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Autor: Landolt, Elias
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Taxonomy and Ecology of the Section *Wolffia* of the Genus *Wolffia* (*Lemnaceae*)

Elias LANDOLT

1. INTRODUCTION

Until 1865 all species of the section *Wolffia* were subsumed under the name *Wolffia arrhiza* (L.) Horkel. KARSTEN collected a flowering and fruiting *Wolffia* in Sta. Martha, Colombia (without date) and described it in 1865 as *W. columbiana*. North American collectors as e.g. G. ENGELMANN recognized that all American samples should be separated from *W. arrhiza* and attributed to this new species. HEGELMAIER (1868) characterized the new species in detail. He distinguished and described again a third species out of this group from Angola which he named *W. cylindracea*. At that time all the Asian and Australian plants from this section were still united with *W. arrhiza*. In his monograph of *Lemnaceae*, DAUBS (1965) recognized only the two species *W. arrhiza* and *W. columbiana* and did not accept *W. cylindracea*. In 1874 W. v. MÜLLER named a specimen from Mt. Emu Creek, Victoria, Australia, as *W. hegelmaieri* (in herb. STU). However, this name was never published validly. In 1878 BENTHAM described the big Australian plants as *W. arrhiza* var. *australiana*. This variety was elevated to the rank of a species (*W. australiana*) by DEN HARTOG and VAN DER PLAS in 1972. In southern and eastern Asia the wide-spread representative of this section was called *Lemna globosa* by ROXBURGH (1832), but not compared with or distinguished from *W. arrhiza*. Under the name of *W. globosa*, DEN HARTOG and VAN DER PLAS (1972) separated all small *Wolffia* plants from *W. arrhiza*. They also united the small African plants (*W. cylindracea*) with *W. globosa*. In 1980 LANDOLT recognized *W. angusta* from Australia as a separate species. In a monograph

(LANDOLT 1986) he accepted the incorporation of *W. cylindracea* into *W. globosa*. Some of the plants from Singapore, Malaysia, southern India, Sri Lanka and Pakistan have been included under *W. angusta*.

The characteristics of the *Wolffia* species which belong to the smallest flowering plants on earth, are very scarce due to the extreme morphological reduction. On the other hand the phenotypic plasticity of these plants is relatively high. This makes it extremely difficult to recognize various species within this group on morphological grounds only. To characterize taxonomic units, the plants must be grown simultaneously under the same conditions. Besides morphological features, ecological, physiological and biochemical characteristics should be included as well.

For the past two years, Prof. Dr. D.J. Crawford, Columbus, Ohio, has been conducting allozyme investigations on *Lemnaceae* using live specimens from our collection in Zürich. He has been able to show that *W. angusta* strains from southern Pakistan have a different enzymatic pattern. The genetic similarity between the two groups is very low. Furthermore, the African plants of *W. globosa* showed a relatively low similarity to the plants from other origins (CRAWFORD and LANDOLT, unpubl.). During a field trip to Zimbabwe and Botswana in 1992 it was observed that the ecology of the African plants is quite different from those of Asia and California (LANDOLT 1994).

It was therefore advisable to more closely investigate the morphology of clones of the section *Wolffia* in our collection in Zürich. *W. arrhiza* which is rather variable was not yet investigated.

Acknowledgment

Thanks are owed to Rizwan Yusuf Hashmi, Karachi, for sending live samples of *W. neglecta*, to Prof. Dr. D.J. Crawford for reading the manuscript and to Anita Hegi, Zürich, for cultivation and preparation of the clones, and for making the drawings and photo plates. René Graf prepared the photographic documentation. The authorities of the herbarium in Firenze (FI) (Dr. Riccardo M. Baldini, curator) kindly sent photographic material of *Lemna arrhiza* from the herbarium of Micheli.

2. MATERIAL AND METHODS

Strains from our collection in Zürich were cultivated under identical conditions: 24°/22° day/night temperature, light intensity 20'000 Lux, day length 12

hours; 1/5 Hutner solution. After two weeks the fronds were photographed and morphological measurements taken. Most of the other data come from literature (LANDOLT 1986, 1994) or from unpublished notes and observations in nature and on herbarium material.

3. DESCRIPTIONS

3.1. DESCRIPTION OF THE SECTION

Type species: *W. arrhiza* (L.) Horkel

The section *Wolffia* is characterized by the following features:

Fronds spherical to ellipsoid or boat-shaped, flat or convex on the upper surface, without prominent papules, but often with small one-celled teeth on the surface or along the margins, the green surface area often pointed at the tip; without pigment cells; forming small light green compact turions under unfavourable conditions which sink to the bottom of the water under unfavourable conditions. Stigma with or without (but anthers always with) pigment cells .

Table 1. Ecological limits of the species of the section *Wolffia*.

