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Clarification of total number of recognized duckweed species

Regarding the number of Duckweed species, Dr. Eric Lam commented recently:

"A recurring question that came up in discussions with colleagues and students is what the true number of recognized species for duckweed. The two numbers that have been quoted are 38 and 37. After a recent discussion with Klaus Appenroth, it is confirmed that it should be 37. Although a paper in 2002 from Don Les' group listed 38 species, *Lemna ecuadoriensis* was combined with *Lemna obscura* by Elias Landolt. This was subsequently supported by the recent AFLP analysis results from the Appenroth lab (Planta 2010) where 84 clones of Lemna were compared. I hope this comment will help to lessen the confusion in the field and stimulate additional discussions if necessary."



Lemna obscura (photo kindly taken by Walter Lämmler)

For more details or discussions about this issue, Dr. Eric Lam (ericl89@hotmail.com) or Dr. Klaus Appenroth (Klaus.Appenroth@uni-jena.de) are the right people.



MamaGrande's news in South America

MamaGrande is a biorefinery social business that promotes a full cycle process to clean water, grow high starch biomass and create local jobs.



In our pilot plant in Argentina (<http://goo.gl/maps/ArNMU>), the 8 experimental canals (10 m² each) are doing great, with *Lemna gibba* growing 60% of it's biomass/day at a minimum of 38% dry weight starch without any optimization.

5 of the 8 experimental canals in MamaGrande's pilot biorefinery in Totoras

If the 4 ha. of wastewater treatment lagoons (41.000 m²) can maintain in summer half of that production, that would mean ~230 kg of starch a day.



Panorama view of the lagoon #2.

$$41.000 \text{ m}^2 * 1 \text{ kg (fresh)}/\text{m}^2 * 30\% \text{ growth/day} = 12300 \text{ kg (fresh)}/\text{day produced}$$

$$12300 \text{ kg (fresh)}/\text{day} * 5\% \text{ (dry weight)} * 38\% \text{ (starch content)} = 233 \text{ kg of starch/day}$$

Not bad, coming from cleaning wastewater...

We are also trying different methods to manage the crop along the full lagoons surface, an interesting challenge, creating lanes to tame the wind effect and move the duckweeds as they grow to the harvesting canal (~150x4.5m=675m²).



Partial view of the harvesting canal filled with Lemna gibba in the back of Federico, Sebastián and Eduardo (cofounders of MamaGrande).

By the way, we are constantly reminded about why Duckweed's name: we have a community of more than 50 (and growing) very happy ducks that must be continually fended off, or they eat a substantial amount of production every day. :)

We are working on solutions for this too.

