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EDITORIAL

After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm?

> Happy is he who in the present period of scientific mass attack on nature still has the opportunity to peacefully enjoy the growth of his 'material' and make observations, asking questions and at the same time answering them, so leading the questioner to enlightment. (H. Burgeff 1954; cited as the final sentence in the monographic study of Landolt & Kandeler 1987;).

There is hardly any need to explain why the production of plant biomass is of increasing importance for the future of our globe (Campbell 2013). It has long been known that duckweeds (Lemnaceae) are suitable for producing large amounts of biomass without competing with the agricultural land that is increasingly required for the production of food plants (Hillman & Culley 1978). A chapter of nearly 40 pages on 'Application and Economic Importance' can be found in the duckweed monograph published over a quarter of a century ago (Landolt & Kandeler 1987). This chapter provided a broad overview of the uses of the plant family, and the authors were aware of the great economic potential of duckweeds. However, at that time not even they could envision the current interest in the little plants, particularly as a source of biofuel or biogas. There seems little doubt that given Elias Landolt's knowledge of the biology of these organisms, and his common sense conservation concerns, that he could have provided valuable perspectives on the economic use of duckweeds well into the future.

The most basic, and in one sense most important, contribution Elias Landolt made with his studies of duckweeds was his thorough understanding and appreciation of the biological and evolutionary complexity of the group. There have been very few other such detailed investigations over decades of a taxonomic group of comparable size, in which an investigator has seen all the species in their native habitats as well as in cultivation. It can be validly argued that with so few morphological characters to study in such reduced, miniaturised flowering plants, detailed dedicated studies using additional sources of data were necessary to infer evolutionary relationships. Elias Landolt's rare combination of keen eye, patience and curiosity led to the amazing insights he achieved in understanding variation and relationships in duckweeds. The two-volume monograph of Lemnaceae (Landolt 1986; Landolt & Kandeler 1987) has to be seen to be appreciated, the two volumes are an amazing compilation of just about everything known about the family at the time, including use of duckweeds in developmental and physiological studies. More than 3,500 references are cited!

Someone with such a taxonomic mastery of the duckweeds could simply have been satisfied with knowing more than anyone else about his little creatures; in other words, he could have been content with universal recognition as the world authority on the group. However, as noted above, Elias Landolt was curious about Lemnaceae in a much broader context than their taxonomy. Part of this curiosity was manifested by his desire to collaborate with others in order to learn more about the duckweeds. For example, while he presented intuitive phylogenies for the duckweeds in his monograph, he was eager to have his hypotheses tested with more 'modern' methods (Les *et al.* 2002; Bog *et al.* 2010, 2013), and he really didn't care whether or not the data supported his ideas: in the true scientific spirit, he just wanted to understand. Although it should be obvious, it is nevertheless important to emphasise that in these collaborations Elias Landolt was generously providing material for other investigators worldwide from the extensive culture collection he maintained in Zurich for many years. It is difficult to overstate the time, effort and expense that went into obtaining and maintaining that material, not to mention the value of his expertise in attaching names to the material. Clearly, in all collaborations, he was the key person because he not only supplied the material, but he interpreted the results within the broad context of his knowledge of the organisms. It should be mentioned that, almost without exception, collaborative studies supported his taxonomic–phylogenetic concepts in Lemnaceae (Borisjuk *et al.* 2015; Tippery *et al.* 2015).