- 1 Lowest mean temperature of the three coolest months in ° C
- 2 Lowest mean temperature of the three warmest months in ° C
- 3 Highest mean temperature of the three warmest months in ° C
- 4 Aridity factor of Martonne:
$$\frac{\text{annual precipitation in cm}}{\text{mean annual temperature in } ^\circ \text{C} + 10}$$
- 5 Duration of water p: permanent s : seasonal

| species | 1 | 2 | 3 | 4 | 5 |
|-----------------------|------|----|------|-----|---|
| <i>W. australiana</i> | + 8 | 18 | 22 | 3-6 | p |
| <i>W. angusta</i> | + 8 | 22 | > 28 | 4-8 | p |
| <i>W. neglecta</i> | + 8 | 22 | > 28 | 2-5 | s |
| <i>W. arrhiza</i> | - 8 | 18 | 28 | 2-5 | p |
| <i>W. cylindracea</i> | +12 | 22 | > 28 | 2-5 | s |
| <i>W. globosa</i> | + 1 | 22 | > 28 | 4-8 | p |
| <i>W. columbiana</i> | - 12 | 18 | 28 | 2-5 | p |

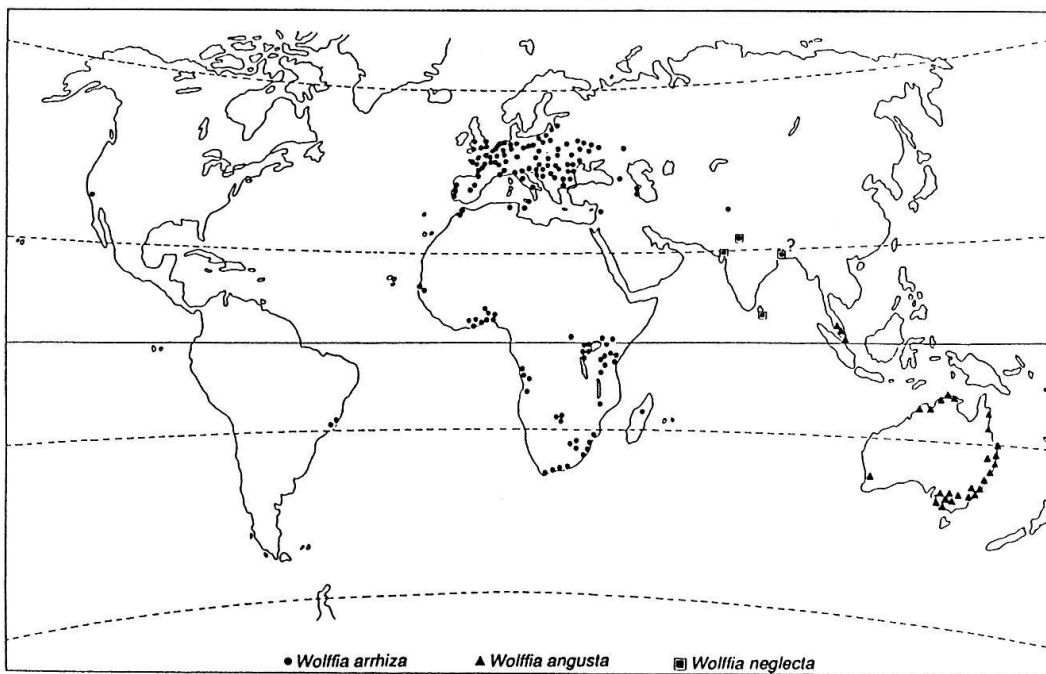
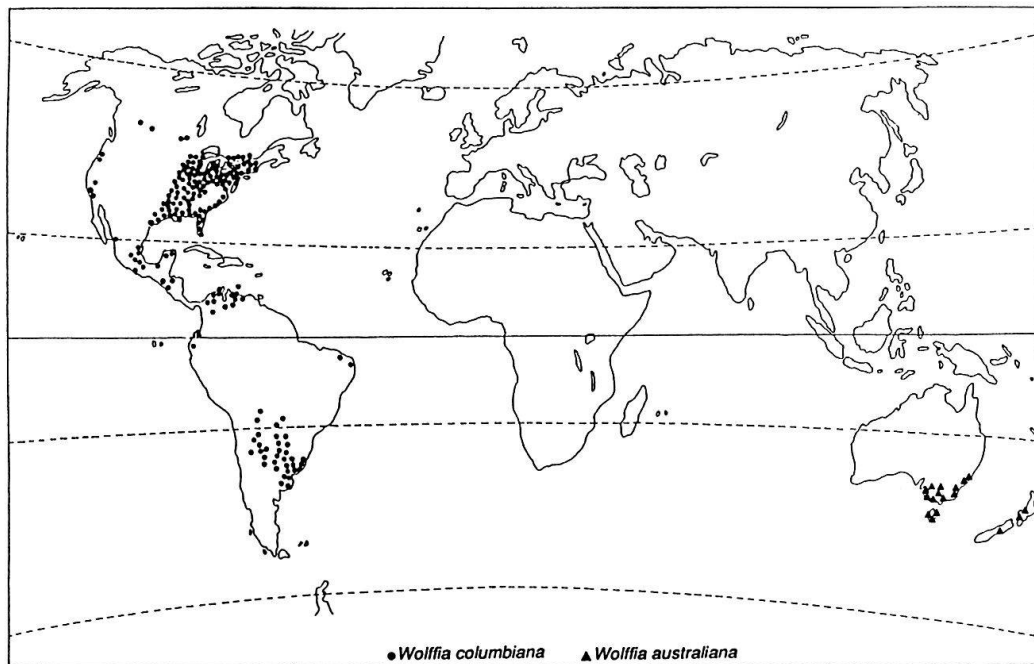