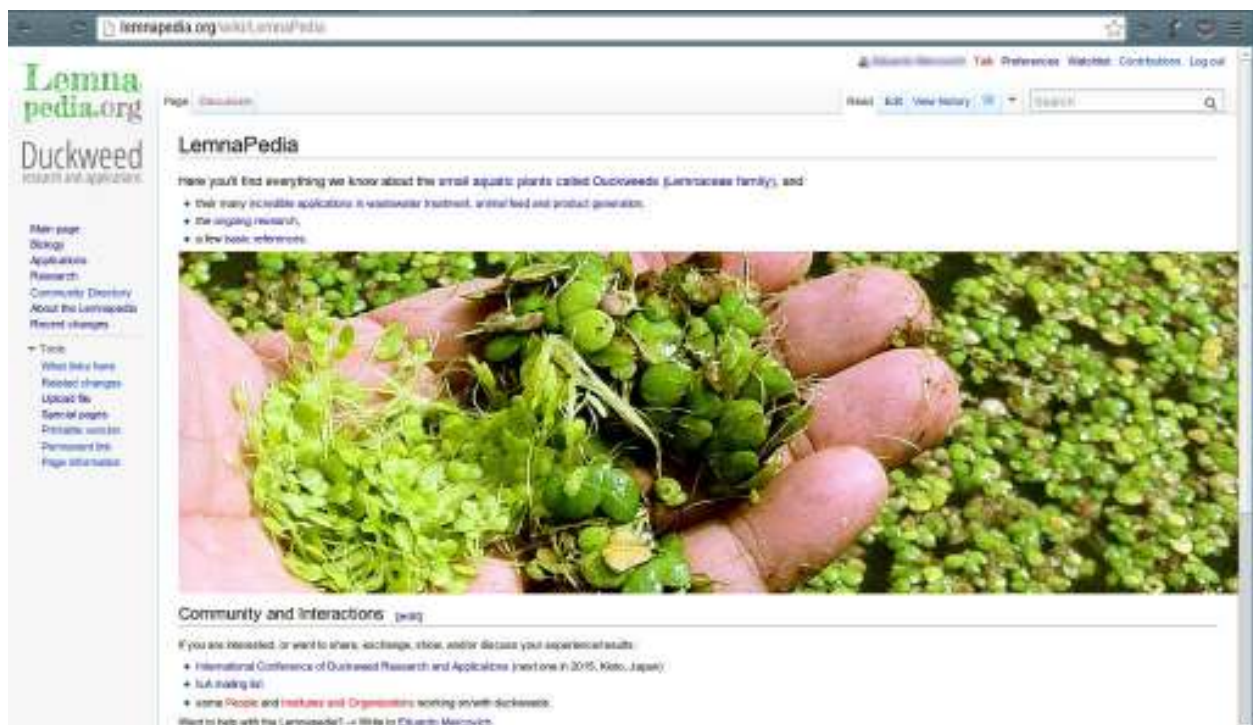
For more information about the MamaGrande pilot, write to info@mamagrande.org.



The Lemnapedia is starting

In the 2nd International Conference on Duckweed Research and Applications (ICDRA), a few of us discussed how could we help to lower the entrance barrier and facilitate knowledge exchange of the field, while showing the world the potential of this incredible plant.

Thus, the idea of a centralized information space in which to integrate all the Lemna related information to facilitate the realization of the Duckweed potential for Humanity was born: <http://Lemnapedia.org>.



Lemnapedia front page.

While it took a few months to start, we are now in the first steps already.

If anyone is interested to help, you are very welcome: just write to Tamra Fakhoorian (tamraf9@gmail.com) or Eduardo Mercovich (eduardo@mamagrande.org).

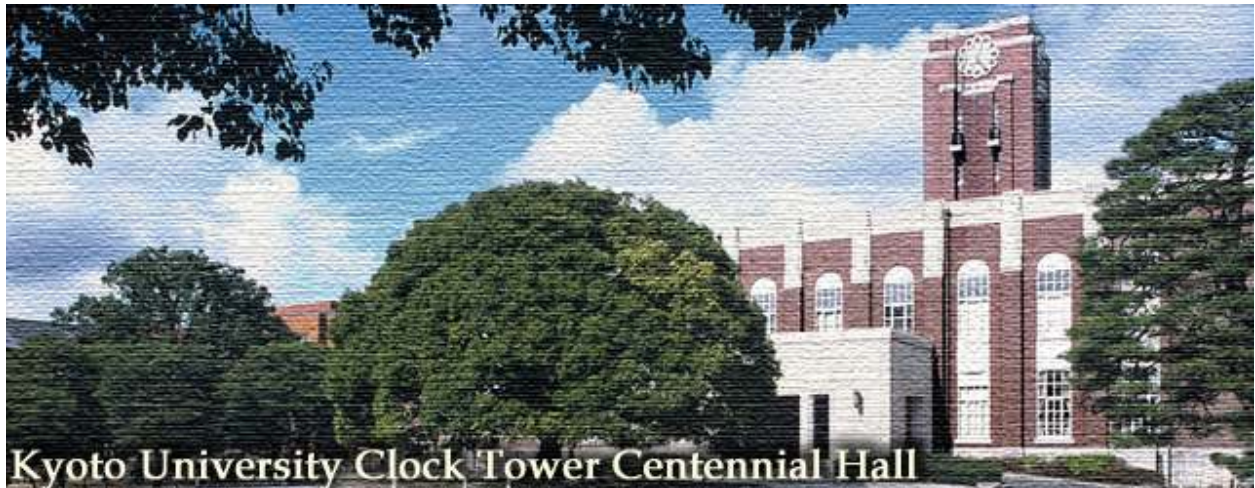


International Conference on Duckweed Research and Applications 2015, Kyoto

Plan your trip to the beautiful Kyoto for 27th (Sat) to 29th (Mon) June 2015: the 3rd International Conference on Duckweed Research and Applications is on its way.

