps. The resulting Sp9509 assembly was the most complete and accurate genome to date with few gaps ("Ns"). We anticipate that this new version of the Sp9509 genome will serve as a gold standard for Lemnaceae genome research to come, in addition to serve as an excellent example of the rapid advances that can be made from collaborative efforts using independent methods. In this new age of genome editing and synthetic biology, a chromosome-resolved and validated genome assembly, which has all bases fully covered, is a necessary starting point for the systematic engineering of the Lemnaceae genome to tap its full potential as a new crop platform.

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Practical Applications of Duckweed: Challenges and Opportunities

On a mission to introduce duckweed as the 21st century cash crop: An update on current active industrial efforts

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Dear readers,

In our previous newsletters' issue 22, we have provided under this section a review on active industrial efforts that are on the way to realize the potential of duckweeds as promising platforms for various practical applications.

As part of this review, we listed currently active industrial entities that are leading breakthrough technologies towards duckweed based new products and/or services.

Since this presented list was by no means final and aimed to extend our dialogue, I was very happy to receive your feedback and valuable input.

Accordingly, below you may find an updated table with current active companies and its subsequent figure. It is notable that the lead field of application is “Human food” and that the highest concentration of operating companies is in the USA

I hope that this section will continue to be a “living publication” providing further updated information to the benefit of you all.

Table 1: Companies that develop duckweed based new products and applications (Sorted per application, and by an alphabetical order within each application). It is notable that the lead field of application is “Human food”.

	Company Name	Field of application	Focused on	Country	Website
1	ABC Kroos	Human food	Plant-based protein ingredients	The Netherlands	http://abc-kroos.nl
2	Aquible/ Plantible Foods	Human food & pet food	Plant-based protein & fiber ingredients	The Netherlands /USA	http://aquible.com

3	Bioponica	Human food	Powder nutrient supplement	USA-	http://bioponica.net/growing-duckweed/; http://bioponica.net
4	Hinoman	Human food	Plant-based protein ingredients	Israel	www.hinoman.biz
5	GreenOnyx	Human food	Fresh green vegetables	Israel	https://www.greenonyx.biz
6	OxyGenesis GmbH	Human food	Plant-based protein ingredients	Germany	https://www.oxygenesis.de/en
7	Parabel	Human food	Plant-based protein ingredients	USA	http://www.parabel.com
8	Aqua Light Ltd.	Water treatment & Air treatment	"Symbiofilter" for purifying air or water	Germany	https://symbiofilter.de/?page_id=77
9	Lyndon Water	Water treatment	Integrating water treatment with aquaculture	UK	http://www.lyndonwaterafrica.com
10	Sulabh Social Service Organization	Water treatment	Local waste water treatment with direct economic returns from pisciculture	India	http://www.sulabhinternational.org/duckweed-based-waste-water-treatment/
11	Agravis Raiffeisen GmbH	Animal feed	Raw material for animal feed	Germany	https://geschaeftsbericht.agravis.de/en/gb/gb15/wir_gestalten_zukunft/wasserlinsen/index.html
12	Drygro	Fish & animal feed	Feed ingredient for farmed fish	UK / Kenya	https://www.undercurrentnews.com/2017/10/23/uk-alternative-feed-ingredient-startup-eyes-aquaculture-entry/
13	LemPro	Animal & Fish feed	Organic protein for fishmeal, feed, & fertilizer	USA	http://www.lempro.net
14	GreenSun Products, LLC	Pet food		USA	https://www.linkedin.com/company/greensun-products-llc/?originalSubdomain=il; http://www.GreenSunProducts.com
15	Biomass Alternative Power	Bioenergy	Produce natural gas	USA	https://biomassalternativepower.com
16	Duckweed USA	Bioenergy		USA	http://duckweedusa.com/index.html

17	FMC	Aquatic ecotoxicology	Chemical manufacturing	USA	http://www.fmc.com
18	Stillmeadow	Aquatic ecotoxicology	Toxicology services: Lemna toxicity tests	USA	http://www.stillmeadow.com
19	Syntech Research	Aquatic ecotoxicology	Research, development & regulatory services	Spain	https://www.syntechresearch.com/news/new-aquatic-toxicology-services-in-europe/
20	Toxi-Coop Ltd.	Aquatic ecotoxicology	Toxicology test services: Lemna growth inhibition	Hungary	https://toxicoop.com
21	Prosys Biorefining Systems	Duckweed growing systems	Animal feed, Human food & Bioenergy	USA	http://www.prosysbiorefining.com/products.html
22	Space Lab Tech	Duckweed growing systems	Space crop	USA	http://www.spacelabtech.com/index.html

Figure 1: Global distribution of companies active in the development of duckweed based new products and commercial platforms, demonstrating a high concentration in the USA, then central Europe (Germany, the Netherlands, UK, Hungary and Spain) followed by Israel, India and Kenya. It is notable that: Bioponica headquarter (HQ) is in Georgia, USA while it operates in Oregon, USA; Aquible, originally from the Netherland, currently operates in California, USA under the name of Plantible Foods; Drygro, is based in the UK with operations in Kenya.

Historical Accounts: Matthias Jacob Schleiden (1804 – 1881) and the beginning of Lemnaceae research

Manfred Eichhorn (dr-manfred-eichhorn@web.de)

Weimar, Germany

Fig. 1. Matthias Jakob Schleiden (undated, photo collection of the city Frankfurt/ Main, Germany)

M. J. Schleiden (Fig. 1) was born on 5th of April 1804 in Hamburg as son of a physician. He studied jurisprudence in Heidelberg and earned his doctorate degree in Law. Thereafter, he worked as barrister-at-law in his hometown of Hamburg. This kind of professional activity apparently created some problems for the cosmopolitan and curiosity-driven Schleiden, resulting in episodes of depression. After a prevented suicide, he started again to study, this time medicine in Göttingen (1832/ 1833). Frustrated also from this topic, he went in 1835 to the University of Berlin. Under the influence of his uncle, Johann Horkel (1769 – 1846; full professor of Plant Physiology), he finally turned to Botany. In 1839, he got an offer from the University of Jena. He accepted the position but applied for a second promotion. In November 1839 – at the age of 35 years - he applied for and received in the same month the certificate of a degree as doctor of philosophy (for that he did not submit any additional manuscript; evidently his fundamental publications of 1837 and 1838 from his time as student were sufficient to fulfill the requirements). One year later (since 1838 a member of the Leopoldina), Schleiden became extraordinary professor at the University of Jena (Jahn 1963). In Jena, he stayed until 1862, finally as Ordinarius (full professor).

From the very day at the University of Berlin onward, he developed an unprecedented scientific productivity in the field of Botany. Initially, he was interested in Lemnaceae. He collected diligently duckweeds in the outskirt of Berlin but also at other places, and noted: “In 1835 I found first *Lemna minor* close to Berlin with flowers and fruits and since that time on all places where I carefully searched for it…In 1836 I found *Lemna trisulca* in blossom and since that time also every year…Only in 1837 I discovered *Lemna gibba* (a rare species in Berlin) with flowers…*Lemna polyrrhiza*, however, beside best care and intention, I could not find in flowers for a long time until I finally managed to discover so-called male flowers close to Wernigerode, i.e. in which the pistil was not completely developed” (Schleiden 1839). Two years after the beginning of his study, i.e. in 1837, he published an essay about developmental history of ovule in flowering plants (Schleiden 1837), and was noted as “a masterpiece of microscopy and its presentation” (Wartenberg 1959). In this paper, he also

mentioned the situation in duckweed that he collected, which he stressed later again: "The three first mentioned species of duckweed [*L. minor, L. trisulca, L. gibba*] I have investigated completely and frequently in all phases of development of flowering, fruit formation and germination" (Schleiden 1839). Also in the paper "Contributions to Phytogenesis", published one year later (Schleiden 1838), his observations of duckweeds were mentioned.

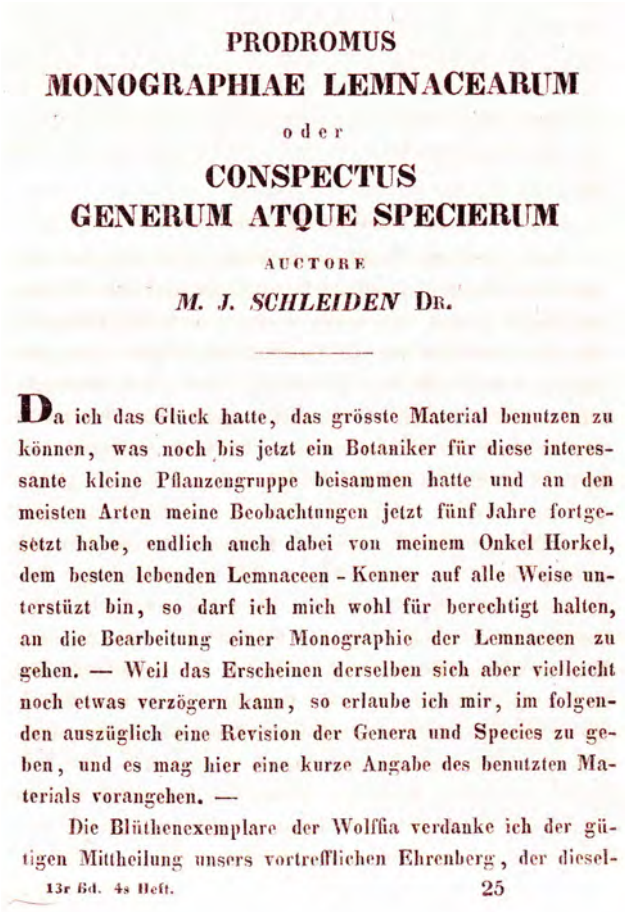
Only toward the end of his time in Berlin, he published a preliminary communication about Systematics of Lemnaceae. He wrote: "As I was in the fortunate position to have available the largest material collection of this small plant group that until now any botanist had, and I have continued my observations for five years, and finally was supported by my uncle Horkel, who is the best present-days specialist of Lemnaceae, I think I am justified to start with the production of a monography of Lemnaceae. However, because its publication might get delayed for some time, I feel encouraged to give in the following some revisions of genera and species." (Schleiden 1839, Fig. 2).