Fig. 1. Distribution of the *Wolffia* species of the section *Wolffia*.

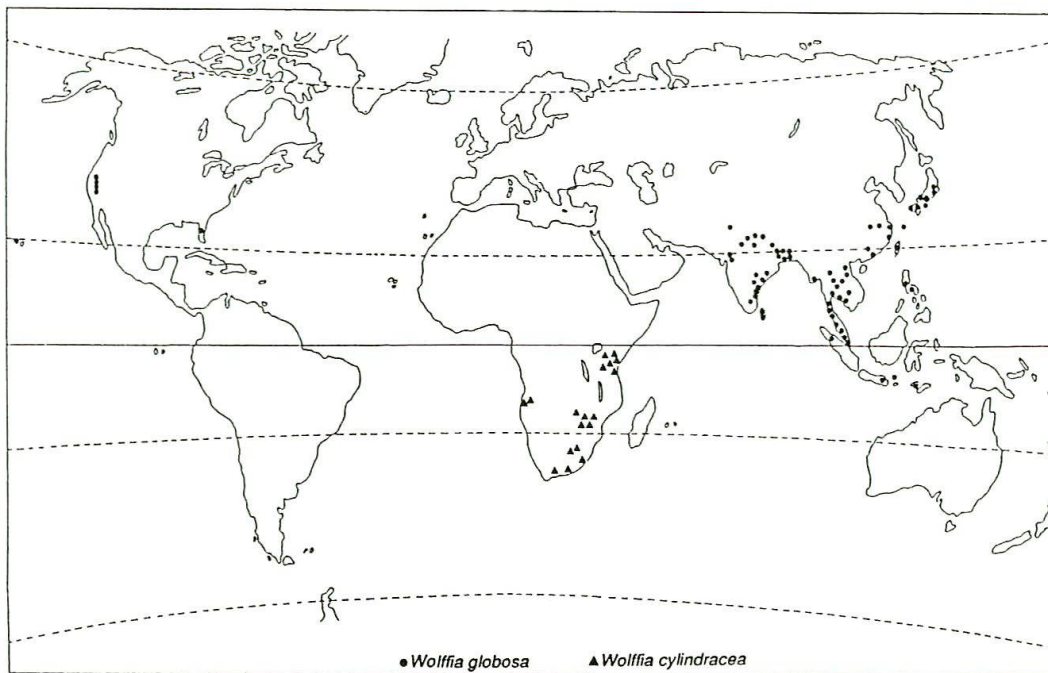


Fig. 1 (contin.). Distribution of the *Wolffia* species of the section *Wolffia*.

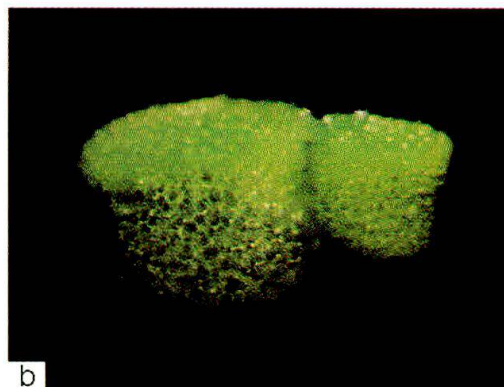
The size of the assimilating small-celled green tissue on the surface in relation to the big-celled translucent tissue below is important for the taxonomy.

The section *Wolffia* consists of 7 partly vicariant species occurring in most parts of the world except in very winter-cold, in summer-cool, in very dry or in very wet regions (Fig. 1).

The ecological distribution of the species is given in Table 1. The species grows only at relatively high temperatures (summer temperatures, mostly above 18° C) and are sensitive to cool temperatures. Temperatures below zero are lethal to growing fronds. Most species can survive cold temperatures as turions at the bottom of the water. *W. cylindracea* and *W. neglecta* are able to grow in seasonal waters and thus occur in winter-dry regions along with *Lemna aequinoctialis*. *W. cylindracea* stands the dry season by forming turions which sink to the bottom and become imbedded in the humous clayey soil. It is not known whether *W. neglecta* produces fruits or turions at the beginning of the dry season.