Kyoto 2015 will be an intense time of exchange, discovery and discussion, with the added pleasure of seeing again known fellows and getting to know new people. It will be, for sure, even better than the previous ones

(http://lemnepedia.org/wiki/International_Conference_of_Duckweed_Research_and_Applications).



As before, it will cover many levels, from biology and botany, to physiology and genetics, passing through applications in bioremediation and biofuels, among others.

If you have any questions, Dr. Masaaki Morikawa (morikawa@ees.hokudai.ac.jp) is one of the organizers.



Spirodela genome published

A few days ago, Wenqin Wang wrote:

“The Spirodela genome is published [...] The transcriptome analysis is online too [...].

So at the earliest time I want to share both the genome and transcriptome data with you. I wish the reference genome and transcriptome will help and accelerate the duckweed research in our community. [...]”

This wonderful news, along with the links to access and download the data are included as the first 2 new publications in this letter.

If you have any questions, please feel free to ask Wenqin Wang at duckweed3@gmail.com.



New research publications

Genome sequencing of the model aquatic plant, *Spirodela polyrhiza*

Wenqin Wang, Joachim Messing (unpublished yet)

Duckweed is a promising source of sustainable energy and also has been used for wastewater treatment. It is also a basal monocot that reveals its important taxonomical rank. Thus its genome sequence is invaluable resource for both comparative genomics and as a new reference for duckweed research. Here, the paper with the title of "The *Spirodela polyrhiza* genome reveals insights into its neotenus reduction, fast growth and aquatic lifestyle" has just been published in Nat. Comm.

(<http://www.nature.com/ncomms/2014/140219/ncomms4311/full/ncomms4311.html>).

It reports the analysis of the 158 Mb *Spirodela* genome that is predicted to contains only 19,623 protein-coding genes from 32 chromosome-sized pseudomolecules. Reduced gene families and missing genes reflect changes consistent with its simple and reduced morphology, forever-young life style and aquatic suspension. It underwent two ancient rounds of large-scale whole-genome duplications and no recent retrotranspositions. Besides the Rutgers group, the authors on the publication also include researchers from MIPS/IBIS, Helmholtz Center Munich, Germany; University of California, Davis; Georgia Institute of Technology; Brookhaven National Laboratory; Donald Danforth Plant Science Center; University of Jena, Germany, HudsonAlpha Institute for Biotechnology; and the Leibniz - Institute of Plant Genetics and Crop Plant Research (IPK), Germany.

There are multiple options to access the data. One can:

- download the data at this link: <http://www.waksman.rutgers.edu/spirodela/home>,
- search online with BLAST directly: <http://pgir.rutgers.edu/blast/blast.html>.

There is also a news release at both Rutgers

(<http://news.rutgers.edu/research-news/pond-dwelling-powerhouses-genome-points-its-biofuel-potential/20140219#.UxnV4OA4p0q>) and JGI webpage (http://www.jgi.doe.gov/News/news_14_02_19.html).

If you are interested in learning more about duckweed research, please check SpirodelaBase: <http://www.waksman.rutgers.edu/spirodela/home>.