In his systematics of *Lemna* species, he based on existing results about Aroideae: "In the categorization of the genera I followed basically the same principles that were fortunately applied already with so much success by Schott and later by Blume to Aroideae, in which Lemnaceae only represents a tribe, and I think that I have guessed with this pretty well to be correct." (Schleiden 1839). His confidants Heinrich Wilhelm Schott (Austrian Botanist, 1794-1865) and Carl Ludwig Blume (Dutch Botanist, 1796-1862) published several systematic papers, e.g. about Aroideae.

Although announced, no further publication followed this preliminary Lemnaceae monography of Schleiden. In addition, also his supporter, Horkel, did not write anything about duckweed. Only Christoph Friedrich Hegelmaier (1833-1906) produced a fundamental duckweed monography (Hegelmaier 1868), which he supplemented later (Hegelmaier 1895). Hegelmaier accepted there widely the systematics of Lemnaceae as developed by Schleiden; only in few points, he criticized morphological observations of Schleiden.

The present standard textbook about Lemnaceae are still the two volumes of Landolt (1986), Landolt and Kandeler (1987): "The family of Lemnaceae – a monographic study". With the name of the genus "Spirodela Schleid." M. J. Schleiden is honoured.

(Translated by Klaus-J. Appenroth)



PRODROMUS
MONOGRAPHIAE LEMNACEARUM
oder
CONSPECTUS
GENERUM ATQUE SPECIERUM
AUCTORE
M. J. SCHLEIDEN Dr.

Da ich das Glück hatte, das grösste Material benutzen zu können, was noch bis jetzt ein Botaniker für diese interessante kleine Pflanzengruppe beisammen hatte und an den meisten Arten meine Beobachtungen jetzt fünf Jahre fortgesetzt habe, endlich auch dabei von meinem Onkel Horkel, dem besten lebenden Lemnaceen-Kenner auf alle Weise unterstützt bin, so darf ich mich wohl für berechtigt halten, an die Bearbeitung einer Monographie der Lemnaceen zu gehen. — Weil das Erscheinen derselben sich aber vielleicht noch etwas verzögern kann, so erlaube ich mir, im folgenden auszüglich eine Revision der Genera und Species zu geben, und es mag hier eine kurze Angabe des benutzten Materials vorangehen. —

Die Blüthenexemplare der Wolffia verdanke ich der gütigen Mittheilung unsers vortrefflichen Ehrenberg, der diesel-

13r Bd. 4s Heft. 25

Fig. 2. Front page of Schleidens´s Lemnaceae publication. In: Linnaea1839, 385-392.

Fig. 3. The author, Dr. sc. rer. nat. Manfred Eichhorn, worked as scientific coworker in the Institute of General Botany and Plant Physiology, Friedrich Schiller University of Jena, Germany (now Matthias Schleiden Institute) from 1966 to 1999.

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Duckweed as an Ideal System for the Study of Flowering: a Historical Perspective

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The duckweeds contain some of the smallest flowering plants in the plant kingdom and many of them rarely flower. Nevertheless, there are several species that have well characterized flowering behavior and under appropriate conditions will flower profusely. The two most commonly studied duckweeds are *Lemna paucicostata* (now redesignated as *L. aequinoctialis*) 6746, which is a short-day plant with a critical daylength of about 15 hours (Hillman, 1959), and *Lemna gibba* G3, which is a long-day plant with a critical daylength of about 10 hours (Cleland and Briggs, 1967).

In *L. gibba* G3 experiments are started with a single 4-frond colony. Cleland and Briggs (1967) showed that in a typical 4-frond colony there are already about 50 fronds that have formed (Figure 1). Many of these fronds are already too large to be induced to flower so flower induction only takes place in fronds that are less than about 0.1 mm in length. Consequently, even when the level of flower induction is 100 per cent, the FL % (number of flowering fronds/total number of fronds) will never exceed about 70-80%.

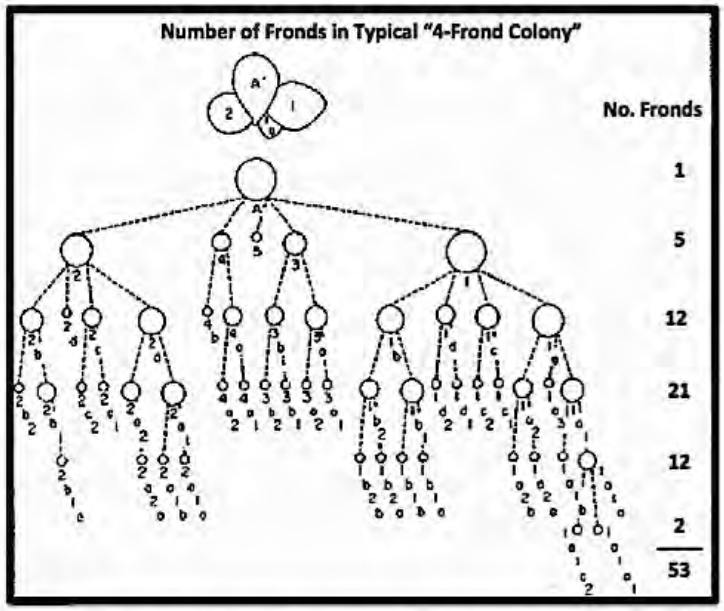

Figure 1. Diagram depicting the number of fronds that was observed in a typical 4-frond colony of *Lemna gibba* G3. Modified from Cleland and Briggs (1967).

Cleland used *L. gibba* G3 as a bioassay system in efforts to identify substances that stimulate or inhibit flowering. The flowering stimulus or florigen is known to move from photoinduced leaves to the stem apices via the phloem. Aphids feed on the phloem and much of what

they take up from the plant is excreted in the form of honeydew. Cleland obtained a population of the aphid *Dactynotus ambrosiae* that feeds on *Xanthium strumarium* and collected honeydew produced by aphids feeding on flowering and vegetative *Xanthium*. When extracts of the aphid honeydew were tested on *L. gibba* G3 one fraction was identified that induced a significant increase in flowering. This fraction was later identified as salicylic acid (SA) and authentic SA was shown to be very effective in causing flowering in *L. gibba* G3 (Cleland, 1974; Cleland and Ajami, 1974).

Since SA was isolated from honeydew produced by aphids feeding on both flowering and vegetative *Xanthium*, it was clear the SA was not a natural flower-inducing substance in *Xanthium*. Nevertheless, the effect of SA on flowering in duckweed can be quite dramatic. For *L. gibba* G3 when plants are grown on the standard E medium addition of SA at 3.2 to 10 uM reduces the critical daylength by 1-2 hours. On a 10L:14D cycle where there is very little flowering in regular E medium, addition of 3.2 uM SA results in nearly 50% flowering (Cleland and Ajami, 1974). When *L. gibba* G3 is grown on half-strength Hutner's medium under continuous light there is no flowering but addition of 10 uM SA leads to a nearly optimal flowering response (Tanaka, et al., 1979). SA is also very effective in stimulating flowering in several other duckweed. For instance, in *L. obscura* 7133 there is no flowering on E medium on short or long days but with 3.2 uM SA a very strong flowering response is obtained (Cleland, et al., 1982) (Figure 2).

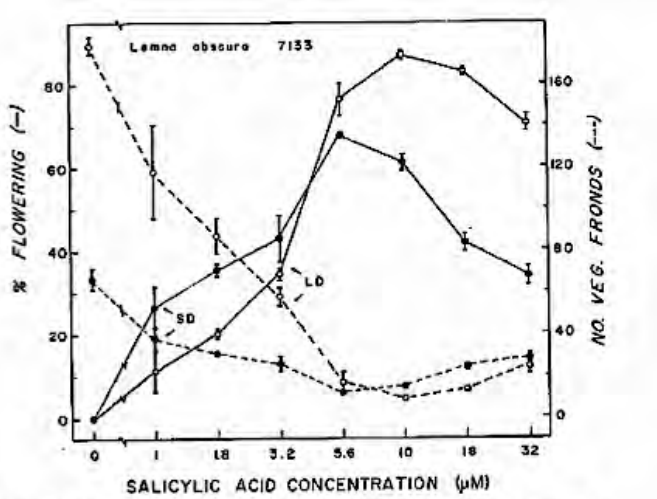

Figure 2. Effects of Salicylic acid on flowering and growth of *L. obscura* 7133. Data and figure from Cleland et al. (1982).

SA has been shown to stimulate flowering in the aquatic plant *Pistia stratiotes* (Pieterse, 1978) but for the most part its stimulating influence on flowering is mostly limited to certain duckweed. SA is also known to induce systemic acquired resistance (SAR), an important plant defense mechanism (Ryals, et al., 1996). SAR can also be induced by benzo (1,2,3)thiadiazole-7-carbothionic acid S-methyl ester (BTH) (Lawton, et al., 1996) and BTH was shown to induce flowering in *L. paucicostata* 151 and *L. gibba* G3 at concentrations as low as 1 uM (Endo, et al., 2009). This raises the possibility that there may be some connection between the SA effect on flowering in duckweed and SAR.