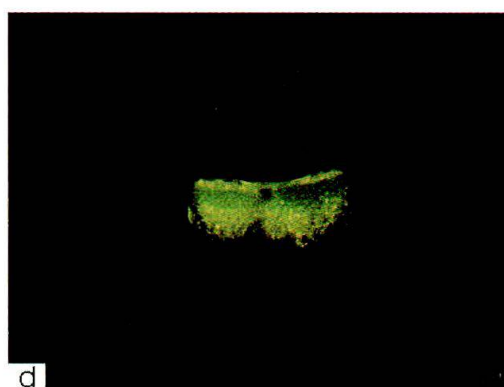
a



b



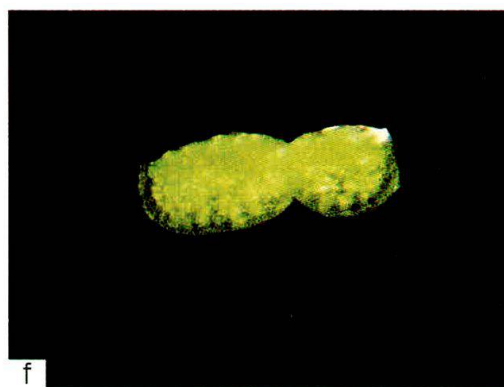
c



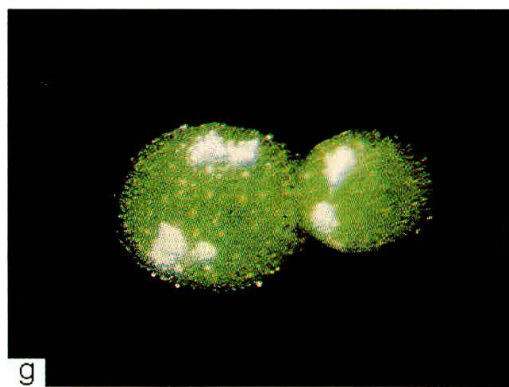
d



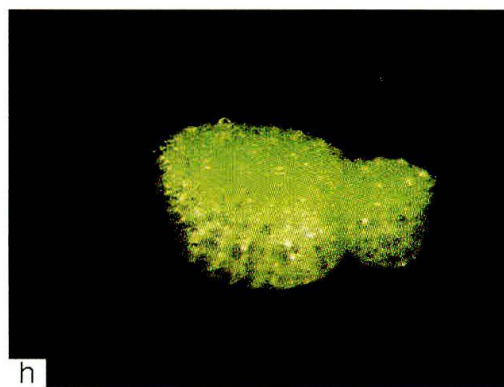
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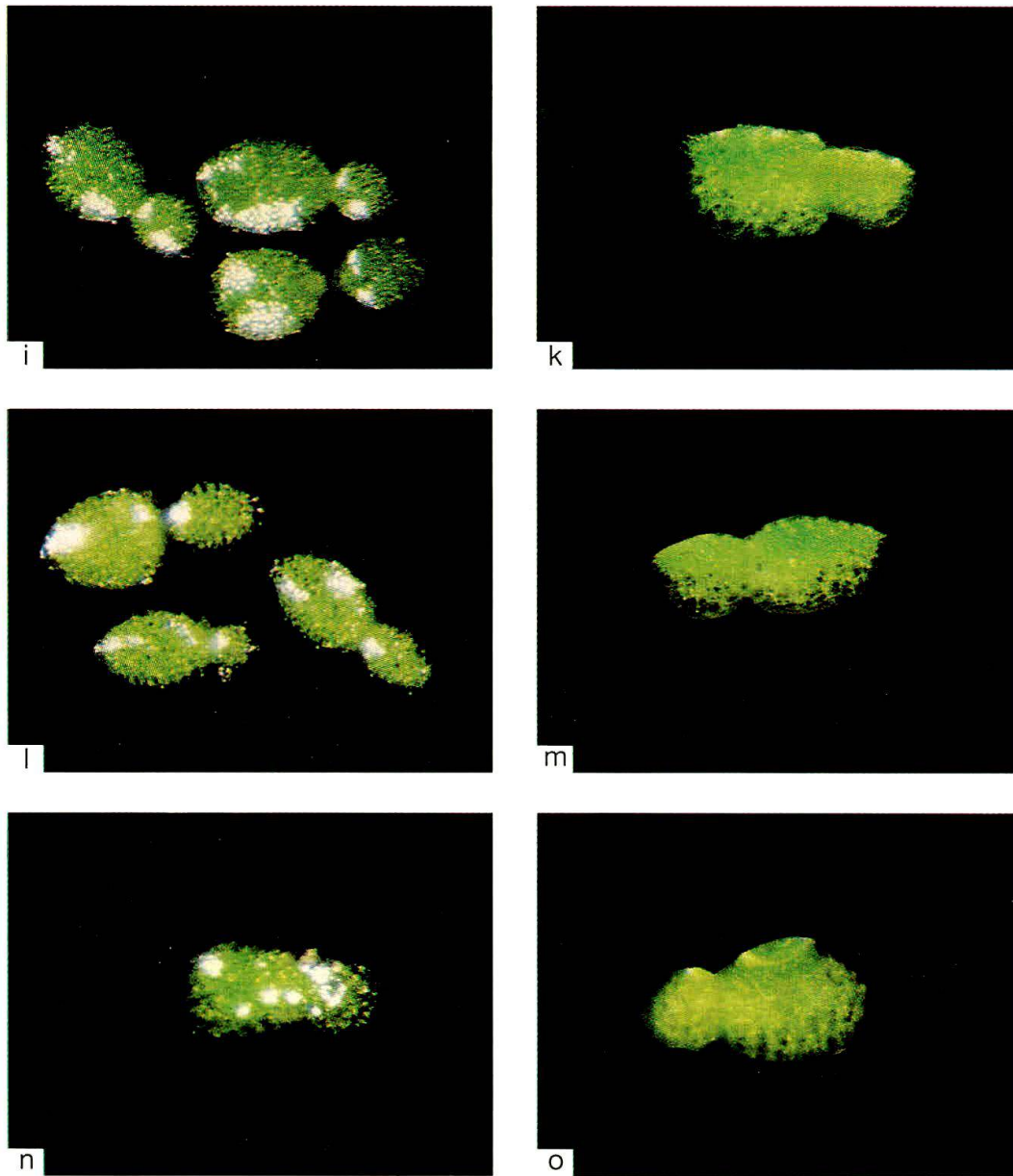