Transcriptome studies uncover insights into dormancy of Greater duckweed

Wenqin Wang, Joachim Messing Waksman (unpublished yet)



Greater Duckweed, *Spirodela polyrhiza*, is an aquatic plant that grows very fast by converting sun energy into biomass. It prefers to propagate clonally by budding fronds rather than through sexual reproduction under favorable conditions. In order to survive under cold winter or shortage of nutrition, it generates the dormant structure of turions that will form fronds again in spring with rising temperature. To understand the developmental change during its life cycle, differential expression of genes can be an important step in understanding processes that underlie this developmental switch. Here, the paper "RNA-Seq transcriptome analysis of *Spirodela* dormancy without reproduction" published in BMC Genomics (<http://www.biomedcentral.com/1471-2164/15/60>), examines quantitatively the transcriptome of turion development using next generation sequencing technology, referred to as RNA-Seq. It reports that the genes involved in the pathways of signal transduction, seed dehydration, carbohydrate and secondary metabolism, and senescence are up-regulated, whereas the genes responsible for rapid growth and biomass accumulation through DNA assembly, protein synthesis and carbon fixation are repressed at the onset of dormancy. Noticeably, it identified tissue-specific promoters of LEA genes and key genes for starch synthesis (APS1, APL3 and GBSSI), which could be used in chimeric gene construction to engineer duckweeds for practical applications easing agricultural production of food crops.

Effect of soluble copper released from copper oxide nanoparticles solubilisation on growth and photosynthetic processes of *Lemna gibba* L

Perreault, Francois; Samadani, Mahshid; Dewez, David

NANOTOXICOLOGY **8**: 374-382 (2014)

Copper oxide nanoparticles (CuO NPs) are used as a biocide in paints, textiles and plastics. Their application may lead to the contamination of aquatic ecosystems, where potential environmental effects remain to be determined. Toxic effects may be related to interactions of NPs with cellular systems or to particles' solubilisation releasing metal ions. In this report, we evaluated CuO NPs and soluble copper effects on photosynthesis of the aquatic macrophyte *Lemna gibba* L to determine the role of particle solubility in NPs toxicity. When *L. gibba* plants were exposed 48 h to CuO NPs or soluble copper, inhibition of photosynthetic activity was found, indicated by the inactivation of Photosystem II reaction centers, a decrease in electron transport and an increase of thermal energy dissipation. Toxicity of CuO NPs was mainly driven by copper ions released from particles. However, the bioaccumulation of CuO NPs in plant was shown, indicating the need to evaluate organisms of higher trophic level.



Bioaugmentation involving a bacterial consortium isolated from the rhizosphere of *Spirodela polyrhiza* for treating water contaminated with a mixture of four nitrophenol isomers

Kristanti, Risky Ayu; Toyama, Tadashi; Hadibarata, Tony; et al.

RSC ADVANCES **4**: 1616-1621 (2014)

A flask-scale laboratory study was performed to assess the bioaugmentation of water contaminated with a mixture of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol by using a bacteria consortium consisting of three nitrophenol-degrading bacteria strains (*Pseudomonas* sp. strain MFR-1, *Pseudomonas* sp. strain PFR-1 and *Rhodococcus* sp. strain DFR-1), reinoculated into the roots of *Spirodela polyrhiza*. The selected strains were colonized into the root at approximately $10(4)$ to $10(6)$ colony-forming units (CFU per plant). The high populations remained stable through five sequential two-days degradation cycles and complete nitrophenol removal was achieved within five-repeated cycles. Hence, inoculation of subjected degraders into the roots of aquatic plants is an effective treatment for nitrophenol-contaminated effluents or aquatic resources.

The Lemna Bioassay: Contemporary Issues as the Most Standardized Plant Bioassay for Aquatic Ecotoxicology

Mkandawire, Martin; Da Silva, Jaime A. Teixeira; Dudel, E. Gert

CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY **44**: 154-197 (2014)

The Lemna bioassay is one of the most standardized higher plant bioassays for assessing the impacts of contaminants in aquatic environments. The simple anatomy and ease with which *Lemna* sp. can be handled makes them ideal test organisms. They have been used to predict the cytotoxic, cytogenetic, and mutagenic effects of several chemical pollutants including, inter alia, polycyclic aromatic hydrocarbons, heavy metals, metalloids, organometallic compounds, pesticides, pharmaceuticals, radionuclides, and pharmaceuticals. However, there is still some contention as to the exact scope of application and definition of the Lemna bioassay regarding its accuracy of prediction and toxicity assessment. In this article, we review some critical issues on the development and effective use of the Lemna bioassay.