Most long-day plants exhibit a rosette growing pattern where flowering is accompanied by rapid stem elongation or bolting. In such plants gibberellin treatment causes bolting and in some cases will also stimulate flowering. *L. gibba* G3 is one of the very few non-rosette plants that exhibit a clear cut qualitative long-day flowering response. Gibberellin will not induce flowering in *L. gibba* G3 and thus the long-day flowering response in *L. gibba* G3 can be studied without any complicating influence of gibberellin.

The duckweeds offer special advantages for studies on flowering. The plants are grown aseptically and because they are aquatic, substances that may influence flowering can be added directly to the medium so they can be taken up by the plants. Due to their small size plants can be grown in 50 or 125 ml Erlenmeyer flasks in bench top growth chambers. For these various reasons duckweed species that exhibit photoperiodic control of flowering are an ideal bioassay system that can be used in future studies on flowering.

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Student Spotlight: Phuong Thi Nhu Hoang

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Gatersleben, Stadt Seeland, Germany

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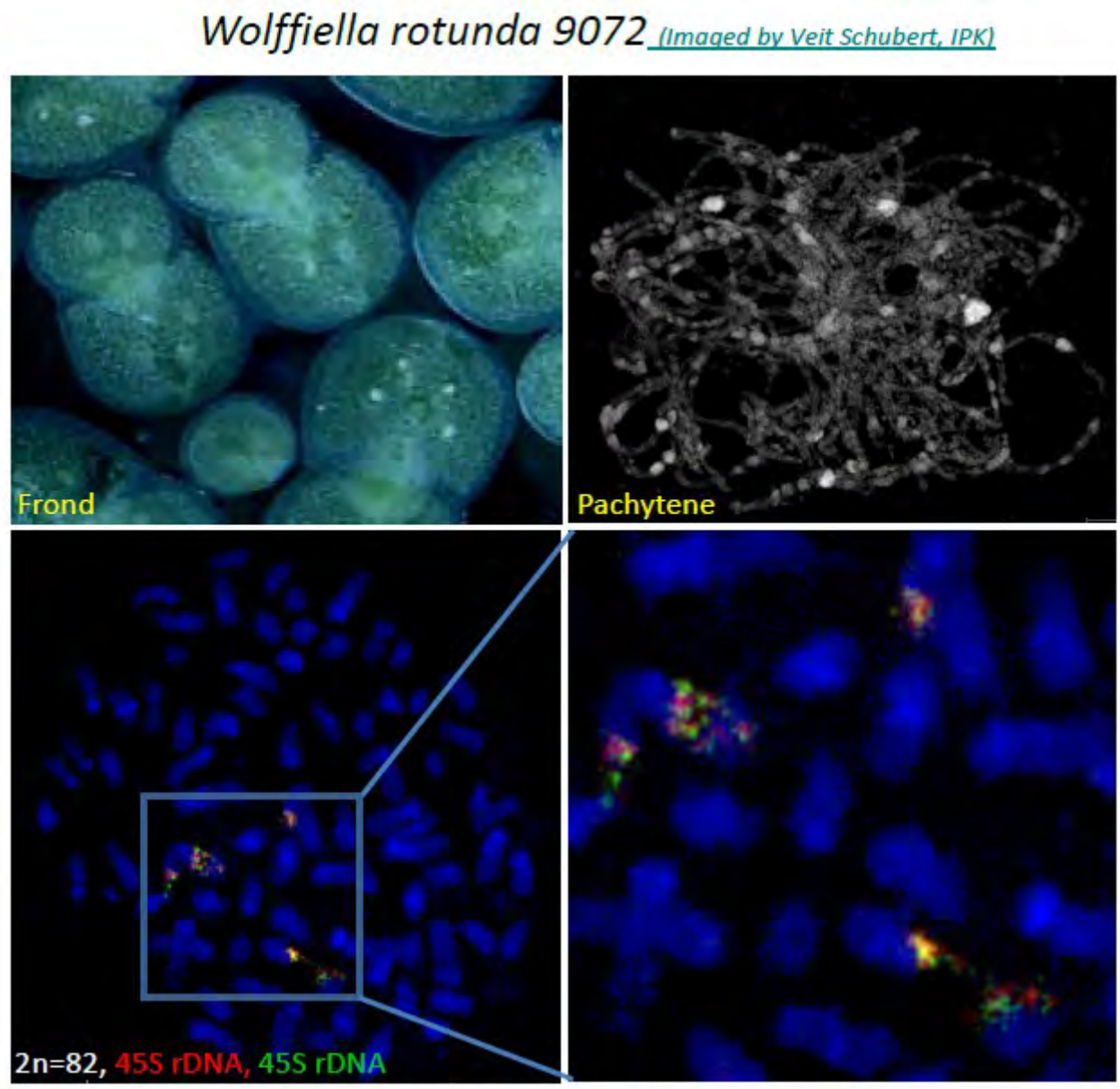
When I completed my Bachelor and Master degrees at Biology Department, Dalat University, Vietnam, I was already interested in plants and their applications in daily life. My Master thesis focused on medical application of *Taraxacum officinale*. It was my dream to study for PhD abroad to improve my knowledge, experimental and language skills. In 2014 I won a scholarship of the Vietnamese Government to go abroad for PhD study. Fortunately, my friends introduced me to Prof. Ingo Schubert and Dr. Hieu Cao, both working on duckweeds – a potential crop for the future. After several rounds of online interviews, I got the opportunity to work in this research group at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben in January 2015. Step by step I entered the colorful world of microscopy and cytogenetics in duckweeds.

Phuong T.N. Hoang in the Schubert lab (left) and at the IPK campus (right)

After overcoming initial technical difficulties with chromosome spreading and cross-species fluorescence in situ hybridization (FISH), I could resolve several open questions in duckweed research. Based on the cytogenetic map of *Spirodela polyrhiza* (Cao *et al.*, 2016) which was established by FISH with 96 bacterial artificial chromosomes (BACs), anchored in the genome assembly of this species (Wang *et al.*, 2014), I studied chromosome homeology and rearrangements between *S. polyrhiza* (2n=40) and *S. intermedia* (2n=36), the only two species of the most ancestral duckweed genus. The results revealed possible scenarios of karyotype evolution between both species (Hoang and Schubert 2017). The cytogenetic map for *S. intermedia* served as a frame for assembling the *S. intermedia* genome using PacBio reads, which is in progress.

Furthermore, I investigated by FISH with suitable BACs the discrepancies between the cytogenetic

map (Cao *et al.,* 2016) and the BioNano map (Michael *et al.,* 2017) of *S. polyrhiza*. In collaboration with Prof. Eric Lam (Rutgers University, USA) and Dr. Todd Michael (J. Craig Venter Institute, USA) an updated map for this species was established combining optical mapping, Oxford Nanopore sequencing and cytogenetics (Hoang *et al.,* 2018). This work illustrated that more than two independent approaches are required for a robust genome assembly, and that cytogenetics could be very useful for validating proposed linkage groups, in particular when genetic maps are not available, as in case of asexually propagating duckweeds.



The first cytogenetic observations for the duckweed *Wolffiella rotunda*

Then, eleven representative species of the five duckweed genera were selected to investigate the correlation of neoteny level and genome size with cell and nuclear volume. In parallel, chromosome number as well as 5S and 45S rDNA loci of these species were determined.

Besides introducing me into plant cytogenetics, my supervisor gave me opportunity to attend international conferences to introduce my work, to receive suggestion from expert scientist and to

advance further collaboration. So I attended the Chromosome painting Workshop, held by Prof. James A. Birchler, in Missouri University on September, 2017. Afterwards I visited Prof. Eric Lam's lab, met his research group and presented my results at Rutgers University. In January 2018 I could deliver a talk at the Duckweed Workshop during the Plant and Animal Genome (PAG) Conference in San Diego, USA and meet expert scientists worldwide especially in duckweed research. It was also great experience for me to attend the cytogenetics conference of the German Society for Plant Breeding (2017), the Plant Science Student Conferences (2016, 2017 and 2018), the Conference on Molecular Biology of Plants (2018), the EMBO workshop "Plant Genome Stability and Change" (2018), all in Germany. In September 2018 I attended the 22nd International Chromosome Conference in Prague, Czech Republic, and presented my data on duckweed chromosomes also there.

I had the chance to meet and work with Prof. Klaus Appenroth (Jena University, Germany), Prof. Nikolai Borisjuk (Huaiyin Normal University, China), Dr. Manuela Bog (University of Greifswald, Germany), Dr. K. Sowjanya Sree (Central University of Kerala, India) and received helpful comments and suggestions for my study.

After defending my thesis, I will continue to investigate duckweed chromosomes at my home University in Dalat, and I hope for continuing intense collaboration with the community of duckweed researchers.

Finally, I would like to take this occasion to express my thanks to my supervisor Prof. Ingo Schubert, to my initial co-supervisor Dr. Hieu Cao, to Prof. Jochen C. Reif, head of Department of Breeding Research at the IPK, who provided me working facilities, and to all friends which supported my work. Last but not least, I would like to express my very deep gratitude to my family, for their immeasurable love, limitless sacrifice, unfailing support and continuous encouragement throughout my years of study.