Fig. 2. The *Wolffia* species of the section *Wolffia* from above (left) and from the side (right) (x 8)

a., b.: *Wolffia australiana*

g., h.: *Wolffia arrhiza*

n., o.: *Wolffia columbiana*

c., d.: *Wolffia angusta*

i., k.: *Wolffia cylindracea*

e., f.: *Wolffia neglecta*

l., m.: *Wolffia globosa*

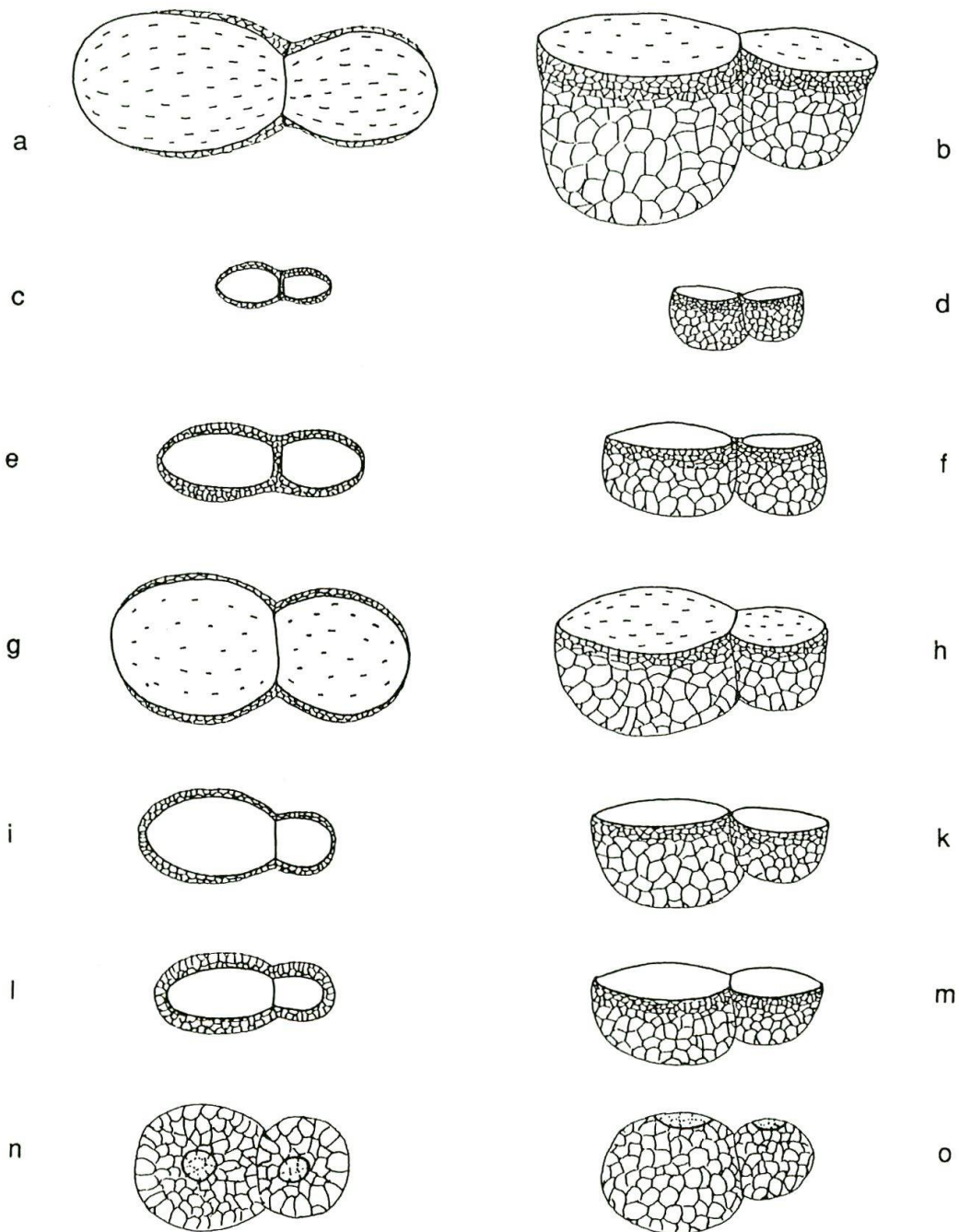


Fig. 3. Drawings of the *Wolffia* species of the section *Wolffia* from above (left) and from the side (right) (x 8)

a., b.: *Wolffia australiana*

c., d.: *Wolffia angusta*

e., f.: *Wolffia neglecta*

g., h.: *Wolffia arrhiza*

i., k.: *Wolffia cylindracea*

l., m.: *Wolffia globosa*

n., o.: *Wolffia columbiana*

3.2. KEY TO THE SPECIES

- Surface of the fronds $1\frac{1}{3}$ - $2\frac{1}{2}$ times as long as wide, $1\frac{1}{2}$ -3 times as deep as wide, with the greatest width at the surface of the water (nearly no translucent edge visible from above); stigma with pigment cells
 - Fronds mostly > 0.9 mm long, with 50-120 stomata *W. australiana*
 - Fronds mostly < 0.9 mm long, with 8-20 stomata
 - Fronds whitish green at the surface with intensely green margins, 2-3 times as deep as wide *W. angusta*
 - Fronds intensely green at the surface without green coloured margins, $1\frac{1}{2}$ -2 times as deep as wide *W. neglecta*
- Surface of the fronds 1 - $1\frac{2}{3}$ times as long as wide, $\frac{3}{4}$ - $1\frac{1}{2}$ as deep as wide, with the greatest width below the surface of the water (at least laterally a translucent edge visible from above); stigma without pigment cells
 - Fronds intensely green and mostly shiny at the surface, with mostly > 30 stomata *W. arrhiza*
 - Fronds not shiny, pale green to rather intensely green, with < 30 stomata
