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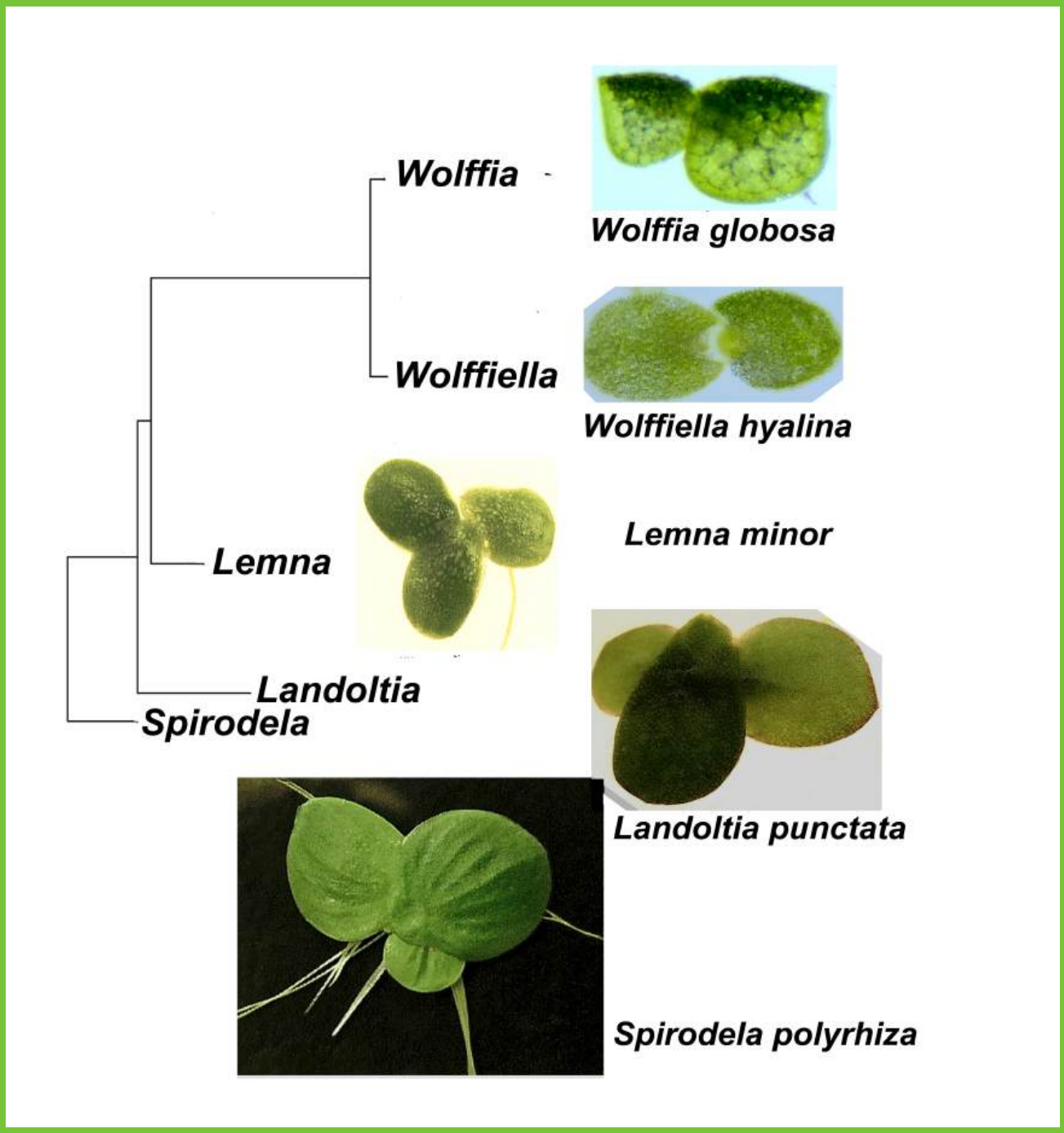

The genera of Lemnaceae (Duckweeds)

Accurate species identity is an important prerequisite for duckweed research

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Biological research deals with organisms or parts of organisms, from molecular, cellular level, to the level of organs or whole plants, in populations or ecosystems. In each case we need to know from which organism the new information has been deduced, which is critical for its independent validation as well as for the incorporation of new information into the body of knowledge in the scientific community. Research in the field of duckweed (or water lentils; family Lemnaceae Martinov; Tippery et al., 2021) is no exception.