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2nd Duckweed Research and Applications Workshop at PAG XXVII

Invitation to submit candidates as cover photo for the Duckweed Forum

We would like to invite readers of the Duckweed Forum to consider submitting a duckweed-related photo as the cover for an issue of our newsletter. After featuring the 37 species of duckweed in the past 10 issues of the Duckweed Forum, it is time to open the Cover Page to highlight input from our community. Please send your favorite duckweed photo to our Steering Committee members to be considered as the next Cover. Ideally it would illustrate an important event, a novel discovery, or an artistic rendition of duckweed's unique characteristics. An image of approximately 27 cm deep by 21 cm wide, at least 300 dpi resolution at that size and preferably in TIFF format would be acceptable. We look forward to receiving contributions from you all.

From the Database

Highlight

Sequence-guided approach to genotyping plant clones and species using polymorphic NB-ARC-related genes.

Chu, P; Wilson, GM; Michael, TP; Vaiciunas, J; Honig, J; Lam, E. (2018) PLANT MOLECULAR BIOLOGY Sep 6. doi: 10.1007/s11103-018-0774-1

Rapid and economical genotyping tools that can reliably distinguish species and intraspecific variations in plants can be powerful tools for biogeographical and ecological studies. Clones of the cosmopolitan duckweed species, *Spirodela polyrhiza*, are difficult to distinguish morphologically due to their highly abbreviated architecture and inherently low levels of sequence variation. The use of plastidic markers and generic Amplification Fragment Length Polymorphism approaches have met with limited success in resolving clones of *S. polyrhiza* from diverse geographical locales. Using whole genome sequencing data from nine *S. polyrhiza* clones as a training set, we created an informatic pipeline to identify and rank polymorphic regions from nuclear-encoded NB-ARC-related genes to design markers for PCR, Sanger sequencing (barcoding), and fragment length analysis. With seven primer sets, we found 21 unique fingerprints from a set of 23 *S. polyrhiza* clones. However, three of these clones share the same fingerprint and are indistinguishable by these markers. These primer sets can also be used as interspecific barcoding tools to rapidly resolve *S. polyrhiza* from the closely related *S. intermedia* species without the need for DNA sequencing. Our work demonstrates a general approach of using hyper-polymorphic loci within genomes as a resource to produce facile tools that can have high resolving power for genotyping applications.

Biochemistry

Structural and Biochemical Properties of Duckweed Surface Cuticle

Borisjuk, N; Peterson, AA; Lv, JY; Qu, GR; Luo, Q; Shi, L; Chen, GM; Kishchenko, O; Zhou, YZ; Shi, JX. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 317

The plant cuticle, which consists of cutin and waxes, forms a hydrophobic coating covering the aerial surfaces of all plants. It acts as an interface between plants and their surrounding environment whilst also protecting them against biotic and abiotic stresses. In this research, we have investigated the biodiversity and cuticle properties of aquatic plant duckweed, using samples isolated from four different locations around Hongze lake in Jiangsu province, China. The samples were genotyped using two chloroplast markers and nuclear ribosomal DNA markers, which revealed them as ecotypes of the larger duckweed, *Spirodela polyrhiza*. Duckweed cuticle properties were investigated by compositional analysis using Gas Chromatography coupled with Mass Spectroscopy (GC-MS) Flame Ionization Detector (GC-FID), and ultrastructural observation by cryo-Scanning Electron Microscopy (cryo-SEM). Cuticle compositional analysis indicated that fatty acids and primary alcohols, the two typical constituents found in many land plant cuticle, are the major duckweed wax components. A large portion of the duckweed wax fraction is composed of phytosterols, represented by campesterol, stigmasterol, sitosterol and their common precursor squalene. The cryo-SEM observation uncovered significant differences between the surface structures of the top air-facing and bottom water-facing sides of the plant fronds. The top side of the

fronds, containing multiple stomata complexes, appeared to be represented by a rather flat waxy film sporadically covered with wax crystals. Underneath the waxy film was detected a barely distinguished nanoridge net, which became distinctly noticeable after chloroform treatment. On the bottom side of the fronds, the large epidermal cells were covered by the well-structured net, whose sections became narrower and sharper under cryo-SEM following chloroform treatment. These structural differences between the abaxial and adaxial sides of the fronds evidently relate to their distinct physiological roles in interacting with the contrasting environments of sunlight/air and nutrients/water. The unique structural and biochemical features of *Spirodela* frond surfaces with their rapid reproductive cycle and readily availability genome sequence, make duckweed an attractive monocot model for studying the fundamental processes related to plant protection against ultraviolet irradiation, pathogens and other environmental stresses.

Correlation of apiose levels and growth rates in duckweeds

Pagliuso, D; Grandis, A; Igarashi, ES; Lam, E; Buckeridge, MS. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 291

The carbon assimilated by photosynthesis in plants can be partitioned into starch, soluble sugars, and cell wall polymers. Higher levels of starch accumulation in leaves are usually correlated with a lower growth capacity. Duckweeds are fast-growing aquatic monocot plants that can accumulate high levels of starch. They are an unusual group because their cell wall has very low levels of lignin while accumulating apiogalacturonan, a pectic polysaccharide that could be involved with boron assimilation. In this work, five duckweed species from different genera (*Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Wolffiella caudata*, and *Wolffia borealis*) were cultivated under two light intensities (20 and 500 µmoles of photons m^{-2} s^{-1}) to evaluate the effects of growth rate on carbohydrate metabolism. A comparative analysis was performed by measuring their relative growth rates (RGR), and their content for starch, as well as soluble and cell wall carbohydrates. We found that the faster-growing species (the Lemnoideae) accumulate lower starch and higher soluble sugars than the slower-growing species within the Wolffioideae. Interestingly, analysis of the cell wall monosaccharides revealed that the slower-growing species displayed lower content of apiose in their walls. Our results indicate that higher accumulation of apiose observed in cell walls of the Lemnoideae species, which likely correlates with a higher proportion of apiogalacturonan, may lead to higher efficiency in the assimilation of boron. This is consistent with the increased RGR observed under conditions with higher apiose in the cell wall, such as higher light intensity. Consistent with their lower growth capacity, the Wolffioideae species we studied shows higher starch accumulation in comparison with the Lemnoideae species. We suggest that apiose levels could be good biomarkers for growth capacity of duckweeds and suggest that boron uptake could be an important factor for growth control in this aquatic plant family.

A new herbicidal site of action: Cinmethylin binds to acyl-ACP thioesterase and inhibits plant fatty acid biosynthesis

Campe, R; Hollenbach, E; Kammerer, L; Hendriks, J; Hoffken, HW; Kraus, H; Lerchl, J; Mietzner, T; Tresch, S; Witschel, M; Hutzler, J. (2018) PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY 148: 116-125

The prevalent occurrence of herbicide resistant weeds increases the necessity for new site of action herbicides for effective control as well as to relax selection pressure on the known sites of action. As a consequence, interest increased in the unexploited molecule cinmethylin as a new solution for the control of weedy grasses in cereals. Therefore, the mechanism of action of cinmethylin was reevaluated. We applied the chemoproteomic approach cellular Target Profiling (TM) from Evotec to

identify the cinmethylin target in *Lemna paucicostata* protein extracts. We found three potential targets belonging to the same protein family of fatty acid thioesterases (FAT) to bind to cinmethylin with high affinity. Binding of cinmethylin to FAT proteins from *Lemna* and *Arabidopsis* was confirmed by fluorescence-based thermal shift assay. The plastid localized enzyme FAT plays a crucial role in plant lipid biosynthesis, by mediating the release of fatty acids (FA) from its acyl carrier protein (ACP) which is necessary for FA export to the endoplasmic reticulum. GC-MS analysis of free FA composition in *Lemna* extracts revealed strong reduction of unsaturated C18 as well as saturated C14, and C16 FAs upon treatment with cinmethylin, indicating that FA release for subsequent lipid biosynthesis is the primary target of cinmethylin. Lipid biosynthesis is a prominent target of different herbicide classes. To assess whether FAT inhibition constitutes a new mechanism of action within this complex pathway, we compared physiological effects of cinmethylin to different ACCase and VLCFA synthesis inhibitors and identified characteristic differences in plant symptomology and free FA composition upon treatment with the three herbicide classes. Also, principal component analysis of total metabolic profiling of treated Lemna plants showed strong differences in overall metabolic changes after cinmethylin, ACCase or VLCFA inhibitor treatments. Our results identified and confirmed FAT as the cinmethylin target and validate FAT inhibition as a new site of action different from other lipid biosynthesis inhibitor classes.

Biotechnology

Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns

Markou, G; Wang, L; Ye, JF; Unc, A. (2018) BIOTECHNOLOGY ADVANCES 36: 1238-1254

Aquatic organisms, such as microalgae (*Chlorella, Arthrospira* (*Spirulina*), *Tetrasselmis, Dunalliela* etc.) and duckweed (Lemna spp., *Wolffia* spp. etc.) are a potential source for the production of protein-rich biomass and for numerous other high-value compounds (fatty acids, pigments, vitamins etc.). Their cultivation using agro-industrial wastes and wastewater (WaW) is of particular interest in the context of a circular economy, not only for recycling valuable nutrients but also for reducing the requirements for fresh water for the production of biomass. Recovery and recycling of nutrients is an unavoidable long-term approach for securing future food and feed production. Agro-industrial WaW are rich in nutrients and have been widely considered as a potential nutrient source for the cultivation of microalgae/duckweed. However, they commonly contain various hazardous contaminants, which could potentially taint the produced biomass, raising various concerns about the safety of their consumption. Herein, an overview of the most important contaminants, including heavy metals and metalloids, pathogens (bacteria, viruses, parasites etc.), and xenobiotics (hormones, antibiotics, parasiticides etc.) is given. It is concluded that pretreatment and processing of WaW is a requisite step for the removal of several contaminants. Among the various technologies, anaerobic digestion (AD) is widely used in practice and offers a technologically mature approach for WaW treatment. During AD, various organic and biological contaminants are significantly removed. Further removal of contaminants could be achieved by post-treatment and processing of digestates (solid/liquid separation, dilution etc.) to further decrease the concentration of contaminants. Moreover, during cultivation an additional removal may occur through various mechanisms, such as precipitation, degradation, and biotransformation. Since many jurisdictions regulate the presence of various contaminants in feed or food setting strict safety monitoring processes, it would be of particular interest to initiate a multi-disciplinary discussion whether agro-industrial WaW ought to be used to cultivate microalgae/duckweed for feed or food production and identify most feasible options for doing this safely. Based on the current body of knowledge it is estimated that AD and

post-treatment of WaW can lower significantly the risks associated with heavy metals and pathogens, but it is yet unclear to what extent this is the case for certain persistent xenobiotics.