 - Fronds mostly < 0.6 mm wide, $1\frac{1}{4}$ - $1\frac{2}{3}$ as long as wide
 - Fronds with no translucent edge at the tip, with 15-30 stomata *W. cylindracea*
 - Fronds with distinct translucent edge at the tip, mostly < 20 stomata *W. globosa*
 - Fronds mostly > 0.6 mm wide, 1 - $1\frac{1}{3}$ as long as wide *W. columbiana*

3.3. DESCRIPTION OF THE SPECIES

Wolffia australiana (Bentham) Den Hartog & Van der Plas (Blumea 20, 151; 1972). Figs. 2a, 3a. (see also LANDOLT 1986, plate XIV a.,b., p. 526)

Wolffia arrhiza var. *australiana* Bentham (Fl. Austr. 7, 162; 1878)

Type locality: Australia, Victoria, Mount Emu Creek; leg. F. v. Müller, 25.2.1874.

Lectotype (DEN HARTOG and VAN DER PLAS 1972): K. Isotypes: BRI, FI, MEL, STU.

Morphology: Fronds boat-shaped with the greatest width at the surface of the water (no translucent edge visible from above), (0.5)1.0-1.3(1.5) mm long, (0.3)0.5-0.7(0.8) mm wide; 11/3-12/3 times as long as wide, 2-3 times as deep as wide, with 60-120 stomata, intensely green and shiny on the surface; 3-5 layers of small cells below the upper surface which are much smaller than the cells at the bottom of the frond; the lower submerged part of the frond shifted towards the distal side. Stigma with pigment cells.

Distribution (Fig. 1a): Southern Australia (NSW, South Australia, Tasmania, Victoria), New Zealand (North Island: region of Wellington; South Island: region of North Canterbury).

In permanent waters of warm temperate regions with mild winters.

Wolffia angusta Landolt (Veröff. Geobot. Inst. ETH, Stiftung Rübel, Zürich, 70, 29; 1980). Figs. 2b, 3b (see also LANDOLT 1986, plate XIV, c., d., p.526)

Type locality: Australia, NSW, NE of Newcastle, ca. 8 km W of Seaham; leg. B.G. Briggs and L.S. Johnson, 15.3.1970. ETH collection No. 7274.