The clonal dependence of turion formation in the duckweed *Spirodela polyrhiza* - an ecogeographical approach



Kuehdorf, Katja; Jetschke, Gottfried; Ballani, Ludwig; Appenroth; Klaus-J.

PHYSIOLOGIA PLANTARUM **150**: 46-54 (2014)

Formation of turions, the vegetative perennation organs, plays an important role in the survival strategy of *Spirodela polyrhiza* (L.) Schleiden. Turion formation [quantified as number of turions formed per frond; specific turion yield (SY)] was investigated in 27 clones collected from a wide geographical range. The Pearson correlation was tested with (1) duration of growing season (monthly average temperature of 10 degrees C), (2) relative growth rate of the fronds, (3) longitude and latitude, and (4) several climatic parameters, in all possible single and multiple regressions. All single coefficients of determination were below 0.10. The highest correlation ($R^2 = 0.61$; adjusted for the number of explaining variables 0.54) was found in a multiple linear regression with the following five parameters: average temperatures over the year and during the growing season, duration of the growing season and precipitation over the year and during the growth period. All these parameters were shown to have significant contributions. This equation was used successfully to predict the SY of five newly isolated clones. Finally, on the basis of all 32 clones the following conclusions were drawn: The mean annual temperature has the highest impact. It is suggested that lower temperatures decrease the survival rate of turions and that adaptation refers to increasing SY. The different levels of SY in the clones (ranging from SY = 0.22 to 5.9) were detected even after several years of in vitro cultivation. It is therefore assumed that these adaptations to the climatic conditions are genetically determined.

Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species and cloning of a Delta 6-desaturase responsible for the production of gamma-linolenic and stearidonic acids in *Lemna gibba*

Yan, Yiheng; Candreva, Jason; Shi, Hai; et al.

BMC PLANT BIOLOGY **13**: 201 (2013)

Background: Duckweeds, i.e., members of the *Lemnoideae* family, are amongst the smallest aquatic flowering plants. Their high growth rate, aquatic habit and suitability for bio-remediation make them strong candidates for biomass production. Duckweeds have been studied for their potential as feedstocks for bioethanol production; however, less is known about their ability to accumulate reduced carbon as fatty acids (FA) and oil.

Results: Total FA profiles of thirty duckweed species were analysed to assess the natural diversity within the *Lemnoideae*. Total FA content varied between 4.6% and 14.2% of dry weight whereas triacylglycerol (TAG) levels varied between 0.02% and



0.15% of dry weight. Three FA, 16:0 (palmitic), 18:2 Delta 9,12 (Linoleic acid, or LN) and 18:3 Delta 9,12,15 (alpha-linolenic acid, or ALA) comprise more than 80% of total duckweed FA. Seven *Lemna* and two *Wolffia* species also accumulate polyunsaturated FA containing Delta 6-double bonds, i.e., GLA and SDA. Relative to total FA, TAG is enriched in saturated FA and deficient in polyunsaturated FA, and only five *Lemna* species accumulate Delta 6-FA in their TAG. A putative Delta 6-desaturase designated LgDes, with homology to a family of front-end Delta 6-FA and Delta 8-sphingolipid desaturases, was identified in the assembled DNA sequence of *Lemna gibba*. Expression of a synthetic LgDes gene in *Nicotiana benthamiana* resulted in the accumulation of GLA and SDA, confirming it specifies a Delta 6-desaturase.

Conclusions: Total accumulation of FA varies three-fold across the 30 species of *Lemnoideae* surveyed. Nine species contain GLA and SDA which are synthesized by a Delta 6 front-end desaturase, but FA composition is otherwise similar. TAG accumulates up to 0.15% of total dry weight, comparable to levels found in the leaves of terrestrial plants. Polyunsaturated FA is underrepresented in TAG, and the Delta 6-FA GLA and SDA are found in the TAG of only five of the nine *Lemna* species that produce them. When present, GLA is enriched and SDA diminished relative to their abundance in the total FA pool.