Development of *Wolffia arrhiza* as a producer for recombinant human granulocyte colony-stimulating factor

Khvatkov, P; Firsov, A; Shvedova, A; Shaloiko, L; Kozlov, O; Chernobrovkina, M; Pushin, A; Tarasenko, I; Chaban, I; Dolgov, S. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 304

To date, the expression of recombinant proteins in transgenic plants is becoming a powerful alternative to classical expression methods. Special efforts are directed to the development of contained cultivation systems based on cell culture or rhyzosecretion, which reliably prevents the heterologous DNA releasing into the environment. A promising object for the development of such systems is the tiny aquatic plant of *Wolffia arrhiza*, which can be used as a dipped culture in bioreactors. Herein we have expressed the human granulocyte colony-stimulating factor (hG-CSF) in nuclear-transformed *Wolffia*. The nucleotide sequence of hG-CSF was optimized for expression in *Wolffia* and cloned into the vector pCamGCSF downstream of double CaMV 35S promoter. *Wolffia* plants were successfully transformed and 34 independent transgenic lines with hG-CSF gene were obtained, PCR and Southern blot analysis confirmed the transgenic origin of these lines. Western blot analysis revealed accumulation of the target protein in 33 transgenic lines. Quantitative ELISA of protein extracts from these lines showed hG-CSF accumulation up to 35.5 mg/kg of *Wolffia* fresh weight (0.194% of total soluble protein). This relatively high yield holds promise for the development of *Wolffia* -based expression system in strictly controlled format to produce various recombinant proteins.

Hydrotreatment of bio-oil distillates produced from pyrolysis and hydrothermal liquefaction of duckweed: A comparison study

Wang, F; Tian, Y; Zhang, CC; Xu, YP; Duan, PG. (2018) SCIENCE OF THE TOTAL ENVIRONMENT 636: 953-962

A comprehensive comparison of hydrothermal liquefaction (HTL) to the pyrolysis of duckweed was conducted to determine the yields and components of the crude bio-oils and their distillates. The upgrading behaviors of the distillates were thoroughly investigated with the use of used engine oil as a solvent. With all other variables fixed, HTL produced crude bio-oil with a lower H/C ratio (1.28 ± 0.03) than pyrolysis did (1.45 ± 0.04). However, its distillates had a higher H/C ratio (1.60 ± 0.05) and total yield (66.1 ± 2.0 wt%) than pyrolysis (1.46 ± 0.04 and 47.2 ± 1.4 wt%, respectively). Phenolics and nitrogenous heterocycles constituted relatively major proportions of the two crude bio-oils and most of their distillates. Obvious differences in molecular composition between the two crude bio-oils and their distillates were ascribed to the distinct impacts of HTL and pyrolysis and were affected by the distillate temperature. Co-hydrotreating with used engine oil (UEO) provided the upgraded bio-oils much higher H/C ratios (similar to 1.78 ± 0.05) and higher heating values (similar to 45.5 ± 1.4 MJ . kg^{-1}), as well as much lower contents of N, O and S compared to their initial distillates. Aromatics and alkanes constituted a large proportion in most of upgraded bio-oils. N removal from the pyrolysis distillates was easier than from the HTL distillates. Distinct differences in yields and molecular compositions for the upgraded bio-oils were also attributed to the different influences associated with the two conversion routes.

Decomposition of *Wolffia arrhiza* residues rapidly increases mineral nitrogen and decreases extractable phosphorus in acidic soils

Chikuvire, TJ; Muchaonyerwa, P; Zengeni, R. (2018) ENVIRONMENTAL MONITORING AND ASSESSMENT 190, Article Number: 510

While nutrient loads from anthropogenic sources upset aquatic ecosystem balance, *Wolffia arrhiza* (duckweed) has capacity to purge nutrient-rich water if continuously harvested. The nutrients accumulated in biomass have potential as soil fertility amendments. The objective of this study was to determine changes in release of nitrogen (N) and phosphorus (P), and the fate of P in soils after duckweed biomass amendment. An incubation experiment was conducted at 25 °C using three soils amended with proportions equivalent to 501, 1002 and 1503 mg N kg^{-1} and 62, 124 and 186 mg P kg^{-1}. Soil samples were collected on 0, 3, 7, 14, 21, 28, 42 and 56 days, for ammonium-N, nitrate-N and extractable-P measurements. At the end of incubation, P pools were determined. At least 25 mg kg^{-1} of ammonium-N was released on day 0, reaching a peak within the first 2 weeks. Nitrate- and mineral-N increased from 14 to 42 days, with a corresponding decrease in ammonium-N. Relatively fertile soil released more mineral-N at higher applied ratios of duckweed than the less fertile. About 10-80 mg kg^{-1} of duckweed P was extractable on day 0 and amounts progressively declined over the incubation period. The combined percentage (0.5%) of tissue aluminium (Al) and iron (Fe) facilitated Al and Fe phosphate accumulation as the proportion of duckweed amendment increased. The results suggested that soil type and elemental composition of duckweed are important determinants for N and P release, and liming could improve P availability in soil.

Ecology

Competition between *Lemna minuta*, *Lemna minor*, and *Azolla filiculoides*. Growing fast or being steadfast?

Paolacci, S; Jansen, MAK; Harrison, S. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 207

A substantial number of Lemnaceae are invasive outside their natural distribution area. *Lemna minuta* is considered invasive in several European countries, where it can occur in the same habitat as invasive *Azolla filiculoides* and native *Lemna minor*. In this study the presence, abundance and growth rates of all three species were monitored across 24 natural ponds and in a series of mesocosms in order to explore the importance of species invasiveness and habitat invisibility. Field monitoring showed that the distribution of the three species of macrophytes is heterogeneous in space and time. However, the data show no association of nutrient or light levels with plant distribution. Indeed, using reciprocal transplanting experiments it was demonstrated that all species are able to grow in all ponds, even ponds where the species do not naturally occur. It is concluded that distribution of *L. minor*, *L. minuta*, and *A. filiculoides* is not limited by the prevailing physicochemical characteristics of the ponds during the summer period. Remarkably, in these experiments *A. filiculoides* displayed the highest RGR, and exerted a negative influence on growth rates and surface cover of *L. minor* and *L. minuta*. Despite such apparent invasiveness, *A. filiculoides* was relatively rare in the study area. Rather, the species most abundant was *L. minor* which has the lowest RGR under field conditions in summer. Therefore, this study shows that the invasiveness of the species during the summer months is not necessarily reflected in the actual distribution pattern in natural ponds. In fact, alien *L. minuta* and *A. filiculoides* are under-represented in the monitored area. It is concluded that the interaction of several factors, including growth under winter-conditions

and/or dispersal after disturbances, is the major determinant of the abundance and heterogeneous distribution of *L. minor*, *L. minuta*, and *A. filiculoides* in the study area.

Ecophysiological characteristics of turions of aquatic plants: A review

Adamec, L. (2018) AQUATIC BOTANY 148: 64-77

Turions or winter/overwintering buds are vegetative, dormant storage organs formed by perennial aquatic plants. They are formed in subtropical to polar zones in response to unfavourable ecological conditions, usually at the beginning of autumn. In this way, fragile shoots of aquatic plants avoid freezing and damage. Turions are formed in at least 14 genera of aquatic vascular plants from nine plant families, mainly in submerged (or amphibious) and free-floating species. Turions are propagules formed by extreme condensation of apical stems (intemodes) with modified attached leaves or scales which are dissimilar to summer leaves; they germinate and sprout after they separate from the mother plant. As opposed to pale subterranean rhizome apices, turions contain chlorophyll and sprouting turions are able to photosynthesize, which gives them an ecological advantage. Here I review various aspects and particulars of turion biology - structure, ecological significance, costs and benefits, turion formation, dormancy, germination, sprouting, metabolism and frost tolerance - from an ecophysiological point of view with an emphasis on ecological and biochemical developmental signals of turion formation and germination or sprouting. All turion forming genera and species are included except Spirodela polyrhiza because of the high ecophysiological specificity of this species. Unpublished data are also included. Most of the *Potamogeton crispus* turions have an inverted environmental regulation of their formation and sprouting as they sustain over the warm summer period in a dormant stage and sprout afterwards (aestivation).

