Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Xuan Hieu Cao
Paul Fourounjian
Wenqin Wang *Editors*

The Duckweed Genomes

Springer

Compendium of Plant Genomes

Series Editor

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Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant Arabidopsis thaliana in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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The Duckweed Genomes

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This book series is dedicated to my wife Phullara and our children Sourav and Devleena
Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F_2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the-then available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology, physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, particularly Dr. Christina Eckey and Dr. Jutta Lindenborn for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

Kalyani, India Chittaranjan Kole

Preface

The duckweed or Lemnaceae family is a collection of five genera (*Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*) and 37 species of the smallest, fastest growing flowering plants living in aquatic environments. Many of these monocotyledonous plants can grow all over the world in a variety of climates. Provided their simplified and neotenous morphology, duckweeds have been researched for several decades as a model species for plant physiology and ecotoxicological research, contributing to the knowledge, e.g., about flowering response, plant circadian system, sulfur assimilation pathways, and auxin biosynthesis. In addition, duckweed-based water treatment has been proven as a feasible and inexpensive solution, especially within developing countries, to remove phosphorus and pharmaceutical chemicals from sewage and wastewater. With a dry mass yield per hectare per year up to 80 tonnes (equivalent to 10 tonnes of protein), duckweed is a promising aquatic crop in new modern and sustainable agriculture. Besides being an excellent primary or supplemental feedstock for production of livestock and fish, duckweed biomass can be utilized as a potential resource for human nutrition, biofuel, or bioplastics, depending on water quality as well as protein or starch accumulating procedures. Those academic and commercial interests led to the international effort to sequence the *Spirodela polyrhiza* genome, the smallest and most ancient genome in the family. *Spirodela* genomes reveal novel insights into the 158-Mbp genome size with less than three quarters number of *Arabidopsis thaliana* protein-coding genes and no signs of recent retrotranspositions.

In view of above, a total of 46 authors, representing 23 academic institutions or companies from five countries, have contributed 18 chapters for this book. This volume in the genome compendium series covers not only the latest findings in modern genetics, phylogenetics (Chaps. 2, 5), epigenetics, cytogenetics (Chap. 4), transcriptomics (Chaps. 12, 13, and 16), proteomics (Chap. 14), and genomics research in all five genera of duckweeds but also efforts toward transformation, genome editing and sequencing of the over one Gigabase *Wolffia* genomes (Chaps. 15, 17), with their large potential impacts on genome evolution and agricultural research. The introductory chapter stresses the importance of duckweeds as an aquatic plant model and as an extensive resource for biotechnological applications. The book tells the tale of the first *Spirodela* genome sequencing adventure (Chap. 7), details the nuclear (Chap. 9) and organelle (Chap. 10) genome sequences of *Spirodela polyrhiza*, which is the smallest, least methylated, and least transposon-rich monocot genome sequenced to date (Chap. 8). It describes the current genomics applications of these findings (genotyping by sequencing in Chap. 11; small RNA in Chap. 16) and the strategies to obtain new genome sequences within the family (Chap. 6). Finally, Chap. 18 is devoted to deeper insights and future perspective of using the duckweed genome information for duckweed research and applications.

It has been a great privilege to work with colleagues of the duckweed research community on this book. We are grateful to all the authors for their contribution in writing chapters of high quality. We are also thankful to the reviewers (Dr. Olaf Barth and Dr. Wiebke Zschiesche from Martin Luther University of Halle-Wittenberg, Germany and Dr. Hien Le Thu from Institute of Genome Research, Vietnam) for helping us in improving the quality of the chapters. The editors would like to express our sincere thanks to Prof. Chittaranjan Kole, Editor-in-Chief, of the Genome Compendium Series for cordial inviting us to contribute on this important masterpiece as well as to Springer, in general, Naresh Kumar Mani and Praveen Anand Sachidanandam, in particular, for constant help and support in publication and promotion of this book. We also appreciate and recognize cooperation and moral support from our family members for sparing us precious time for writing and editorial work.

We hope that our efforts in compiling the information on different aspects of duckweed will help the duckweed research and application community in enhancing better understanding about the duckweed biology and developing an extensive resource for biotechnological applications. This book will also benefit students, scientists both in academia and industry, and policy-makers in updating their knowledge on the importance and recent advances of duckweeds as an aquatic plant model.