Sulfidation of Silver Nanoparticles: Natural Antidote to Their Toxicity

Levard, Clement; Hotze, Ernest M.; Colman, Benjamin P.; et al.

ENVIRONMENTAL SCIENCE & TECHNOLOGY **47**: 13440-13448 (2013)

Nanomaterials are highly dynamic in biological and environmental media. A critical need for advancing environmental health and safety research for nanomaterials is to identify physical and chemical transformations that affect the nanomaterial properties and their toxicity. Silver nanoparticles, one of the most toxic and well-studied nanomaterials, readily react with sulfide to form Ag(0)/Ag₂S core-shell particles. Here, we show that sulfidation decreased silver nanoparticle toxicity to four diverse types of aquatic and terrestrial eukaryotic organisms (*Danio rerio* (zebrafish), *Fundulus heteroclitus* (killifish), *Caenorhabditis elegans* (nematode worm), and the aquatic plant *Lemna minuta* (least duckweed)). Toxicity reduction, which was dramatic in killifish and duckweed even for low extents of sulfidation (about 2 mol % S), is primarily associated with a decrease in Ag⁺ concentration after sulfidation due to the lower solubility of Ag₂S relative to elemental Ag (Ag⁰). These results suggest that even partial sulfidation of AgNP will decrease the toxicity of AgNPs relative to their pristine counterparts. We also show that, for a given organism, the presence of chloride in the exposure media strongly affects the toxicity results by affecting Ag speciation. These results highlight



the need to consider environmental transformations of NPs in assessing their toxicity to accurately portray their potential environmental risks.

Cadmium and nickel: Assessment of the physiological effects and heavy metal removal using a response surface approach by *L. gibba*

Demim, S.; Drouiche, N.; Aouabed, A.; et al.

ECOLOGICAL ENGINEERING **61**: 426-435 (2013)

In this study, the Response surface methodology (RSM) was used to describe the individual and interactive effects of two variables - initial Cd²⁺ concentration and initial Ni²⁺ concentration - at five levels. They were combined, under controlled conditions, according to a Central Composite Design (CCD) of the duckweed *L. gibba*. Parallely, the assessment regarding the removal of the aforementioned compounds took place. The effects of initial Cd²⁺ and initial Ni²⁺ concentrations on the growth parameters, the photosynthetic pigment and the removal of heavy metals were part of the study. The results were statistically analyzed using JMP 9.0.2 (SAS Institute) software. A second-order quadratic model could approximate the mathematical relationship between responses and two significant independent variables which showed the influence of each variable and their interactions. The analysis of variance (Anova) revealed the relative significance of the process parameters in responses. Moreover, a high correlation was found between the experimental and the predicted results, reflected by R² (coefficient of determination). This study has demonstrated that RSM can be applied to predict the physiological responses of *L. gibba* and the heavy metal removal. The method applied for this research allowed obtaining the maximum of the information counting with a limited set of laboratory experiments.

Bioremoval of C.I. Basic Red 46 as an Azo Dye from Contaminated Water by *Lemna minor* L.: Modeling of Key Factor by Neural Network

Movafeghi, A.; Khataee, A. R.; Torbati, S.; et al.

ENVIRONMENTAL PROGRESS SUSTAINABLE ENERGY **32**: 1082-1089 (2013)

This study was carried out to investigate the potential of duckweed (*Lemna minor* L.) for bioremediation of C.I. Basic Red 46 (BR46) solution. Our results revealed the notable ability of the plant species in biological degradation of BR46. The reusability of the plant in long-term repetitive operations was also confirmed. The decolorization efficiency was extremely dependent on different operational parameters such as initial dye concentration, pH, temperature, and amount of plant. Interestingly, activities of superoxide dismutase and peroxidase as antioxidant enzymes were increased in



different concentrations of the dye. In contrast, catalase activity declined in duckweed plants exposed to the dye. An artificial neural network (ANN) model was developed to predict the biological decolorization efficiency. The obtained data indicated that ANN provide realistic predictive performance ($R^2 = 0.981$).