Plant response to overcrowding - *Lemna minor* example

Kufel, L; Strzalek, M; Przetakiewicz, A. (2018) ACTA OECOLOGICA-INTERNATIONAL JOURNAL OF ECOLOGY 91: 73-80

Plants adopt various strategies in response to increasing density. We tested that response in two populations of *Lemna minor* L. - a free floating aquatic plant that frequently experiences intraspecific competition for space. Surface area of fronds and colonies, colony size (the number of fronds per colony), the rate of reproduction (based on the number of produced fronds) and growth rate (enlargement of surface area of all colonies) were the analysed factors presumably affected by density. The study was performed in natural stands and in experimental conditions with the use of two contrasting plant densities. Plants growing in natural conditions produced fronds of smaller and less variable surface area as a response to overcrowding but the number of fronds per colony was unrelated to plant density. Stable experimental conditions facilitated formation of fronds and colonies larger than in the field but frond detachment decreasing colony size was more intensive at high than at low density. This strategy allowed plants to more efficiently occupy limited available space. No self-thinning was observed during experimental cultures. Due to increasing frond area in cultures, growth rate was always higher than the rate of plant reproduction. Both were strongly negatively affected by high density. Performed calculations indicate that density-dependent growth inhibition starts when *L. minor* colonies cover the available water surface with a mono-layer mat. Some types of responses were found to significantly differ between analysed populations, which was also shown by genetic differences tested with the ISSR-PCR technique. Possible causal relationship between plant strategies and their genomic structure needs, however, further studies.

Feed & Food

The influence of aquaponically grown duckweed (*Lemna minuta* Kunth) used for composition of sustainable diets on hydrochemical and technological parameters in carp (*Cyprinus carpio* L.)

Sirakov, I; Velichkova, K. (2018) TURKISH JOURNAL OF FISHERIES AND AQUATIC SCIENCES 18: 1037-1044

The aim of the current study was to investigate the influence of aquaponically grown duckweed (*Lemna minuta*) used as part of a biofilter in recirculation aquaculture systems, when its included in the composition (10 and 30% content of daily feed ratio) of sustainable diets on hydrochemical and technological parameters in common carp (*Cyprinus carpio* L) fingerlings cultivated in recirculation aquaponic systems. The inclusion of *L. minuta* in diets for carp fingerlings influenced the hydrochemical parameters and decreased the quantity of nitrogen and phosphorus compounds in water of tanks where carps were fed with feed containing duckweed, but difference was statistically significant only for ammonium (0.087 ± 0.008)($P \leq 0.05$). The carps fed with a diet containing 30% (L30) duckweed of their daily feed ratio showed better survival but similar growth and FCR than fish fed with L0 and differences were not significant ($P \geq 0.05$). The lower growth and higher FCR were measured in carps fed with L10 compared to the values of these parameters in carp's fingerlings fed with L0 diet and the differences were significant ($P \leq 0.05$). The duckweed presents cheap and easy accessible ingredients for feeding of carp. Furthermore, it could be used for treatment of wastewater in recirculating aquaculture systems this way increasing their sustainability.

Interaction with microorganisms

Bacterial production of indole related compounds reveals their role in association between duckweeds and endophytes

Gilbert, S; Xu, J; Acosta, K; Poulev, A; Lebeis, S; Lam, E. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 265

Duckweed farming can be a sustainable practice for biofuel production, animal feed supplement, and wastewater treatment, although large scale production remains a challenge. Plant growth promoting bacteria (PGPB) have been shown to improve plant health by producing phytohormones such as auxin. While some of the mechanisms for plant growth promotion have been characterized in soil epiphytes, more work is necessary to understand how plants may select for bacterial endophytes that have the ability to provide an exogenous source of phytohormones such as auxin. We have isolated and characterized forty-seven potentially endophytic bacteria from surface-sterilized duckweed tissues and screened these bacterial strains for production of indole related compounds using the Salkowski calorimetric assay. Indole-3-acetic acid (IAA), indole-3-lactic acid (ILA), and indole produced by various bacterial isolates were verified by mass spectrometry. Using the Salkowski reagent, we found that 79% of the isolated bacterial strains from our collection may be capable of producing indole related compounds to various extents during in vitro growth. Of these, bacteria that are producing indole related compounds, 19% are additionally producing indole. There is an apparent correlation between the type of indole related compound produced by a particular bacteria and the duckweed genus from which the bacterial strain is derived. These results suggest the possible association between different duckweed genera and endophytes that are producing distinct types of secondary metabolites. Understanding the role of indole related compounds during

interaction between endophytes and the plant host may be useful to help design synthetic bacterial communities that could target specific or multiple species of duckweed in the future to sustainably enhance plant growth.

Mission possible: diatoms can be used to infer past duckweed (lemnoid Araceae) dominance in ponds

Emson, D; Sayer, CD; Bennion, H; Patmore, IR; Rioual, P. (2018) JOURNAL OF PALEOLIMNOLOGY 60: 209-221

Compared to larger lakes, ponds have rarely been the focus of palaeoecological studies. A common feature of ponds, especially those subject to eutrophication, is mass surface coverings of lemnoid Araceae (duckweed) which have severe implications for ecological processes in small waterbodies, in particular lowered oxygen content. To help understand the implications of duckweed dominance for the long-term ecology of ponds, and to determine the potential for palaeoecological studies in ponds more generally, we develop a new diatom-based *Lemna*-indicator metric. Recent studies of diatom host-plant relationships have shown significant associations between duckweed and the epiphytes *Lemnicola hungarica* and *Sellaphora saugerresii* (formally known as Sellaphora seminulum). To determine the potential of these species as palaeo-indicators of long-term duckweed dynamics in ponds, we investigated the diatom composition of surface sediment assemblages in sets of duckweed and non-duckweed-dominated ponds in Norfolk, eastern England. In addition, we undertook diatom analysis of two cores from a small farmland pond (Bodham Rail Pit) subject to a known duckweed dominance event (1999-2005). Both *L. hungarica* and *S. saugerresii* were significant predictors of past *Lemna* dominance in the surface sediments. Further, in the core study, both diatom species accurately and closely tracked the documented "on-off" duckweed cycle. Our study suggests huge potential for using ponds in palaeoecological studies and for diatom-based investigations of floating plant histories.

Molecular Biology

Generating a high-confidence reference genome map of the Greater Duckweed by integration of cytogenomic, optical mapping, and Oxford Nanopore technologies.

Hoang, PNT; Michael, TP; Gilbert, S; Chu, P; Motley, ST; Appenroth, KJ; Schubert, I; Lam, E. (2018) PLANT JOURNAL Jul 28. doi: 10.1111/tpj.14049.

Duckweeds are the fastest growing angiosperms and have the potential to become a new generation of sustainable crops. Although a seed plant, *Spirodela polyrhiza* clones rarely flower and multiply mainly through vegetative propagation. Whole-genome sequencing using different approaches and clones yielded two reference maps. One for clone 9509, supported in its assembly by optical mapping of single DNA molecules, and one for clone 7498, supported by cytogenetic assignment of 96 fingerprinted bacterial artificial chromosomes (BACs) to its 20 chromosomes. However, these maps differ in the composition of several individual chromosome models. We validated both maps further to resolve these differences and addressed whether they could be due to chromosome rearrangements in different clones. For this purpose, we applied sequential multicolor fluorescence in situ hybridization (mcFISH) to seven *S. polyrhiza* clones, using 106 BACs that were mapped onto the 39 pseudomolecules for clone 7498. Furthermore we integrated high-depth Oxford Nanopore (ON) sequence data for clone 9509 to validate and revise the previously assembled chromosome models. We found no major structural rearrangements between these

seven clones, identified seven chimeric pseudomolecules and Illumina assembly errors in the previous maps, respectively. A new *S. polyrhiza* genome map with high contiguity was produced with the ON sequence data and genome-wide synteny analysis supported the occurrence of two Whole Genome Duplication events during its evolution. This work generated a high confidence genome map for *S. polyrhiza* at the chromosome scale, and illustrates the complementarity of independent approaches to produce whole-genome assemblies in the absence of a genetic map.

Development of efficient protocols for stable and transient gene transformation for *Wolffia globosa* using *Agrobacterium*

Heenatigala, PPM; Yang, JJ; Bishopp, A; Sun, ZL; Li, GJ; Kumar, S; Hu, SQ; Wu, ZG; Lin, W; Yao, LG; Duan, PF; Hou, HW. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 227

Members of the *Wolffia* genus are fascinating plants for many biologists as they are the smallest flowering plants on Earth and exhibit a reduced body plan that is of great interest to developmental biologists. There has also been recent interest in the use of these species for bioenergy or biorefining. Molecular and developmental studies have been limited in *Wolffia* species due to the high genome complexity and uncertainties regarding the stable genetic transformation. In this manuscript, we present new protocols for both stable and transient genetic transformation for *Wolffia globosa* using *Agrobacterium tumefaciens*. For the transient transformation, we used *Wolffia* fronds whereas we used clusters for the stable transformation. As proof of concept we transformed two synthetic promoter constructs driving expression of the GUS marker gene, that have previously been used to monitor auxin and cytokinin output in a variety of species. Using these approaches, we obtained a Transformation Efficiency (TE) of 0.14% for the stable transformation and 21.8% for the transient transformation. The efficiency of these two methods of transformation are sufficient to allow future studies to investigate gene function. This is the first report for successful stable transformation of *W. globosa*.