The systematic collection and typing of several thousand defined duckweed strains for more than 50 years by Elias Landolt provided an incomparable resource to the community. Dr. Landolt's generous attitude in sharing this resource and his knowledge of duckweeds also facilitated the development of systematics and biogeographic studies of the family, as well as an annotated source of plant material for a myriad of experimental studies. Indeed, several of the papers in this special issue cite Landolt collections as the sources of experimental material. Both his collection and his taxonomic expertise, combined with his readiness to share his resources with the duckweed community, laid the foundation for the current activity using duckweeds for basic and applied research. This activity is documented through the first International duckweed meetings in Chengdu, China, in 2011 and New Brunswick, New Jersey, in 2013 (Zhao *et al.* 2012; Lam *et al.* 2014). The physiological basis of the attractiveness of duckweeds as experimental organisms and for applications is mainly the very rapid vegetative growth rate of many of the 37 species of the family. It has been shown for the first time that duckweed clones represent the fastest growing of all flowering plants (Ziegler *et al.* 2015; see also the review of Kutschera & Nikals 2015). Interestingly, the variation in growth rates was demonstrated to be primarily at the clonal level (*i.e.* locally-adapted ecotypes) and not at the species level. The rapid production of biomass by duckweeds, together with the very small genome size in *Spirodela polyrhiza* (158 Mb; Wang *et al.* 2011; Wang & Messing 2015), were the reasons for selecting one clone (7498; Durham, NC, USA) for genome sequencing (Wang *et al.* 2014a,b). Genomic sequence data of another clone of *Spirodela polyrhiza* (9509; Jena, Germany) had previously been obtained, and a similar quality of genome assembly achieved for clone 7498 (Eric Lam, personal communication) was obtained. By cross-referencing and integrating the two genome drafts, more robust reference genome sequence maps can be expected. Also, for *Lemna gibba* and *Lemna minor* (Rob Martienssen, personal communication) genome assembly and gene annotation are already at an advanced stage and the data will soon be available. The strong impact of these results on fundamental research can be seen already (Wang *et al.* 2014a,b; Cao *et al.* 2015; cf. review of Wang & Messing 2015). One important step is the availability of an effective method for genetic transformation, and this has now been achieved (Vunsh *et al.* 2007; Cantó-Pastor *et al.* 2015). Polyploidisation is another interesting tool for genetic modification of the used duckweeds (Li *et al.* 2004; Vunsh *et al.* 2015).

Beside the high potential for biomass production, duckweeds have other important advantages. First, duckweeds can grow in eutrophic water and take up essential nutrients like phosphate and nitrate. This has the effect of cleaning wastewater (Zhao *et al.* 2015 and references therein), and contrasts with the effect of modern agriculture, which results in eutrophication because of the use of high amounts of fertilisers. Under optimal growth conditions, duckweed plants have a high protein content (Cheng & Stomp 2009; Anderson *et al.* 2011), making them valuable as food for animals and even for human consumption (Landolt & Kandeler 1987; Anderson *et al.* 2011; Xu & Shen 2011). Alternatively, reduced growth conditions result in a decrease in the protein content but high accumulation of starch (Sree & Appenroth 2014), which can then be used for bioethanol or butanol production (Cui & Cheng 2015). For both applications, the results of several pilot-scale investigations are already published (Cui & Cheng 2015; Zhao *et al.* 2015).

As already mentioned, Elias Landolt was originally mainly interested in the taxonomy of Lemnaceae (Landolt 1986), but he was always eager to have his hypotheses tested with more 'modern; methods. Meanwhile, molecular taxonomy made great progress (for review see Appenroth *et al.* 2013) but Borisjuk *et al.* (2015) were not able to identify each of the species using DNA barcoding (cf. also Wang *et al.*, 2010). While presently, only 30 of the 37 species can be identified with varying confidence, in most cases results from molecular methods are highly concordant with species as defined by Elias Landolt (1986). This holds true also for the first report concerning the use of nuclear sequence data (Tippery *et al.* 2015) for constructing a phylogenetic hypothesis, as prior studies almost exclusively used plastid sequence data. Moreover, characterisation and identification at the level of clones is still in its infancy (Bog *et al.* 2013). Wiersema (2015) argues cogently that, in contrast to a recent suggestion, *Landoltia punctata* (G. Meyer) Les & Crawford should be maintained. It is worth mentioning that most (but not all) of the contributions keep the term Lemnaceae, considering them as a plant family in contrast to a subfamily (*Lemnoideae*). Since Lemnaceae (or *Lemnoideae*) are nested in Araceae in molecular phylogenetic studies (Cusimano *et al.* 2011; Nauheimer *et al.* 2012), whether or not the duckweeds are recognised at the familial or subfamilial level depends on whether one accepts paraphyletic groups. To circumvent this rather philosophical debate, one can separate the small group of Protoaraceae together with the group of Lemnaceae from the 'true Araceae' (as these two groups are the most basal elements in this group), which results in three monophyletic plant families, *i.e.* true Araceae, Lemnaceae and Protoaraceae. Arguments for keeping the term Lemnaceae instead of *Lemnoideae* were summarised by Appenroth *et al.* (2013) after thorough discussion with Elias Landolt. It should be added that the number of presently accepted species of Lemnaceae is 37, as Landolt (2000) placed *Lemna ecuadoriensis* in synonymy with *Lemna obscura* (for discussion see Bog *et al.* 2010).