Holotype (LANDOLT 1980): ZT. Isotype: NSW.

Morphology: Fronds boat-shaped with the greatest width at the surface of the water (nearly no translucent edge visible from above, but distinct green margins present), 0.5-0.8 mm long, 0.2-0.4 mm wide, 12/3-21/2 times as long as wide, 2-3 times as deep as wide, with 8-20 stomata, whitish green on the surface with more intensely green margins; the cells below the epidermis much smaller than the cells at the bottom of the frond; the lower submerged part of the frond shifted towards the distal side. Stigma with pigment cells.

Distribution (Fig. 1b): Australia (except Tasmania, southern Victoria, dry regions of the Center and West); Singapore, Malaysia (western coastal area);

India (Calcutta? the sample from the herb. CAL might belong to *W. neglecta*).

In permanent waters of warm temperate to tropical regions with relatively warm winters.

***Wolffia neglecta* sp. nov.** Figs. 2c, 3c

Latin diagnosis: Frondes summa aqua natantes, cumbulaeformes cum magna latitudine longitudineque in summa aqua, 0.6-0.9 mm longae, 0.4-0.6 mm latae, $1\frac{1}{2}$ - $1\frac{2}{3}$ partibus longiores quam latae, superficie viride uniformiter. Stomata 8-20. Stigma cum cellulis pigmentatis. Differt ab *W. angusta* superficie viride (nec alboviride cum margine viride), ab *W. globosa* et *W. cylindracea* stigma cum cellulis pigmentatis. Habitat in Pakistania, Sri Lanka et India occidentalis.

Type locality: Pakistan, Karachi, Rashid Minhas Road near Nipa; leg. A. Hussain, 4.2.1986. ETH collection No. 8917.

Holotype: ZT. *Isotype:* KUH

Morphology: Fronds ellipsoid to boat-shaped, with the greatest width at the surface of the water (nearly no translucent edge visible from above), 0.6-0.9 mm long, 0.4-0.6 mm wide, $1\frac{1}{2}$ - $1\frac{2}{3}$ times as long as wide, $1\frac{1}{2}$ -2 times as deep as wide, with 8-20 stomata, intensely green on the surface; the cells below the epidermis much smaller than the cells at the bottom of the frond; the lower submerged part of the frond pointing straight down. Stigma with pigment cells.

Distribution (Fig. 1c): India (Rajasthan; Calcutta ?, the sample might belong to *W. angusta*); Pakistan (Karachi, Hyderabad); Sri Lanka (Southern Prov.).

Herbarium specimens seen: *Pakistan:* Karachi, Drigh Colony Railway Station (10.4.1985, 4.2.1986, 21.8.1993, A. Hussain : KUH, ZT); Karachi, University Camp, near Biological Research Centre (R. Yusuf 75: KUH); Karachi, Rashid Minhas Road near Nipa (A. Hussein 4.2.1986: ZT); Hyderabad, National Highway to Matari (S. Omer and R. Yusuf 1944: KUH). *India:* Rajasthan, Kota (Cook and Frey 4584); ? West Bengal, Calcutta, Royal Bot. Garden (28.7.1949, Nashar: CAL)? (this specimen might belong to *W. angusta*). *Sri Lanka*, Southern Prov., Tongalla - Beliatta (1985, Kasselman 1985: ZT). Probably only in seasonal waters of winter-dry subtropical to tropical regions.

***Wolffia arrhiza* (L.) Horkel ex Wimmer** (Fl. Schles. ed.3, 140; 1857) Figs. 2d, 3d (see also LANDOLT 1986, plate XV, a., b., p. 527)

Lemna arrhiza L. (Mant. 2, 294; 1771)
Wolffia michelii Schleid. (Beitr. Bot. 233; 1844)
Wolffia delilii Miq. (Nederl. Kruidk. Arch. 3, 428; 1855)
Bruniera vivipara Franch. (Billotia 1, 25; 1864).

Type locality: There is no sample of *W. arrhiza* in the Linnaean herbaria of London, Stockholm and Helsinki. The name *Lemna arrhiza* dates back to Micheli. Therefore, it is obvious to choose a specimen from the herbarium of Micheli as a neotype. There are two sheets neither of which has any indication of origin. However it is supposed that they were collected in Italy (perhaps near Pisa which is a well-known station of *W. arrhiza*). The smaller sample is proposed as neotype.

Neotype: The smaller sample of *Lemna arrhiza* in the herbarium of Micheli in Firenze (FI).

Morphology: Fronds spherical to ellipsoid, with the greatest width just below the surface of the water (laterally a translucent edge visible from above), (0.5) 0.7-1.3 (1.5) mm long, (0.4) 0.6-1.0 (1.2) mm wide, 1-1 1/3 as long as wide, 1-1 1/3 as deep as wide, with (20) 30-120 stomata; intensely green and mostly shiny on the surface; the cells below the epidermis smaller than the cells at the bottom of the frond; the lower submerged part of the frond pointing straight down. Stigma without pigment cells.