Genomes and Transcriptomes of Duckweeds

An, D; Li, CS; Zhou, Y; Wu, YR; Wang, WQ. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 230

Duckweeds (Lemnaceae family) are the smallest flowering plants that adapt to the aquatic environment. They are regarded as the promising sustainable feedstock with the characteristics of high starch storage, fast propagation, and global distribution. The duckweed genome size varies 13-fold ranging from 150 Mb in *Spirodela polyrhiza* to 1,881 Mb in *Wolffia arrhiza*. With the development of sequencing technology and bioinformatics, five duckweed genomes from *Spirodela* and *Lemna* genera are sequenced and assembled. The genome annotations discover that they share similar protein orthologs, whereas the repeat contents could mainly explain the genome size difference. The gene families responsible for cell growth and expansion, lignin biosynthesis, and flowering are greatly contracted. However, the gene family of glutamate synthase has experienced expansion, indicating their significance in ammonia assimilation and nitrogen transport. The transcriptome is comprehensively sequenced for the genera of *Spirodela, Landoltia*, and *Lemna*, including various treatments such as abscisic acid, radiation, heavy metal, and starvation. The analysis of the underlying molecular mechanism and the regulatory network would accelerate their applications in the fields of bioenergy and phytoremediation. The comparative genomics has shown that duckweed genomes contain relatively low gene numbers and more contracted gene families, which may be in parallel with their highly reduced morphology with a simple leaf and primary roots. Still, we are waiting for the advancement of the long read sequencing technology to resolve the complex genomes and transcriptomes for unsequenced *Wolffiella* and *Wolffia* due to the large genome sizes and the similarity in their polyploidy.

Physiology

Boron accumulation by *Lemna minor* L. under salt stress

Liu, CG; Gu, WC; Dai, Z; Li, J; Jiang, HR; Zhang, Q. (2018) SCIENTIFIC REPORTS 8: Article Number: 8954

Excess boron (B) is toxic to aquatic organisms and humans. Boron is often present in water with high salinity. To evaluate the potential of duckweed (*Lemna minor* L.) for removing B from water under salt stress, we cultured duckweed in water with 2 mg/L of B and sodium chloride (NaCl) concentrations ranging from 0 to 200 mM for 4 days. The results show that with increasing salinity, the capacity of *L. minor* to accumulate B initially decreased and then increased. *L. minor* used different mechanisms to accumulate boron at lower and higher levels of salt stress. The growth and chlorophyll synthesis of *L. minor* were significantly inhibited when the concentration of NaCl reached 100 mM. Our results suggest that *L. minor* is suitable for the accumulation of B when NaCl salinity is below 100 mM.

Improving biomass and starch accumulation of bioenergy crop duckweed (*Landoltia punctata*) by abscisic acid application

Liu, Y; Chen, XY; Wang, XH; Fang, Y; Huang, MJ; Guo, L; Zhang, Y; Zhao, H. (2018) SCIENTIFIC REPORTS 8: Article Number: 9544

Duckweed is a valuable feedstock for bioethanol production due to its high biomass and starch accumulation. In our preliminary experiment, we found that abscisic acid (ABA) could simultaneously increase starch and biomass accumulation of duckweed, but the mechanisms are still unclear. The results showed that the biomass production of duckweed reached up to 59.70 and 63.93 g m^{-2} in 6 days, respectively, with an increase of 7% ($P < 0.05$) compared to the control. The starch percentage increased from 2.29% up to 46.18% after 14 days of treatment, with a total of starch level 2.6-fold higher than that of the control. Moreover, the level of endogenous ABA, zeatin-riboside (ZR) and indole-3-acetic acid (IAA) increased, while gibberellins (GAs) decreased. Notably, ABA content in treated samples reached 336.5 mg/kg (fresh weight), which was 7.5-fold greater than that of the control. Importantly, the enzyme activities involved in starch biosynthesis increased while those catalyzing starch degradation decreased after ABA application. Taken together, these results indicated that ABA can promote biomass and starch accumulation by regulating endogenous hormone levels and the activity of starch metabolism related key enzymes. These results will provide an operable method for high starch accumulation in duckweed for biofuels production.

Retrospective analyses of archive phytotoxicity test data can help in assessing internal dynamics and stability of growth in laboratory duckweed cultures

Olah, V; Hepp, A; Vaca, NYG; Tamas, M; Meszaros, I. (2018) AQUATIC TOXICOLOGY 201: 40-46

High growth potential of duckweed species (Lemnaceae family) has been utilized in wide range of research and practical applications. Based on literature data, however, it can be assumed that duckweed populations maintain constant growth rates only when short periods are considered but can vary over longer time scales. This intrinsic instability in growth can affect the interpretation of growth data. Duckweed phytotoxicity tests are usually performed according to highly standardized protocols. Therefore the archive data provide an opportunity for retrospective comparisons. In the present study we collected growth (frond number- and frond area-based relative growth rates) and

morphology (average frond and colony sizes) data from control treatments of phytotoxicity tests. All the analyzed tests were carried out with the same *Spirodela polyrhiza* (L.) Schleid. (giant duckweed) clone (RDSC ID No. 5501) under the same experimental conditions over more than four years. We aimed to assess the overall variability of the above parameters and to test if intrinsic growth patterns affect growth data in short-term. In general, the results reflected high stability of the measured parameters in long term but also indicated that some temporal variability is inevitable which can bias the comparability of growth tests. The frond area-based relative growth rate resulted in smaller coefficient of variation than the usually preferred frond number-based one. The results also revealed a negative correlation between mean growth rates and their coefficients of variation. Therefore, it would be advisable to introduce higher minimal growth rates and/or maximized tolerable coefficients of variation for control cultures into the standard duckweed growth inhibition tests. Analyses of growth data aggregated on seasonal basis indicated faster growth and larger mean frond size in laboratory duckweed cultures from mid-autumn till mid-spring than during summer and early autumn. But, in shorter term (similar to 50 days) we did not observe distinct trends in growth suggesting that the successive frond generations have no effect on growth traits within this time-scale. Our results point to the importance of assessing intrinsic growth dynamics in duckweed cultures and also to the re-usability of the already collected phytotoxicity data in addressing new research questions.

Effect of exogenous general plant growth regulators on the growth of the duckweed *Lemna minor*

Utami, D; Kawahata, A; Sugawara, M; Jog, RN; Miwa, K; Morikawa, M. (2018) FRONTIERS IN CHEMISTRY 6: Article Number: 251

Gibberellic acid (GA_3), indole-3-acetic acid (IAA), salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA) 1-amino cyclopropane-1-carboxylic acid (ACC) and aminoethoxyvinylglycine (AVG) are popular growth regulators of plants. However, the effects of their exogenous addition on the biomass production of aquatic plants, including Lemnoideae plants, "duckweeds," are largely unknown. In this study, the growth of *Lemna minor* was tested for 10 d in Hoagland medium containing each compound at different concentrations of 0-50 mu M. GA_3, IAA, and SA were found to have no apparent positive effect on the growth at all concentrations tested. Conversely, ACC and JA moderately and AVG and ABA severely inhibited the growth of L. minor. Among the tested compounds, ascorbic acid had an apparent growth-promoting effect.

Phytomedicine

Spirodela polyrhiza and its chemical constituents exert anti-allergic effects via ORAI1 channel inhibition

Nam, YR; Kim, HJ; Nam, JH; Kim, WK. (2018) ALLERGY 73: 734-734. Supplement: 105. Special Issue: SI. Meeting Abstract: 1453

No abstract available.

Phytoremediation

Preliminary assessment of heavy metals in water, sediment and macrophyte (*Lemna minor*) collected from Anchar Lake, Kashmir, India

Showqi, I; Lone, FA; Naikoo, M. (2018) APPLIED WATER SCIENCE 8: Article Number: UNSP 80

Water samples, sediments and free floating macrophytic plant, *Lemna minor* specimens were collected from five designated sites in Anchar lake (Srinagar, J&K, India) to assess its heavy metal (Cu, Cr, Zn, Ni, Cd, Pb) load and changes on seasonal basis. The concentration of heavy metals was determined using atomic absorption spectroscopy. Most of the samples were found within limits of maximum permissible concentrations as recommended by WHO (Guidelines for drinking water quality, pp 491-493, 2006). During all the seasons, highest concentration of all heavy metals (Cu, Cr, Zn, Ni, Cd, Pb) was recorded at highly polluted sites of the lake viz. near agricultural fields (S-1), near settlements (S-3) and SKIMS (S-4). These sites received huge agrochemical run-off from the surrounding agricultural fields, solid and liquid wastes from the nearby catchment areas and effluents from Sher-e-Kashmir Institute of Medical Sciences (SKIMS) compared to control site lake centre (S-5). Furthermore, most of the metals in water and sediment were found with highest concentration during autumn (Viz., Cu-1.5 ppm; Zn-0.38 ppm; Ni-1.89 ppm; Pb-0.84 ppm in water and Cu-26.9 ppm; Zn-13.6 ppm; Pb-4.33 ppm in sediment) and summer (Viz., Cr-0.68 ppm in water and Ni-4.8 ppm; Cd-2.6 ppm; Cr-8.01 ppm in sediment) seasons. Also in *Lemna minor* plant highest concentration was observed during summer season (Cu-29.09 ppm; Zn-19.11 ppm; Ni-5.7 ppm; Cd-1.34 ppm; Cr-9.18 ppm and Pb-9.77 ppm). From these observations, it was found that the sources of heavy metals in Anchar lake were both natural and anthropogenic ones. This study recommended that continuous monitoring of heavy metals (Viz; Cu, Cr, Zn, Ni, Cd and Pb) in water, sediment and other aquatic biota of Anchar lake should be directed to protection of ecological status of the lake and its surrounding area.