Duckweeds are gaining some of their physiological importance as model organisms, which was lost with the arrival of the *Arabidopsis* era. Several physiological mechanisms await molecular explanations. For many years a group from Kyoto University has investigated circadian clocks in duckweeds (Miwa *et al.* 2006). In their newest paper this group reports that circadian rhythms show diversity in period length and sustainability in a broad range of duckweed species, suggesting that circadian clock mechanisms are somewhat diversified among duckweeds (Muranaka *et al.* 2015).

Duckweeds are very often used for investigations into stress physiology, *e.g.* phytotoxicity. Horemans *et al.* (2015) report the different effects of uranium and cadmium on oxidative stress response in *Lemna minor*, while Monselise *et al.* (2015) demonstrated, using NMR measurements, the presence of alanine as a universal stress marker, here after UV irradiation of *Landoltia punctata* (formerly *Spirodela oligorrhiza*). The transfer of zinc into the food chain was investigated by the group of M. A. K. Jansen (Lahive *et al.* 2015) using the interaction partners *L. minor* and *Gammarus pulex* (L.). Kuehdorf *et al.* (2014) showed the different capacity of clones of *Spirodela polyrhiza* to form dormant organs (turions) as an adaptation to climate conditions at the place of collection. Appenroth & Adamec (2015) demonstrated that the molecular mechanism is based on changes in the threshold value for the external phosphate concentration at which turion formation is induced. Clones with higher turion yield have higher threshold values for the formation of turions. An interesting ecological contribution comes from Coughlan *et al.* (2015), showing that long-distance transport of *Lemna minuta* is possible *via* mallard ducks, because the drought resistance is sufficiently high for *Lemna* to survive between the bird feathers. This result also likely applies to other duckweed species, as well as transport by other water birds.

While this special issue celebrates the prolific and multi-faceted contributions of Elias Landolt to our broad understanding of the biology of Lemnaceae, his bibliography compiled by Walter Laemmler (Data S1) elegantly documents that Landolt's scholarship and interests extended well beyond the duckweeds. Besides being impressed by the breadth of topics covered in his published work, one must also marvel at his sustained record of productivity over a period of more than six decades. His enthusiasm for and dedication to scholarly activity never waned up until the end of his life. His bibliography also demonstrates that he was much more than an 'ivory tower' academic. He was genuinely, indeed passionately, concerned about the conservation of the native flora and plant communities of the Alps. The crowning achievements of his floristic elements were the many editions of '*Our Alpine Flora*', which, with several different collaborators, went through numerous German editions, several French editions and two English versions. His other crowning achievement was his flora of Zürich, which was a true labour of love.

We dedicate this Special Issue, After the genome sequencing of duckweed – how to proceed with research on the fastest growing angiosperm, to the memory of Elias Landolt (21 July 1926–1 April 2013). For his complete scientific bibliography, see Data S1.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Data S1. Bibliography of Elias Landolt.

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