A Single-Cell Bioluminescence Imaging System for Monitoring Cellular Gene Expression in a Plant Body

Muranaka, Tomoaki; Kubota, Saya; Oyama, Tokitaka

PLANT AND CELL PHYSIOLOGY **54**: 2085-2093 (2013)

Gene expression is a fundamental cellular process and expression dynamics are of great interest in life science. We succeeded in monitoring cellular gene expression in a duckweed plant, *Lemna gibba*, using bioluminescent reporters. Using particle bombardment, epidermal and mesophyll cells were transfected with the luciferase gene (luc+) under the control of a constitutive [Cauliflower mosaic virus 35S (CaMV35S)] and a rhythmic [Arabidopsis thaliana CIRCADIAN CLOCK ASSOCIATED 1 (AtCCA1)] promoter. Bioluminescence images were captured using an EM-CCD (electron multiply charged couple device) camera. Luminescent spots of the transfected cells in the plant body were quantitatively measured at the single-cell level. Luminescence intensities varied over a 1,000-fold range among CaMV35S::luc+-transfected cells in the same plant body and showed a log-normal-like frequency distribution. We monitored cellular gene expression under light-dark conditions by capturing bioluminescence images every hour. Luminescence traces of ≥ 50 individual cells in a frond were successfully obtained in each monitoring procedure. Rhythmic and constitutive luminescence behaviors were observed in cells transfected with AtCCA1::luc+ and CaMV35S::luc+, respectively. Diurnal rhythms were observed in every AtCCA1::luc+-introduced cell with traceable luminescence, and slight differences were detected in their rhythmic waveforms. Thus the single-cell bioluminescence monitoring system was useful for the characterization of cellular gene expression in a plant body.

Occurrence and habitat preference of aquatic macrophytes in a large river channel

Engloner, A. I.; Szalma, E.; Sipos, K.; et al.

COMMUNITY ECOLOGY **14**: 243-248 (2013)

This paper explores correlations between macrophyte occurrence and environmental characteristics recorded in a more than 350 rkm long segment of the main Danube



channel in Hungary. The selected river section belongs entirely to the lowland part of the Middle Danube, but it is separated into the mostly gravelly upper and the sandy lower river sections. Two markedly different groups of macrophytes correlated with this separation; the mostly perennial, rooting species (*Myriophyllum spicatum*, *Potamogeton crispus*, *P. nodosus*, *P. pectinatus*, *P. perfoliatus* and *Zannichellia palustris*) preferred the gravelly habitats, while the non-rooting, free-floating macrophytes (*Ceratophyllum demersum*, *Lemna minor*, *Salvinia natans* and *Spirodela polyrhiza*) occurred mainly in the sandy stretches. Based on current velocity and Secchi transparency, these stretches seemed to provide "more lotic" and "rather lentic" habitats. Data evaluation also revealed that the closer are the river stretches to a water course discharging upstream the more free-floating aquatic plants occur in the main Danube channel.

Studies on the toxicity of an aqueous suspension of C-60 nanoparticles using a bacterium (gen. *Bacillus*) and an aquatic plant (*Lemna gibba*) as in vitro model systems

Santos, Sandra M. A.; Dinis, Augusto M.; Rodrigues, David M. F.; et al.

AQUATIC TOXICOLOGY **142**: 347-354 (2013)

The increasing use of C-60 nanoparticles and the diversity of their applications in industry and medicine has led to their production in a large scale. C-60 release into wastewaters and the possible accumulation in the environment has raised concerns about their ecotoxicological impact. In the present study, an aqueous suspension of C-60 nanoparticles was prepared and its potential toxicity studied in laboratory, using a bacterium (*Bacillus stearothermophilus*) and an aquatic plant (*Lemna gibba*) as model systems. C-60 nanoparticles inhibited the growth of *L. gibba*, in contrast to that of the bacterium. Consistently, the ultrastructure and respiratory activity of bacterial cells were not affected by C-60, but the contents of chlorophylls a and band chloroplast oxygen production decreased considerably in *L. gibba*. Altogether, our results suggest that C-60 aqueous dispersions must be viewed as an environmental pollutant, potentially endangering the equilibrium of aquatic ecosystems.

(Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids

Peric, Brezana; Sierra, Jordi; Marti, Esther; et al.

JOURNAL OF HAZARDOUS MATERIALS **261**: 99-105 (2013)

Ionic liquids (ILs) are a promising group of compounds with a large variety of possible



structures and uses. They are considered as a potential "green" replacement for traditional volatile organic solvents, but their impact on the environment is often neglected or not studied enough. In the present study, selected representatives of two ILs groups were analyzed: a new family of protic ILs (derived from aliphatic amines and organic acids) and some frequently used aprotic ILs (substituted imidazolium and piridinium chlorides). The aquatic toxicity (test organisms *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Lemna minor*) and biodegradability tests were carried out. The additional tests with enzyme (acetylcholinesterase) and leukemia rat cells (IPC-81) provided more in-depth evaluation of toxicity. In our comparative hazard assessment protic ILs have EC50 values >100 mg L⁻¹ in all of the tests performed, except in the case of three representatives toward *Lemna minor*. They also show good biodegradability rates. The EC50 values for aprotic ILs are various orders of magnitude lower than the ones for protic ILs in most of the tests and they show a lower biodegradability potential. These findings indicate that protic ILs can be considered as environmentally safer alternatives for more toxic ILs and organic solvents. *Rhizobium paknamense* sp nov., isolated from lesser duckweeds (*Lemna aequinoctialis*).

***Rhizobium paknamense* sp nov., isolated from lesser duckweeds (*Lemna aequinoctialis*)**

Kittiwongwattana, Chokchai; Thawai, Chitti

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY **63**:
3823-3828 (2013)

A Gram-stain-negative, rod-shaped bacterium was isolated and designated strain L6-8(T) during a study of endophytic bacterial communities in lesser duckweed (*Lemna aequinoctialis*). Cells of strain L6-8(T) were motile with peritrichous flagella. The analysis of the nearly complete 16S rRNA gene sequence indicated that strain L6-8(T) was phylogenetically related to species of the genus *Rhizobium*. Its closest relatives were *Rhizobium borbori* DN316(T) (97.6 %), *Rhizobium oryzae* Alt 505(T) (97.3 %) and *Rhizobium pseudoryzae* J3-A1 27(T) (97.0 %). The sequence similarity analysis of housekeeping genes *recA*, *glnII*, *atpD* and *gyrB* showed low levels of sequence similarity (<91.5 %) between strain L6-8(T) and other species of the genus *Rhizobium* with validly published names. The pH range for growth was 4.0-9.0 (optimum 6.0-7.0), and the temperature range for growth was 20-45 degrees C (optimum 30 degrees C). Strain L6-8(T) tolerated NaCl up to 2 % (w/v) (optimum 1 % NaCl). The predominant components of cellular fatty acids were C-19:0 cyclo omega 8c (31.32 %), summed feature 8 (C-18:1 omega 7c and/or C-18:1 omega 6c; 25.39 %) and C-16:0 (12.03 %). The



DNA G + C content of strain L6-8(T) was 60.4 mol% (T-m). *nodC* and *nifH* were not amplified in strain L6-8(T). DNA-DNA relatedness between strain L6-8(T) and *R. borbori* DN316(T), *R. oryzae* Alt505(T) and *R. pseudoryzae* J3-A127(T). was between 11.2 and 18.3 %. Based on the sequence similarity analyses, phenotypic, biochemical and physiological characteristics and DNA-DNA hybridization, strain L6-8(T) could be readily distinguished from its closest relatives and represents a novel species of the genus *Rhizobium*, for which the name *Rhizobium paknamense* sp. nov. is proposed.