Phytoremediation of tetracycline and degradation products from aqueous solutions

Topal, M; Obek, E; Senel, GU; Topal, EIA. (2018) POLLUTION 4: 471-480

The present study aims at phytoremediation of *Lemna gibba* L. in aqueous solutions with different concentrations of TC and Degradation Products (DPs). It also tries to determine whether there are differences in TC, ETC, EATC, and ATC levels, accumulated by *Lemna gibba* L. Exposure concentrations of 50, 100, and 300 ppb have been selected for TC and DPs, showing that the highest TC50, TC100, and TC300 concentrations in the plant have been 23.5 ± 1.1, 80.1 ±3.9, and 274 ± 13 ppb, respectively, while the highest ETC50, ETC100, and ETC300 have proven to be 39.5 ± 1.9, 47.8 ± 2.4, and 168 ± 8.4 ppb, respectively. The highest EATC50, EATC100, and EATC300 concentrations in the plant have been 45.3 ± 2.3; 65 ± 3.0 and 173 ± 9.0 ppb, respectively, whereas the highest ATC50, ATC100, and ATC300 concentrations in *Lemna gibba* L. have been 34.7 ± 1.7, 39.6 ± 0.2, and 114 ± 5.6 ppb, respectively. TC, ETC, EATC, and ATC concentrations in *Lemna gibba* L. have increased with the increase of initial TC, ETC, EATC, and ATC concentration.

Can aquatic macrophytes be biofilters for gadolinium based contrasting agents?

Braun, M; Zavanyi, G; Laczovics, A; Berenyi, E; Szabo, S. (2018) WATER RESEARCH 135: 104-111

The use of gadolinium-based contrasting agents (GBCA) is increasing because of the intensive usage of these agents in magnetic resonance imaging (MRI). Waste-water treatment does not reduce anthropogenic Gd-concentration significantly. Anomalous Gd-concentration in surface waters have been reported worldwide. However, removal of GBCA-s by aquatic macrophytes has still hardly been investigated. Four aquatic plant species (*Lemna gibba, Ceratophyllum demersum, Elodea nuttallii, E. canadensis*) were investigated as potential biological filters for removal of commonly used but structurally different GBCA-s (Omniscan, Dotarem) from water. These plant species are known to accumulate heavy metals and are used for removing pollutants in constructed wetlands. The Gd uptake and release of the plants was examined under laboratory conditions. Concentration-dependent infiltration of Gd into the body of the macrophytes was measured, however significant bioaccumulation was not observed. The tissue concentration of Gd reached its maximum value between day one and four in *L. gibba* and *C. demersum*, respectively, and its volume was significantly higher in *C. demersum* than in *L. gibba*. In C. demersum, the open-chain ligand Omniscan causes two-times higher tissue Gd concentration than the macrocyclic ligand Dotarem. Gadolinium was released from Gd-treated duckweeds into the water as they were grown further in Gd-free nutrient solution. Tissue Gd concentration dropped by 50% in duckweed treated by Omniscan and by Dotarem within 1.9 and 2.9 days respectively. None of the macrophytes had a significant impact on the Gd concentration of water in low and medium concentration levels (1-256 μg L^{-1}). Biofiltration of GBCA-s by common macrophytes could not be detected in our experiments. Therefore, it seems that in constructed wetlands, aquatic plants are not able to reduce the concentration of GBCA-s in the water. Furthermore, there is a low risk that these plants cause the accumulation of anthropogenic Gd in the food chain.

Phytotoxicology

Physiological responses in *Lemna minor* frond to high concentrations of zinc, copper and chromium

Peng, X; Guo, XY; Ding, ZH; Xin, GR. 2018. PAKISTAN JJOURNAL OF BOTANY 50: 2151-2157

Robust, rapid bioindicators of heavy metal water pollution, which are responsible for increasing environmental threats globally, are required. In the present study, we investigated the possibility of using short-term (≤ 12 hour) physiological responses of *Lemna minor* to high concentrations (up to 10 mM) of zinc (Zn), lead (Pb), copper (Cu) and chromium (Cr) for this purpose. The results showed that (a) increase in Pb, Zn, Cu, and Cr levels increased fronds' malonaldehyde (MDA) contents, whereas increase in Pb, Cu, and Cr levels also reduced peroxidase activity (POD), although some of these effects were only observed at high concentrations, (b) high Cu and Cr levels reduced fronds' chlorophyll contents, but Zn increased chlorophyll content from 0.0016 mM; (c) all four heavy metals induced frond abscission, and the percentage of frond abscission remain stable (except for Pb) after exposure for 10 h. The maximal concentrations of Zn, Cu and Cr resulted in > 50% frond abscission rates (EFAC50) within 10 h, but Pb induced much weaker responses. Hence frond abscission would not be a suitable short-term indicator of Pb pollution.

Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L.

Zicari, MA; d'Aquino, L; Paradiso, A; Mastrolitti, S; Tommasi, F. (2018) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 163: 536-543

An increasing input rate of rare earth elements in the environment is expected because of the intense extraction of such elements form their ores to face human technological needs. In this study *Lemna minor* L. plants were grown under laboratory conditions and treated with increasing concentrations of cerium (Ce) ions to investigate the effects on plant growth and antioxidant systems. The growth increased in plants treated with lower Ce concentrations and reduced in plants treated with higher concentrations, compared to control plants. In plants treated with higher Ce concentrations lower levels of chlorophyll and carotenoid and the appearance of chlorotic symptoms were also detected. Increased levels of hydrogen peroxide, antioxidant metabolites and antioxidant activity confirmed that higher Ce concentrations are toxic to *L. minor*. Ce concentration in plant tissues was also determined and detectable levels were found only in plants grown on Ce-supplemented media. The use of duckweed plants as a tool for biomonitoring of Ce in freshwater is discussed.

The effect of hazardous pollutants from coal combustion activity: Phytotoxicity assessment of aqueous soil extracts

Radic, S; Medunic, G; Kuharic, Z; Roje, V; Maldini, K; Vujcic, V; Krivohlavek, A. (2018) CHEMOSPHERE 199: 191-200

Airborne fly ash and related hazardous particles derived from coal combustion contaminate soil and groundwater, negatively affecting ecosystems. The aim of this study was chemical and toxicological evaluation of aqueous extracts of soil collected from the vicinity of a coal-fired Plomin power plant (PPP), using *Lemna* (*Lemna minor* L.) bioassay and additional biochemical indicators - photosynthetic pigments, lipid peroxidation, antioxidative enzymes and glutathione. Topsoil samples were collected from distances of 200,300, 400 and 800 m from the PPP in accordance with the prevailing SW wind direction. Elevated levels of polycyclic aromatic hydrocarbons (up to 15,765 ng L^{-1}) and potentially toxic trace elements were detected in the Plomin soil extracts (PEs) in comparison to control soil extract (CE). Trace elements accumulated in L minor were mostly in accordance with their concentrations in PEs. The results demonstrate that PEs induced significant growth inhibition and other phytotoxic effects. Those effects can be related to damage caused by increased production of reactive oxygen species and impaired antioxidant levels. The connection among the phytotoxicity, a distribution of analyzed contaminants, and distances from the PPP is clearly established.

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

Contributions to DF must be written in English, although they may be submitted by authors from any country. Authors who are not native English speakers may appreciate assistance with grammar, vocabulary, and style when submitting papers to the DF.
DF is currently arranged in sections, which may be chosen by a prospective author(s) to contribute to: Main text, Opinion paper, Discussion corner, Useful methods, Student experiments, Student spotlight, Science meets art, and Cover photo(s). 1,000 words are suggested as the upper limit for each contribution, but can be extended on request to the Steering Committee if the reason for the waiver request is warranted.

Presubmissions

In addition to invitees by a Duckweed Steering Committee member, if you are considering submitting a contribution to DF but are unsure about the fit of your idea, please feel free to contact one of the members in the Duckweed Steering Committee in order to obtain feedback as to the appropriateness of the subject for DF. Please include a few sentences describing the overall topic that you are interested to present on, and why you think it is of interest to the general duckweed community. If you have the abstract or draft text prepared, please include it. The Duckweed Steering Committee will discuss the material in one of its meetings and the decision to formally invite submission will be given shortly afterwards.

Copyright and co-author consent

All listed authors must concur in the submission and the final version must be seen and approved by all authors of the contribution. As a public forum, we do not carry out any Copyright application. If you need to copyright your material, please do so beforehand.

Formatting requirements:

- A commonly used word processing program, such as Word, is highly recommended.

- Formatting requirements: 8.5-by-11-inch (or 22 cm-by-28 cm) paper size (standard US letter).
- Single-spaced text throughout.
- One-inch (or 2.5 cm) left and right, as well as top and bottom margins.
- 11-point Times New Roman font.
- Number all pages, including those with figures on the bottom and center of each page.

Title:

- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
- Should avoid acronyms or abbreviations aside from the most common biochemical abbreviations (e.g., ATP). Other acronyms or abbreviations should either:
    - be introduced in their full form (e.g., Visualization of Polarized Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) Activity in Live Cells by Fluorescence Resonance Energy Transfer (FRET) Imaging); or
    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

Authors:

- All authors are responsible for the content of the manuscript.
- Provide the complete names of all authors.
- Identify which author will receive correspondence regarding the contribution.
- Provide the corresponding author's name, telephone number, and current e-mail address.

Image resolution and submission:

It is extremely important that figures be prepared with the proper resolution for publication in order to avoid inaccurate presentation of the data. The minimum acceptable resolution for all figures is 300 dpi. Excessive file compression can distort images, so files should be carefully checked after compression. Note that figures that contain both line art (such as graphs) and RGB/grayscale areas (such as photographs) are best prepared as EPS (vector) files with embedded TIFF images for the RGB/grayscale portions. The resolution of those embedded TIFF images should be at least 300 dpi. Original images should be submitted as a separate file to the text file. It would be helpful to insert the intended into the Word file as well, if desired, to indicate the location for it. The legend to the image/figure should be added at the end of the text file and labeled as "Legend to Figures".

Links for Further Reading

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

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