Halle (Saale), Germany — Xuan Hieu Cao
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Importance of Duckweeds in Basic Research and Their Industrial Applications

1

Paul Fourounjian, Tamra Fakhoorian and Xuan Hieu Cao

Abstract

The Lemnaceae family, commonly called duckweeds, is 37 species of the smallest and simplest flowering plants found floating on nutrient-rich waters worldwide. Their small size and rapid clonal growth in aseptic conditions made them a stable and simple model for plant research especially from 1950 to 1990, when they were used to study plant physiology and biochemistry including auxin synthesis and sulfur metabolism. Duckweed research then saw a resurgence in 2008 when global fuel prices rose and the US Department of Energy funded the sequencing of the *Spirodela polyrhiza* genome. This launched not only the genomic investigations detailed in this book, but the regrowth of duckweed industrial applications. Thanks to their ability to quickly absorb nitrogen, phosphorous, and other nutrients while removing pathogens and growing at a rate of 13–38 dry tons/hectare year in water treatment lagoons, scientists are currently exploring ways that duckweed can convert agricultural and municipal wastewater into clean water and a high-protein animal feed. The potential of these plants for phytoremediation of heavy metals and organic compounds also allows the possibility to clean the wastewater from heavy industry while providing biofuels and even plastics. Finally, thanks to their superb nutritional profile *Wolffia* species grown in clean conditions promise to become one of the healthiest and most environmentally friendly vegetables. Given the importance of these incredible plants, it is no wonder researchers are investigating the genetic mechanisms that make it all possible.

This chapter was revised and significantly expanded upon, with the guidance of T. F., from the chapter “The Importance and Potential of Duckweeds as a Model and Crop Plant for Biomass-Based Applications and Beyond,” in the Handbook on Environmental Materials Management, which X. H. C. and P. F. wrote for Springer Nature a year ago (Cao et al. 2018). We hope this chapter thoroughly explains non-genomic research and application topics, especially for those who are unfamiliar with the family.

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1.1 Introduction

Duckweed (known as monocotyledon family *Lemnaceae* or recently classified as subfamily *Lemnoideae* in the arum or aroid family *Araceae*) is a small group of aquatic plants with only five genera (*Spirodela*, *Landoltia*, *Lemna*, *Wolffia*, and *Wolffiella*) and 37 species (see Landolt 1986; Nauheimer et al. 2012; Sree et al. 2016). Except for *Wolffiella* (commonly named as bogmat) that is restricted to the Americas and Africa, species of other duckweed genera occur around the whole world. Although highly adaptable across a broad range of climates, most diverse species of duckweed appear in the subtropical or tropical zones. Duckweed species tend to be associated with nutrient-rich or eutrophic freshwater environments with quiet or slow-moving flow. However, they are extremely rare in deserts and are absent in the cold polar regions (Arctic and Antarctica).

Duckweed species are the smallest flowering plants with minute sizes from 0.5 mm to less than two cm (Landolt 1986). Species of duckweed can be easily distinguished morphologically from species of any other flowering plants, even closely related aquatic plants, due to their highly reduced body structure. The leaflike body of the duckweed species, sometimes called a frond or thallus, is a modified stem with only few cellular differentiations (Fig. 1.1). The growth of duckweed vegetatively occurs by budding within the pouches or cavities of the basal sections of the fronds. Each daughter frond emerging from the pouch of mother bud already contains two new generations of daughter fronds. Therefore, under optimal conditions, the growth rate of duckweed is nearly exponential. The frond number of fast-growing species (e.g., *Lemna aequinoctialis*, *Wolffiella hyalina*, and *Wolffia microscopica*) almost doubles within 24 h (Ziegler et al. 2015; Sree et al. 2015b), presenting the fastest growing flowering plants. With a miniaturized body plan and such rapid growth leading to maximum fitness, duckweed has arguably been interpreted as an example of the hypothetical Darwin–Wallace Demon for the lifetime reproductive success (Kutschera and Niklas 2015).

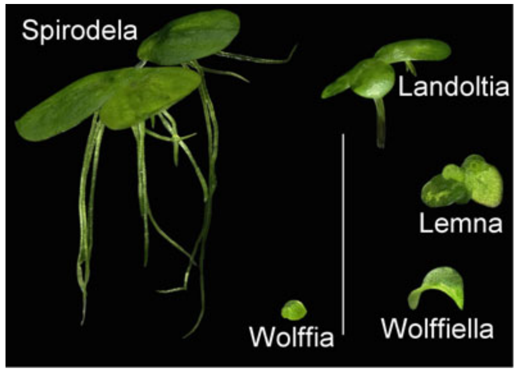

Fig. 1.1 Morphology of five representative species for duckweed genera. Spirodela: *Spirodela polyrhiza*; Landoltia: *Landoltia punctata*; Lemna: *Lemna minor*; Wolffiella: *Wolffiella lingulata*; Wolffia: *Wolffia arrhiza*. Bar: 1 cm

Only occasionally or very rarely, several species of duckweeds produce microscopic flowers in nature as well as under in vitro conditions (Fu et al. 2017; Schmitz and Kelm 2017; Sree et al. 2015a). In *Spirodela* and *Lemna* (belonging to the subfamily *Lemnoideae*), the flowering organs (1 membranous scale, 2 stamens, and 1 pistil) originate in the same pouches in which the daughter fronds are normally formed. In the subfamily *Wolffioideae* (consisting of *Wolffiella* and *Wolffia*), generative and vegetative reproductions are spatially separated occupying the floral cavity on the upper surface of the frond and the budding pouch, respectively.