Species identity has often been facilitated by the use of morphological markers and more so in the current times by molecular barcoding. For duckweeds, the use of morphological features could tell some species apart (Hegelmaier, 1868; Landolt, 1986; Bog et al., 2020). However, duckweeds being small in size and having reduced structural and developmental complexities, the efficient use of these morphological markers has been challenging even for a trained researcher. As Elias Landolt (Appenroth et al., 2023) once mentioned, often, common workers in the field tend to identify *Lemna* species as *Lemna minor* because it is small (the meaning of "minor" is "small") and *Wolffia* species as *Wolffia arrhiza* because the plants do not have roots (the meaning of "arrhiza" is "without roots"), although this is a general characteristic of the whole genus *Wolffia* (Personal communication to KJA).

What can be done to solve this problem? The first step is, of course, using a key of species determination which is based on morphological markers (Bog et al., 2020). With a good understanding of this key, the genera can be identified with accuracy. There are five genera: *Spirodela, Landoltia, Lemna, Wolffiella* and *Wolffia*. For more details, see Bog et al. (2020). The main morphological markers are the following:

1. Genus *Spirodela*: Fronds have 7 to 21 roots (take care not to count the roots per colony!), many but never all of them perforate (pierce through) the scale-like leaflet; fronds 1 to 2 cm large, 1 – 2 times as long as wide.
2. Genus *Landoltia*: Fronds have 2 to 7 roots (rarely more than 7), all of them perforate the scale-like leaflet; normally not larger than 1 cm, 1.5 – 2 time as long as wide; with prominent papillae along the median line of the frond surface (this marker gave the name of the single species *L. punctata*).
3. Genus *Lemna*: One single root per frond
4. Genus *Wolffiella*: No roots; fronds flat with air spaces; daughter fronds emerging from a terminal flat pouch at the base of the mother frond; shape depends on the species.
5. Genus *Wolffia*: No roots; fronds three-dimensional (globular, ellipsoid, cylindrical, conical, naviculate (boat-shaped) or semi-spherical); daughter fronds emerging from a single terminal conical cavity at the base of the mother frond; colonies normally consist only of one mother and one daughter frond.

What can be done for identification at the species level? In total there are presently 35 species of Lemnaceae (Bog et al., 2020; Appenroth et al., 2024), while new hybrid species are also increasingly being identified (Braglia et al., 2021). The method of choice is plastidic DNA barcoding (Bog et al., 2019): Fragments of the plastidic DNA can be amplified by PCR and sequenced to deduce the species identity through comparison to a previous database of known sequences generated for all known duckweed species (Borisjuk et al., 2015). Alternatively, higher resolution for species distinction can be obtained by the method of Tubulin-Based Polymorphism Fingerprinting, a nuclear DNA-based technique, which permits additionally the identification of interspecific hybrids from different fragment size and number after PCR amplification (Braglia et al., 2021). These methods are now routine in many labs with modern molecular biology equipment and training. Sequencing is usually carried out by commercial vendors or institutional core facilities for very reasonable fees. Laboratories that do not have the capacity to carry out these experiments may seek cooperation or could contact one of the duckweed stock collections (Appenroth, 2025) across the globe for help. These methods and resources should make it possible in most cases to identify the species, or at the least narrow down to a few possible candidates.

It is an essential requirement for the proper species identity and source of the duckweed used in the research to be known before the results can be accepted for publication. Otherwise, the reported observations cannot be compared with other findings. We ask not only authors to take this requirement seriously, but its enforcement by reviewers and editors will be especially critical. One criterion of good scientific practice is the opportunity that other researchers could reproduce the published results. Without knowing at least the species identity, this will be impossible. And reproducibility is a key determinant for the reliability of good science in general, and also critical for maintaining public confidence.

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