Distribution (Fig. 1d): Europe (except northern and north eastern part); Africa (except the very dry regions); Asia: Iran, Israel, Georgia, India (Jammu and Kashmir), introduced in Japan (Okinawa: Otaki in lit.); South America: Brazil (region of Rio de Janeiro); North America: introduced in southern California (ARMSTRONG 1989).

In permanent waters of temperate to tropical regions.

Wolffia cylindracea Hegelm. (Lemnaceen, 123; 1868).

Figs. 2e, 3e (see also LANDOLT 1986, plate V, e.,f., p. 527, named as *W. globosa*)

Type collection: Angola, Distr. Libongo, in stagnis limpidissimis montium petrosorum de Libongo; leg. F. Welwitsch, end of September 1858.

Holotype (LANDOLT 1986): STU. *Isotype*: BM.

Morphology: Fronds ellipsoid, with the greatest width just below the surface of the water (laterally and proximally a translucent edge visible from above) (0.4) 0.5-0.8 (0.9) mm long, (0.3) 0.4-0.6 (0.7) mm wide, 1 1/4-1 1/2 times as long as wide, 1-1 1/2 times as deep as wide, with 15-30 stomata, rather intensely

green on the surface; cells below the epidermis smaller than the cells at the bottom of the frond; the lower submerged part of the frond pointing straight down. Stigma without pigment cells.

Distribution (Fig. 1e): Africa (south of the equator).

In seasonal waters of winter-dry subtropical to tropical regions.

Wolffia globosa (Roxburgh) Den Hartog & Van der Plas (*Blumea* 18, 367; 1970). Figs. 2f, 3f (see also LANDOLT 1986, plate XII e./3, p. 524)

Lemna globosa Roxburgh (Fl. Ind. 3, 565; 1832)

Wolffia schleideni Miq. (Nederl. Kruitk. Arch. 3, 428; 1855)

No specimens seen by Roxburgh could be found (neither in herb. BR nor in herb. CAL where plants of Roxburgh are present). Therefore a specimen was chosen as neotype which originates from a region where Roxburgh collected plants and which was studied in culture morphologically, cytologically and alloenzymatically.

Type collection: India, West Bengal, Rajnagar; leg. S.N. Mitra 979, Nov. 13, 1969. ETH collection No. 7214.

Neotype: ZT

Morphology: Fronds ellipsoid, with the greatest width distinctly below the surface of the water (all around a translucent edge visible from above), (0.4)0.5-0.8(0.9) mm long, (0.3)0.4-0.6 mm wide, 1 1/3-1 2/3 times as long as wide, 3/4 - 1 1/3 as deep as wide, with 8-25(35) stomata, pale green on the surface; cells below the epidermis only slightly smaller than the cells at the bottom of the frond; the lower submerged part of the frond pointing straight down. Stigma without pigment cells.

Distribution (Fig. 1f): Southern and eastern Asia (northwards to the Himalaya, southern and eastern China, Honshu; westwards to western India); Hawaii; North America: California (Armstrong 1984), Florida (introduced).

In permanent waters of warm temperate (winter- mild) to tropical regions.

Wolffia columbiana Karsten (Bot. Unters. 1, 103; 1865). Figs. 2g, 3g (see also LANDOLT 1986, plate V, c., d., p. 527).

Type collection: Columbia, Santa Marta; leg. H. Karsten s.d.

Lectotype (LANDOLT 1986): STU.

Morphology: Fronds nearly spherical, with the greatest width distinctly below the surface of the water (all around a translucent edge visible from above), (0.5)0.7-1.2(1.4) mm long, (0.5)0.6-1.1(1.2) mm wide, 1-1 1/3 as long

as wide, 1-1 1/3 as deep as wide, with rounded tip, with 1-15 (30) stomata, pale green on the surface; cells below the surface not smaller than the cells at the bottom of the frond; lower submerged part of the frond pointing straight down. Stigma without pigment cells.

Distribution: North America (northwards to southern Canada: DORE 1957, LOOMAN 1983 as *W. arrhiza*; GRIFFITH and GRIFFITH 1990); South America (southwards to southern Buenos Aires and Ecuador).

In permanent waters of temperate to tropical regions.

SUMMARY

The section *Wolffia* of the genus *Wolffia* (*Lemnaceae* family) consists of 7 morphologically very similar species with different ecological (Table 1) and geographical (Fig. 1) distribution and relatively low genetic identity: *W. australiana*, *W. angusta*, *W. neglecta*, *W. arrhiza*, *W. columbiana*, *W. globosa* and *W. cylindracea*. *Wolffia neglecta* from Southern Asia is newly described.

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Address of the author: Elias LANDOLT
Geobotanisches Institut ETH
Zürichbergstr. 38
CH-8044 Zürich