Duckweed fronds are free floating on or near the surface of the water, often forming dense mats in suitable climatic and nutrient conditions. In unfavorable weather, such as drought or freezing winter seasons, in addition to flowering, several duckweed species are able to form special "resting fronds" (in the dormant phase) to persist until conditions return that can support growth. In place of a frond, the greater duckweed (*Spirodela polyrhiza*) produces a starch-rich tissue called a turion, which sinks to the bottom of the water. Turion production has been reported also for *Lemna turionifera*, *L. aequinoctialis*, *Wolffia brasiliensis*, *Wolffia borealis*, *Wolffia angusta*, *Wolffia australiana*, *Wolffia arrhiza*, *Wolffia columbiana*, and *Wolffia globosa*. These turions do not grow any further but can germinate and start a new life cycle from the bottom of the water body or mud when the water temperature reaches about 15 °C. In addition, resting fronds of the ivy duckweed (*Lemna trisulca*) and *Wolffiella gladiata* with reduced air spaces can accumulate starch and still rather slowly grow on the bottom of the water, forming new but similar fronds. However, the common duckweed (*Lemna minor*), gibbous duckweed (*Lemna gibba*), *Lemna perpusilla*, and some strains of *Lemna japonica* produce starch-rich fronds that do not sink to the bottom of the water but are just pressed down under the ice cover during freezing temperatures. Interestingly, formation of turions as a survival and adaptive capacity of *S. polyrhiza* strains collected from a wide geographical range seems to be genetically determined and highly influenced by the mean annual temperature of habitats (Kuehdorf et al. 2013). Furthermore, the family displays significant inter- and intraspecies differences of cell physiology (e.g., starch, protein, and oil contents) together with duckweed potential for industrial applications (Alvarado et al. 2008; Appenroth et al. 2017; Hou et al. 2007; Mkandawire and Dudel 2005; Tang et al. 2015; Yan et al. 2013; Zhang et al. 2009).

Due to their small and abbreviated structures, morphological and physiological classification of the 37 duckweed species (*Spirodela*: 2 species; *Landoltia*: 1; *Lemna*: 13; *Wolffiella*: 10; *Wolffia*: 11) can be challenging. In the past decade, for species assignment as well as resolving intraspecies differences, several attempts have been carried out to employ molecular genotyping techniques, including random amplified polymorphic DNA (RAPD; Martirosyan et al. 2008), inter-simple sequence repeats (ISSR; Fu et al. 2017; Xue et al. 2012), simple sequence repeats (SSR; Feng et al. 2017), amplified fragment length polymorphism (AFLP; Bog et al. 2010, 2013), and DNA barcoding using plastid sequences (Borisjuk et al. 2015; Wang et al. 2010) or nuclear ribosomal sequences (Tippery et al. 2015). Although DNA barcoding using two plastidic barcodes aids in identifying most duckweed species (at least 30 among 37 species) in a quite simple and straight forward manner, combination of different techniques or using additional barcodes may help to unambiguously and economically assign remaining duckweed species.

The Lemnaceae family was one of the earliest model plants due to their ease of aseptic cultivation in the laboratory and simple morphology. The second volume of Landolt and Kandeler's 1987 monographic study contains 360 pages dedicated to the physiological research of the family in particular and plants as a whole (Landolt and Kandeler 1987). The professors who organized the first duckweed conference summed up the duckweed research stating that duckweeds were the main model for plant biology from 1950 to 1990, when Arabidopsis and rice were used for their sexual reproduction and applicability to terrestrial crops (Zhao et al. 2012). In that time, investigations of duckweeds revealed the tryptophan-independent synthesis of auxin (Baldi et al. 1991), translational regulation in eukaryotes (Slovin and Tobin 1982), and seven of the first stable plant mutants (Posner 1962). Today, physiological studies continue largely in the fields of circadian rhythm research, xenobiotic plant–microbe interactions, and phytoremediation and toxicology. Starting in 2011, a biannual series of international duckweed conferences in research and applications has connected and helped expand this research community and increased public awareness and recognition of duckweed economic and environmental importance (Zhao et al. 2012; Lam et al. 2014; Appenroth et al. 2015). Together with the completion of the *Spirodela* genome in the year 2014 and rapid advances in sequencing technologies, this resurgence of interest has resulted in a proliferation of genome and transcriptome sequences for duckweed species and ecotypes discussed in the remainder of this book.

One of the largest fields of duckweed research is ecotoxicology, where the widely distributed *Lemna* species *minor* and *gibba* serve as model plants to determine the effect of a compound on an ecosystem. These growth tests have been standardized in the International Organization for Standardization's protocol ISO 20079 which